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Woodbridge, VA 22192-575.5
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Since 1973
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December 29, 2020

David Edwards, Director
Division of Animal Feeds (HFV- 220)
Center for Veterinary Medicine
Food and Drug Administration
7519 Standish Pl.
Rockville, MD 20855

Subject: Filing of Animal GRAS Notification for Xylanase prepared from *Komagataella phaffii* expressing the gene encoding xylanase from *Orpinomyces sp.* for the use in swine and poultry feed

Notifier: BioResource International, Inc.
4222 Emperor Blvd., Suite 460
Durham, NC USA 27703

Dear Dr. Edwards:

On behalf of BioResource International, Inc., I am providing a copy of their animal General Recognized as safe notice for the use of Xylanase prepared from *Komagataella phaffii* expressing the gene encoding xylanase from *Orpinomyces* sp. for use in poultry and swine diets. The submission is compliant with 21CFR 570.210-255. The GRAS conclusion is based on scientific procedures.

Should you have any questions on the filing, please contact me directly.

Sincerely,

Kristi Smedley

Digitally signed by Kristi Smedley
DN: cn=Kristi Smedley, o=Center for Regulatory Services, Inc., ou, email=smedley@cfr services.com, c=US
Date: 2020.12.29 14:34:08 05'00'

Kristi O, Smedley
Consultant to BioResource International, Inc.

RECEIVED DATE
JAN 11, 2021

Cc: Rasha Qudsieh, BRI

ATTACHMENTS:

BioResource International, Inc. Letter of Representation
GRAS Notice Xylanase
All Reference materials

Cerrito, Chelsea

From: Kristi Smedley <smedley@cfr-services.com>
Sent: Wednesday, December 30, 2020 11:51 AM
To: Animalfood-premarket
Subject: New AGRN--Xylanase filed
Attachments: SmedleyGRASRepresentationAuthorizationLetter.pdf

Good Morning,

I have received confirmation that FEDEX has delivered the new Animal GRAS notice for Xylanase as filed by BioResource International, this morning.

I just noted that the letter of representation was not included on the CD. I am attaching the signed letter of representation.

I apologize for that oversight.

Kristi O. Smedley, Ph.D.

Center for Regulatory Services, Inc.
5200 Wolf Run Shoals Rd.
Woodbridge, VA 22192

Ph. 703-590-7337
Cel (b) (6)
Fax 703-580-8637

December 18, 2020

David Edwards
Director
Division of Animal Feeds, HFV 220
Center for Veterinary Medicine
Food and Drug Administration
7519 Standish Place
Rockville, MD 20855

Subject: Authorization for Representation—Kristi O. Smedley for
GRAS Notice submission for xylanase enzyme (matter)
BioResource International, Inc. (company)

Dear Dr. Edwards:

We are authorizing Kristi Smedley to act on our behalf to represent BioResource International, Inc. in the matter of submitting GRAS notice for BioResource International xylanase enzyme.

Her contact information:

Kristi Smedley, Ph.D.
Center for Regulatory Services, Inc.
5200 Wolf Run Shoals Rd.
Woodbridge, VA 22192
703 590 7337
Fax 703 580 8637
Smedley@cfr services.com

Please contact the undersigned with any questions.

Sincerely,

(b) (6)

Jeng-Jie Wang

Senior Vice President and Chief Technology Officer

GRAS Notice for:
Endo-1,4-β-xylanase from a genetically modified strain of *Komagataella phaffii (Pichia pastoris)* for use in poultry and swine feed

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Part 1: Signed Statements and Certification

1.1. GRAS Notice Submission

BioResource International, Inc. (BRI) is hereby establishing an independent GRAS (Generally Recognized As Safe) conclusion in accordance with subpart E of part 570.

1.2. Name and Address of Notifier

Firm:	BioResource International, Inc.
	4222 Emperor Blvd., Suite 460,
	Durham, NC USA 27703
Contact person:	Dr. Jeng-Jie Wang, Sr. VP and Chief Technology Officer
	Email: jwang@briworldwide.com
	Phone: +1-919-993-3389 Ext. 202

1.3. Name of Notified Substance

The appropriately descriptive term for this notified substance is: endo-1,4-β-xylanase prepared from *Komagataella phaffii* formerly classified as *Pichia pastoris* expressing the gene encoding xylanase from *Orpinomyces sp*. This specific dried fermentation product is an enzyme commonly known as xylanase, other designations include β-1,4-xylanase, endo-1,4-xylanase, β-xylanase, endo-(1→4)-β-xylanohydrolase; endo-1,4-β-D-xylanase, β-D-xylanase.

Chemical Name: Xylanase, endo-1,4-β

CAS Registry Number: 9025-57-4

E.C. Number: 3.2.1.8

1.4. Intended Conditions of Use

BioResource International's *Komagataella phaffii (P. pastoris)* enzyme preparation (endo-1,4-β-xylanase or xylanase) is used for the hydrolysis of xylans , a component of hemicellulos in poultry and swine feed.

1.5. Statutory Basis for GRAS status

BioResource International's GRAS determination for the intended use of xylanase enzyme is based on scientific procedures.

1.6. Premarket Exempt Status

The intended use of xylanase is not subject to the premarket approval requirements of the Federal Food, Drug and Cosmetic Act based on BRI's determination that it is GRAS.

1.7. Data Availability

The package provides a summary of the information utilized to support BioResource International's GRAS conclusion of the notified substance. Complete data and information the GRAS conclusion is based upon is available to the Food and Drug Administration for review and copy during customary business hours at BioResource International, Inc. at the address above, or will be sent to FDA upon request.

1.8. Freedom of Information Act Statement

This GRAS notice contains confidential business information (CBI) exempt from disclosure under the Freedom of Information Act per 5 U.S.C. § 552(b)(4). Information listed in Appendix 1 through Appendix 11 are considered confidential.

1.9. Certification

To the best of our knowledge, this GRAS notice is a complete, representative, and balanced submission that includes all available information, known to us and pertinent to the evaluation of the safety and GRAS status of the intended use of xylanase in poultry and swine feed.

1.10. Name, Position, and Signature of Notifier

(b)(6)

December 29th 2020

_____ _____

Dr. Jeng-Jie Wang — Date

Sr. VP and Chief Technology Officer

Part 2: Identity, method of manufacture, specifications, and physical or technical effect

2.1. Identity of the Notified Substance

The trade name of BRI's *Komagataella phaffii (Pichia pastoris)* endo-1,4-β-xylanase preparation, described below, is Xylamax. The product is a low odor, water soluble powder with a light gray color. The carrier for the enzyme is limestone. Limestone is affirmed as GRAS for human food use at 21 CFR § 184.1409, and is authorized for animal feed use by the AAFCO, OP, Section 6 Definition 57.9. The xylanase enzyme is extracted and purified after the fermentation process of a genetically modified strain of *Komagataella phaffii (P. pastoris)* expressing a xylanase from *Orpinomyces*. The host organism, *Komagataella phaffii (P. pastoris)*, has a long history of safe industrial use to produce enzymes used in human and animal feed and has been routinely used as a host for recombinant enzymes.

The *Komagataella phaffii (P. pastoris)* xylanase enzyme preparation that is the subject of this GRAS notification is an endo-1,4-β-xylanase as defined by the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (IUBMB).

Systematic Name	Endo-1,4-β-xylanase
CAS Registry Number	9025-57-4
E.C. Number	3.2.1.8
Chemical Name	Xylanase, endo-1,4-β
Common Name	β-1,4-xylanase, endo-1,4-xylanase, β-xylanase, endo-(1→4)-β-xylanohydrolase; endo-1,4-β-D-xylanase, β-D-xylanase

2.1.1. Enzyme Specificity

The enzyme endo-1,4-β-xylanase is a glycosidase which hydrolyzes 1→4-β-D-xylosidic linkages in xylan (E.C. 3.2.1.8, CAS 9025-57-4). The endo-1,4-β-xylanase is used for partial or extensive hydrolysis of the polysaccharide xylan, in animal feedstuffs (such as corn, barley, rye, wheat, grain sorghum, triticale, oats). These xylans are key components of hemicellulose, the major component of plant cell walls. Xylans are a type of non-starch polysaccharide (NSP), found as a structural component of the cell walls of grains. NSP content in feed grains can represent up to one-quarter of the total dry content of poultry and swine feed. Xylans are the most prevalent type of NSP in poultry and swine feed grains. A typical plant-based feed may contain ~2.3–3.8% of xylans (Knudsen, 2014; Jaworski et al., 2015). The substrate for xylanase is naturally occurring in animal feeds.

2.1.2. Amino Acid Sequence and Molecular Mass

(b) (4)

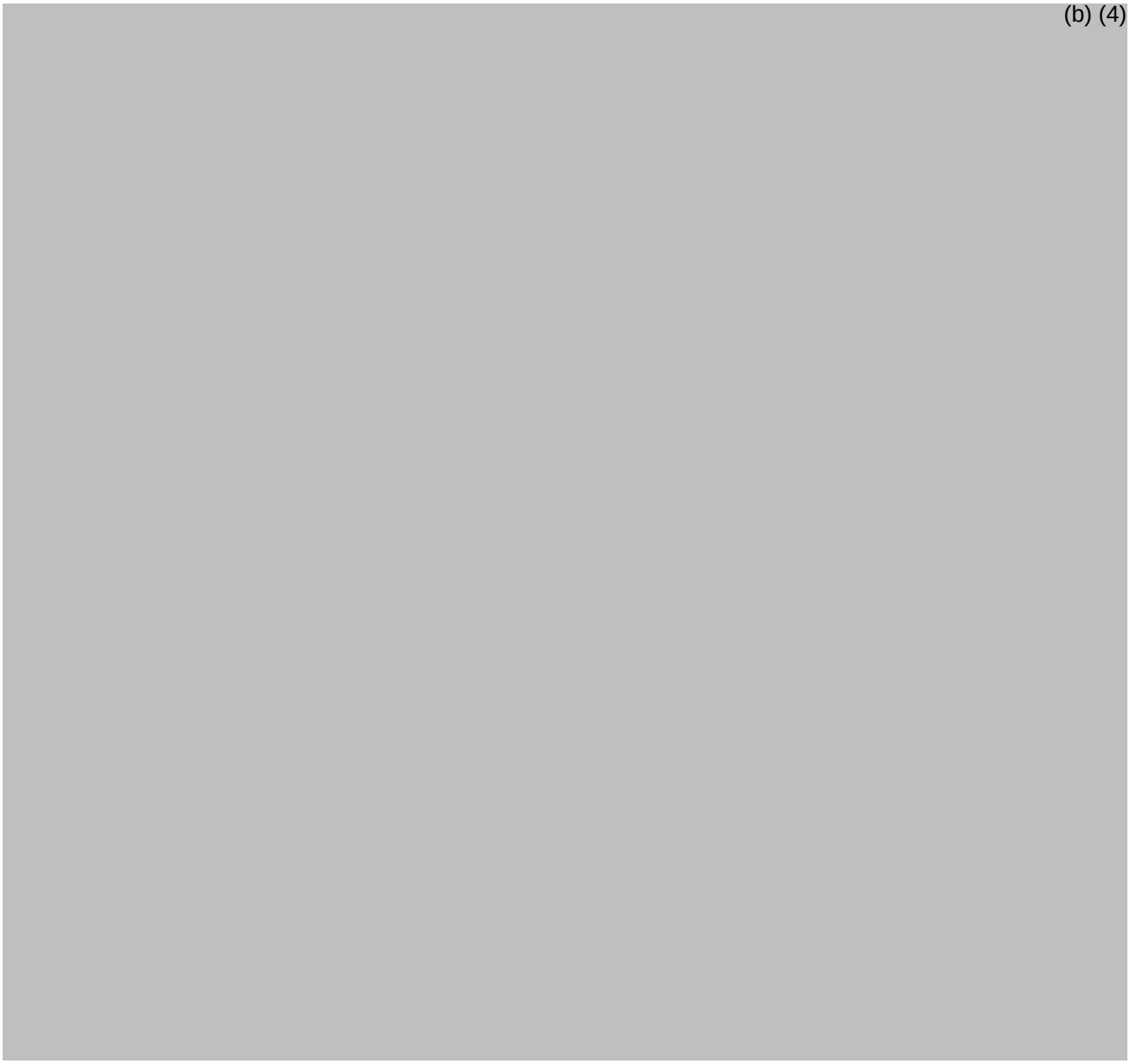
Figure 1. Full length amino acid sequence of xylanase with signal peptide in box.

(b) (4)

Sequence comparison with representative endo-β-1,4-xylanases demonstrates that the xylanase is comparable in size and composition with no extraneous amino acid sequences (Figure 2).

Figure 2. (b) (4)

.



2.1.3. Enzyme Activity

The main enzymatic activity of the xylanase enzyme preparation is endo-1,4-β-xylanase. This feed enzyme catalyzes the hydrolysis of xylosidic linkages in an arabinoxylan backbone (and other β-1,4-linked xylans) depolymerizing arabinoxylan into smaller oligosaccharides.

Catalyzed reaction: Endohydrolysis of (1->4)-beta-D-xylosidic linkages in xylans

Reaction product: xylose

Site of enzyme activity: feed/digesta

The method to analyze the activity of the xylanase enzyme is company specific and capable of (b) (4) activity as defined by IUBMB classification (Appendix 19). (b) (4)

2.2. Identity of the Source

2.2.1. Production Strain

The production organism is a strain of *Komagataella phaffii (Pichia pastoris)* (b) (4) (b) (4), (b) (6)), which has been genetically modified by expression of the endo-β-1,4-xylanase gene from a strain of *Orpinomyces* for the production and secretion of xylanase enzyme. *Komagataella phaffii* (*Pichia pastoris*) (b) (4) was derived from *Komagataella phaffii* CBS (b) (4), which is classified as a Biosafety Level 1 (BSL 1) microorganism by the American Type Culture Collection (ATCC) based on assessment of potential risk using U.S. Department of Public Health guidelines with assistance provided by ATCC scientific advisory committees, and is also complies with the OECD(Organization for Economic Co-operation and Development) criteria for Good Industrial Large-Scale Practice (GILSP) worldwide. It also meets the criteria for a safe production organism as described initially by Pariza and Foster (1983) and Later by Pariza and Johnson (2001).

2.2.2. Host Strain

The host microorganism *Komagataella phaffii* (b) (4) was derived from *Komagataella phaffii* (*Pichia pastoris*) BG08 by removing cytoplasmic killer plasmids using Hoechst dye selection. *Komagataella phaffii* (*Pichia pastoris*) (b) (4) was a single colony isolated from the *Komagataella phaffii* CBS (b) (4). *Pichia pastoris* has been reassigned to the genus *Komagataella* (Kurtzman, 2009). This change is reflected in the ATCC strain documentation and subsequent submissions elsewhere in the literature. The taxonomic information of *Komagataella phaffii* is as follows:

Komagataella phaffii (Pichia pastoris) taxonomy:

Phylum:	Ascomycota
Class:	Saccharomycetes
Order:	Saccharomycetales
Family:	Saccharomycetaceae
Genus:	*Komagataella*
Species:	(*pastoris) phaffii*
Previous or another name:	*Pichia pastoris*

Komagataella phaffii (Pichia pastoris) has a history of safe use as an animal feed additive (CFR 21 Sec. 573.750 *Pichia pastoris* dried yeast) as well as an enzyme production strain see Phytase as described in AAFCO Section 6, table 30.1 Also it is a source of enzymes intended for human use (GRAS Notice 000204). For example, *P. pastoris* GS115 (ATCC® 20864™) is identified as a nontoxigenic, non-pathogenic, histidinol dehydrogenase (HIS4 gene) mutant derived from strain *P. pastoris* NRRL Y-11430 (ATCC® 76273™, also known as CBS (b) (4)). The strain *P. pastoris* SMD 1168, derived from strain *P. pastoris* GS115, has been cited in GRAS Notice 000204 (FDA, 2006) for safe use in enzyme production as well as GRN 764 (https://www.fda.gov/media/126904/download). Additionally, a soybean leghemoglobin protein derived from *P. pastoris* has been cited as safe under GRAS Notice 000540 (FDA, 2015). Per the definitive source of yeast taxonomy (Kreger-van Rij, 1984) as well as a thorough literature search, there are no indications that *Komagataella phaffii (Pichia pastoris)* has been associated with animal or human illness. The use of *Komagataella phaffii (Pichia pastoris)* as a host expression should therefore pose no safety risks in the proposed application. Given the historical use of this strain of *Komagataella phaffii (Pichia pastoris)* in expression systems, production of toxic metabolites is not a

concern. Furthermore, *Komagataella phaffii (Pichia pastoris)* has been found to be safe by EFSA for enzyme products (EFSA Journal 2014; 12(5):3667; EFSA BIOHAZ Panel, 2013). A database literature search showed no reports of *Komagataella phaffii (Pichia pastoris)* as pathogenic, toxic, or unsafe. The Google Scholar search for *Komagataella phaffii* and toxic yielded 1040 hits, the top 5 ones are listed in the summary table below. These findings mainly address engineering of the *Komagataella phaffii (Pichia pastoris)*. In an attempt to provide antibacterial properties to *Komagataella phaffii (Pichia pastoris)*, genetic engineering was used to introduce toxin genes so *Komagataella phaffii (Pichia pastoris)* where these toxins can be used as natural antimicrobials. In the production of Xylanase, BRI used a naturally occurring *Komagataella phaffii (Pichia pastoris)*, as such this potential safety concern would not be an issue. Summary of search is shown in Table below.

Database	Date	Keywords	Hits	Results (Top 5)
PUBMED	10/31/20	*Komagataella phaffii* AND pathogen	0	None
PUBMED	10/31/20	*Komagataella phaffii* AND pathogenic	4	• Production of a lyophilized ready-to-use yeast killer toxin with possible applications in the wine and food industries. Int J Food Microbiol 2020; 335: 108883. • YEASTRACT+: a portal for cross-species comparative genomics of transcription regulation in yeasts. Nucleic Acids Res 2020; 48(D1):D642-D649 • Evolution from covalent conjugation to non-covalent interaction in the ubiquitin-like ATG12 system. Nat Struct Mol Biol 2019; 26:289-296 • Biotechnological exploitation of Tetrapisispora phaffii killer toxin: heterologous production in Komagataella phaffii (Pichia pastoris) Appl Microbiol Biotechnol. 2017; 101(7):2931-2942.
PUBMED	10/31/20	*Komagataella phaffii* AND toxic	12	• Safety and efficacy of fumonisin esterase from Komagataella phaffii DSM 32159 as a feed additive for all animal species. EFSA J. 2020;18(7):e06207 • Safety and efficacy of APSA PHYTAFEED ® 20,000 GR/L (6-phytase) as a feed additive for pigs for fattening. EFSA J. 2020;18(1):e05979 • Safety and efficacy of APSA PHYTAFEED ® 20,000 GR/L (6-phytase) as a feed additive for piglets (suckling and weaned) and growing minor porcine species. EFSA J. 2019;17(11):e05894 • Safety and efficacy of APSA PHYTAFEED ® 20,000 GR/L (6-phytase) as a feed additive for turkeys for fattening, turkeys reared for breeding and minor poultry species. EFSA J. 2019;17(11):e05893. • Engineered Deregulation of Expression in Yeast with Designed Hybrid-Promoter Architectures in Coordination with Discovered Master Regulator Transcription Factor. Adv Biosyst. 2020; 4(4):e1900172
PUBMED	10/31/20	*Komagataella phaffii* AND	0	None

		unsafe		
Google Scholar	12/15/20	*Komagataella phaffii* AND toxic	1040	• Biotechnological exploitation of Tetrapisispora phaffii killer toxin: heterologous production in Komagataella phaffii (Pichia pastoris) Appl Microbiol Biotechnol. 2017 Apr;101(7):2931-2942. • Modulation of acetate utilization in Komagataella phaffii by metabolic engineering of tolerance and metabolism. Biotechnol Biofuels. 2019 Mar 21;12:61. • A Synthetic Malonyl-CoA Metabolic Oscillator in Komagataella phaffii. ACS Synth Biol. 2020 May 15;9(5):1059-1068. • N-Glycosylation Engineering to Improve the Constitutive Expression of Rhizopus oryzae Lipase in Komagataella phaffii. J Agric Food Chem. 2017 Jul 26;65(29):6009-6015. • Recombinant O-mannosylated protein production (PstS-1) from Mycobacterium tuberculosis in Pichia pastoris (Komagataella phaffii) as a tool to study tuberculosis infection. Microb Cell Fact. 2019 Jan 19;18(1):11.

The original strain of (b) (4), *Komagataella phaffii* (CBS (b) (4)) has another name; strain Y-11430, which is deposited in the collection at the Northern Regional Research Laboratories (NRRL). The lineage of *Komagataella phaffii* strain CBS (b) (4), or NRRL Y-11430 is detailed below. The first *Komagataella phaffii* strains were isolated from an oak tree and a chestnut tree and were deposited in the collection at the Northern Regional Research Laboratories (NRRL). Yeast strains screened by Phillips Petroleum for growth on methanol included two *Komagataella phaffii* strains, designated NRRL Y-1603 (*Komagataella pastoris* (ATCC® 28485™), and NRRL YB-4290 (NCAUR, 2006). Phillips Petroleum identified a *Komagataella phaffii* strain with improved growth characteristics. The strain was designated *Pichia pastoris* (Culture 21-1) and deposited at NRRL, as NRRL Y-11430 (Wegner, 1986; Patent 4617274). This strain is now available from ATCC as 76273 (*Komagataella phaffii* (ATCC® 76273™)).

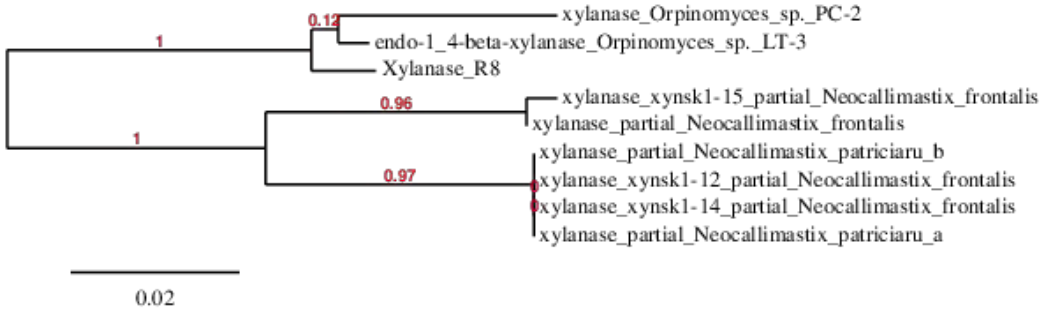
2.2.3. Donor Strain

The xylanase gene described in this submission was isolated from mixed genomic DNA of rumen fungi. It is not possible to recover the original donor phylogenetic information because the DNA extraction method has broken the links between the phylogenetic genes (b) (4)) and the xylanase gene. The best way to identify the origin of the xylanase gene is toblast the sequences and find the closest phylogenetic neighbor, and then judge the phylogenetic information from the closest neighbor's owner organism.

BioResource International has performed the blast of the xylanase gene and found that its closest neighbor was endo-1,4-beta-xylanase from *Orpinomyces* sp. LT-3. The sequence identity comparison to BRI's xylanase was 98%. The next closest was a xylanase from *Orpinomyces* sp. PC-2, with a sequence identity comparison of 96%. The third closest was a xylanase from *Neocallimastix patriciarum*, where the sequence identity comparison suddenly dropped to 88%. The top 8 sequences were selected and a phylogenetic tree was

created (Figure 3, made with http://www.phylogeny.fr/). From the tree, it is very clear that BRI's xylanase gene is close to its *Orpinomyces* relatives and is distant to its *Neocallimastix* counterparts. Therefore, it can be concluded that BRI's xylanase gene is from the *Orpinomyces* genera.

Figure 3. Xylanase gene phylogenic tree



Based on BLAST sequence analysis, the isolated gene is an endo-1,4-β-xylanase from a strain of *Orpinomyces*. The phylogeny of this xylanase gene sequence is as follows:

Phylum: Neocallimastigomycota

Class: Neocallimastigomycetes

Order: Neocallimastigales

Family: Neocallimastigaceae

Genus: *Orpinomyces*

2.2.4. Expression Cassette **(CONFIDENTIAL)**

(b) (4)

(b) (4)

.

(b) (4)

2.2.4.1. Xylanase gene amino acid sequence **(CONFIDENTIAL)**

Figure 4. (b) (4).

(b) (4).

Figure 5. (b) (4)

2.2.4.2. Starch binding domain sequences **(CONFIDENTIAL)**

(b) (4)

(b) (4)

.

2.2.4.3. MFα signal peptide sequence **(CONFIDENTIAL)**

(b) (4)

(b) (4)

.

2.2.5. Codon-Optimized Endo-1,4-β-xylanase Expression Plasmid Construction **(CONFIDENTIAL)**

(b) (4)

(b) (4)

.

Figure 6. Fusion gene sequence **(CONFIDENTIAL)**

2.2.6. Construction of the Recombinant Production Strain **(CONFIDENTIAL)**

2.2.7. Structure of Vector Remaining in the Production Strain **(CONFIDENTIAL)**

(b) (4)

(b) (4)

Figure 7. (b) (4) **(CONFIDENTIAL)**


(b) (4)


(b) (4)

(b) (4)

2.2.8. Bioinformatic Analyses of Parent and Host Whole Genome Sequences **(CONFIDENTIAL)**

(b) (4)

(b) (4)

2.2.9. ORF analysis of the genomic region containing the inserted sequences **(CONFIDENTIAL)**

(b) (4)

2.2.10. Stability of the Introduced Genetic Sequences

The genetic stability of the production strain was determined by Southern Blot analysis. The master cell bank strain was compared to the production strain isolated after four fermentations. DNA was prepared from these cells and the integration pattern of the xylanase gene examined by Southern Blot using digoxigenin-labelled SBD-xylanase gene as probe. The band profiles in each of the isolated samples was identical to one another indicating that the production strain is genetically stable. Report attached as Appendix 5.

2.2.11. Antibiotic Resistance Gene

(b) (4).
(b) (4)

An antibiotic transferability assay using *E. coli* as the target organism was used to verify that no resistance determinants could be transferred to non-resistant entities. The results of this work indicated that there is no evidence of transferable antibiotic resistance gene DNA elements in xylanase product. Details of this procedure are in Appendix 4.

2.2.12. Presence of Production Strain DNA in Finished Product

The presence of the production strain DNA is an established specification for the commercial product. As part of the quality assurance program, the presence of the xylanase production strain DNA is tested in received lots. Three separate lots of product (Lot #s XY20203; XY20210; and XY20217) were tested for presence of production strain DNA in final product, and report is provided in Appendix 6 to confirm that no DNA was present in the final product.

2.2.13. Spill over assessment

Information listed in Appendix 1 and Appendix 2 was used to assess any possible unintended impacts the genetic material introduction may have introduced. The whole genome sequencing and bioinformatics were performed for *Komagataella phaffii* strain (b) (4); the parent strain of the genetically modified *Komagataella phaffii* production strain (Appendix 1). The analysis showed that *Komagataella phaffii* strain (b) (4) was not genetically modified and qualified as a Qualified Presumption of Safety (QPS) when used for enzyme production. Then, whole genome sequencing was used for taxonomic identification and description of genetic modification in the *Komagataella phaffii* production strain (Appendix 2). Based on this analysis, there was one genetic modification cassette of 10 kb in the production strain genome located in chromosome 4. The location of

this modification is not expected to interrupt any metabolic pathways thus not expected to raise any safety concerns.

2.3. Method of Manufacture **(CONFIDENTIAL)**

(b) (4)

(b) (4)

2.3.1. Raw Materials **(CONFIDENTIAL)**

(b) (4)

(b) (4)

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2.3.2. Cell Bank Development and Maintenance **(CONFIDENTIAL)**

(b) (4)

2.3.3. Fermentation Process **(CONFIDENTIAL)**

(b) (4)

2.3.4. Recovery Process **(CONFIDENTIAL)**

(b) (4)

2.3.5. Formulation and Packaging **(CONFIDENTIAL)**

(b) (4)

(b) (4)

2.4. Composition and Specifications of the GRAS substance

2.4.1. Quantitative Composition

The composition of the enzyme product is as detailed in Table 1. Various commercial formulations exist, with a minimum enzyme activities of 150,000 XU/g. one XU (enzyme activity unit) is defined as the amount of enzyme needed for the release of 1 nanomole of reducing sugars (xylose equivalents) per second from 0.5% beechwood xylan at 50°C in 50 mM trisodium citrate buffer pH 6.0. As international units (IU), one XU unit is 0.06 micromole/minute.

Table 1. Product composition

Component	CAS Number	Percent (w/w)
Calcium Carbonate ($CaCO_3$)	1317-65-3	70-90
Xylanase, endo-1,4-β	9025-57-4	10-30

2.4.2. Specifications

The final xylanase enzyme product is analyzed in accordance with the general specifications for enzyme preparations used in food processing as established by the Joint FAO/WHO Expert Committee on Food Additives (JEFCA) in 2006. Specifications for the enzyme preparation and frequency of testing are illustrated in Table 2. Three different batches of endo-1,4-β-xylanase were assessed based upon these specifications and are summarized in Appendix 12.

Table 2. Enzyme production specification and frequency of testing

Property	Specification	Method	Test Frequency
Color	Light Grey powder	Visual inspection	Per lot
Uniformity	No visible impurities	Visual inspection	
Moisture	< 3 %	Loss on drying assay (BRI SAP)	
Xylanase activity	≥ 150,000 XU/g	DNS reducing sugar assay (BRI SAP)	
Mold	$\leq 10^3$ CFU/g	E-Cultural (Chromogenic Media) / FDA BAM Chapter 18	
Coliforms	≤10 CFU/g	E-Cultural (Non-chromogenic Media) / VRB+MUG FDA BAM Chapter 4	
Escherichia coli	≤ 10 CFU/g		
Salmonella	Not detected per 25 g	RT-PCR AOAC-RI 121501	

Heavy metals: Arsenic	< 2 mg/kg	ICP-MS AOAC 2013.06	Annually
Heavy metals: cadmium	< 2 mg/kg		
Heavy metals: mercury	< 0.5 mg/kg		
Heavy metals: lead	< 10 mg/kg		
Mycotoxins: aflatoxin B1, B2, G1, G2	≤ 0.5 µg/kg	LC-MS/MS: detection in ppb (1 ppb = 1 µg/kg)	
Mycotoxins: fumonisin B1 and B2	≤ 25 µg/kg		
Mycotoxins: zearalenone	≤ 30 µg/kg		
Mycotoxins: deoxynivalenol	≤ 100 µg/kg		
Mycotoxins: Ochratoxin	≤ 1 µg/kg		
Dioxins	≤ 1 ng/kg TEQ	QL005: HR GC/MS – PCDD/F (EPA 1613B October 1994)	
Dioxin & Dioxin-Like PCBs	≤ 1.5 ng/kg TEQ	QL005: HR GC/MS – PCDD/F (EPA 1613B October 1994)	
		QL006: HR GC/MS – 12 WHO PCBs (EPA 1668 mod)	
Non-Dioxin-Like PCBs	≤ 10 µg/kg TEQ	GC-HRMS	
Genetically modified organisms	Absent	PCR	

2.5. Intended Technical Effects and Use

This section is specific to the requirements as provided in 21 CFR 570.230(d):

> 21 CFR 570.230 (d) When necessary to Demonstrate safety, relevant data and information bearing on the physical or other technical effect the notified substance is intended to produce, including the quantity of the notified substance required to produce such effect.

Xylanase is an enzyme that is known to hydrolyze xylans, which a component of hemicellulose found in most plant stuffs. The American Association of Feed Control Officials Publications, Chapter 6 (section 30.1) lists a number of sources of Xylanase that are used to hydrolyze xylans in typical animal feedstuffs. The xylanase that is the subject of the GRAS notice is intended to hydrolyze hemicelluloses which may escape digestion, hence permitting additional nutritional value (typically energy from carbohydrates) from feed ingredients that are typically used in animal feed. The use of xylanase in feedstuffs is a "value-added" type use, that is it permits additional nutrition from existing feed. Use of Xylanase is recommended as an additive to nutritionally complete feed.

The GRAS Final Rule (81 FR 54960) provides interpretation of this regulation specific to animal feed ingredients in response to comment 144: "We agree that data and information bearing on the physical or other technical effect the notified substance is intended to produce are only necessary when they bear on safety." A product like phytase would require data, however, the intended purpose of supplementation of Xylanase is to augment

normal digestion of hemicelluloses found in plant feedstuffs. As such, BioResource International, Inc. has determined that the technical effect of *Xylanase inclusion* when fed to swine or poultry does not have a bearing on safety. Thus, data and information demonstrating the intended effect of Xylanase in the feed of poultry and swine are not required as part of this GRAS notice.
We have, as supportive information, provided two published studies that address the intended use issue in vivo (described in section 2.5.3 and 2.5.4). However, these studies are only provided as support and not pivotal data, as it is not required in this GRAS notice.

2.5.1. Mode of Action

As noted earlier, the main reaction of endo-1,4-β-xylanase is the endohydrolysis of (1→4)-β-D-xylosidic linkages in xylans (various 1,4-β-D-xylo-oligosaccharides).

Non-Starch Polysaccharides (NSPs) are considered anti-nutritional factors because they are not well digested, bind nutrients reducing their availability and utilization, increase digesta viscosity which reduces absorption of nutrients, and thereby decrease animal performance. Soluble xylans, or xylans that are highly branched with arabinose, form a very thick solution which increases digesta viscosity. Endo-xylanases catalyze the endo-hydrolysis of 1,4-β-D-xylosidic linkages in xylans. Therefore, it is hypothesized that when xylanase is supplemented to animal feed containing grains such as: corn, barley, rye, wheat, grain sorghum, triticale, and/or oats, the enzyme hydrolyzes xylosidic linkages. As the linkages are hydrolyzed, the chains become shorter and less viscous, and digesta viscosity is reduced. It has been shown that supplementation of xylanase-based feed additives, which degrade various NSPs in the cell wall causing a reduction in digesta viscosity, is an effective way to mitigate the anti-nutritive properties of NSPs in grains, as reported in research published by Choct (1997) and Kiarie et al. (2014).

Results from broiler research have been shown to prove the effect of xylanase on ileal digesta viscosity of broilers fed a corn and soybean meal diet (Flores et al, 2017). Xylanase reduces digesta viscosity of animal feed containing grains (i.e. corn, soy, wheat, etc.). This reduction in viscosity has been shown to increase Body Weight and improve feed efficiency as well as nutrient utilization. The overall conclusion is that xylanase reduces ileal digesta viscosity, which is a target parameter in showing the functionality of xylanase.

2.5.2. Use Levels

This xylanase enzyme is intended for use in poultry, swine and monogastric feeds. The standard minimum recommended dose of the product is at 10,000 XU/Kg feed to a maximum of 50,000 XU/kg feed. The dosage applied in practice depends on formulation and ingredients. One XU activity is defined as the amount of enzyme needed for the release of 1 nanomole of reducing sugars (xylose equivalents) per second from 0.5% beechwood xylan at 50°C in 50 mM trisodium citrate buffer pH 6.0.

2.5.3. Utility Trial in Poultry

In addition to the published studies with other xylanase sources, Appendix 14 is a published paper specific to the notified substance (Appendix 14). This study is only provided as supportive information. The notified substance Xylanase enzyme preparation at inclusion levels up to 40,000 XU/kg feed (Flores et al., 2017) in broilers raised to 42 day of age. Inclusion levels investigated were 10,000, 20,000, and 40,000 XU/kg feed. Results showed that energy digestibility was improved by xylanase inclusion with no adverse effects observed at 40,000 XU/kg.

2.5.4. Utility Trial in Swine

The notified substance, xylanase enzyme, was included in diets of nursery pigs and its effect was evaluated on digesta viscosity. Historically digestive viscosity is used as a marker to demonstrate hemicellulose degradation. We have provided one published study, as supportive information, that demonstrates that Xylanase addition to feed decreased digesta viscosity ($P<0.02$) (The detailed published manuscript is listed in Appendix 15—Duarte et al., 2019). We note that under the GRAS notice regulations as the use Xylanase enzyme does not confer a safety risk, data demonstrating intended use is not required.

2.6. Stability of Enzyme in Product and Feed

2.6.1. Shelf-life and Stability of the Enzyme

The company guarantees that the minimum activity as given on the label is present in the product at the end of the indicated shelf life provided that product is stored in the unopened bag and the storage temperature does not exceed 25°C (77°F). The Xylanase has a minimum guaranteed activity of 150,000 XU/g product when stored at recommended conditions.

For the xylanase feed additive, an accelerated shelf-life stability study of the xylanase enzyme was completed, and the 6-months of data is available in Appendix 16. Another shelf-life stability study was also conducted, and 24 months of data has been reported in Appendix 17. In both studies, xylanase activity was determined according to analytical method detailed in Appendix 19. Based on the available data, it can be concluded that in the standard packaging, the product will maintain the declared activity for at least 24 months.

2.6.1.1. Stability of Enzyme in Product

Stability testing was conducted at two temperatures. The first stability testing was conducted on three lots of xylanase product at storage temperatures of 40°C and 75% humidity for 6 months (Appendix 16). The testing procedures used HDPE lined bags, which is the type that will be the packaging used for the marketed product as illustrated in section 2.3.5. formulation and packaging. All three lots exhibited excellent stability under each of

the defined testing conditions with an average residual activity of 100%, detailed results are listed in Appendix 16.

The second stability testing was conducted on xylanase product at storage temperatures of 30°C for 24 months, details are listed in Appendix 17. The testing procedures used HDPE lined bags, which is the type that will be the packaging used for the marketed product as illustrated in section 2.3.5. formulation and packaging. Xylanase product exhibited stability under the defined testing conditions. Based on activity analyses over the period of 24 months, it can be concluded that xylanase product is stable at temperature of 30°C. furthermore, when xylanase is stored under recommended conditions, xylanase will maintain declared activity of a minimum of 150,000 XU/g product for at least 24 months.

2.6.2. Stability of Enzyme in Feed

2.6.2.1. Stability of Enzyme in Mash Feed

To ensure that xylanase is stable in complete feed for an extended period of time, an in-feed stability study was performed. Poultry feed was used to demonstrate in-feed stability. The in-feed mixing and stability report can be found in Appendix 18, study 1. Xylanase activity in feed samples were determined following analytical assay developed at BioResource International and details can be found in Appendix 20. Results for activity are expressed on basis of XU/g of feed. Stability testing was conducted on the complete feed containing xylanase at room storage temperatures for 6 months. Xylanase exhibited stability under the defined testing conditions with an average residual activity > 80%. Therefore, results showed that storage stability of xylanase is acceptable at 30°C. Xylanase will maintain minimum activity for at least 6 months when stored under recommended conditions.

2.6.2.2. Recovery of Enzyme in Pelleted Feed

To ensure that the xylanase enzyme can survive the pelleting process, a pelleting study was conducted, details are listed in Appendix 18, study 2. Poultry feed was used to demonstrate enzyme recovery post pelleting. Xylanase activity in both mash and pelleted feed samples were determined following analytical assay developed at BioResource International and details can be found in Appendix 20. Results for activity are expressed on basis of XU/Kg of feed. Feed was pelleted at 85°C. Xylanase recovery was 70%. Therefore, results showed that xylanase product can survive pelleting process at 85°C.

2.6.3. Homogeneity of Product in Mash and Pelleted Feed

Homogeneity of xylanase enzyme in mash and pelleted feed was tested in poultry feed to demonstrate homogeneity upon mixing. Details are listed in Appendix 18, study 2. Xylanase activity in both mash and pelleted feed samples were determined following analytical assay developed at BioResource International and details can be found in Appendix 20. Results for activity are expressed on basis of XU/Kg of feed. The coefficient of variation in mash

feed was 6% while in pelleted feed was 1.93% indicating that xylanase product can be mixed homogeneously with other feed ingredients.

Part 3: Target Animal and Human Exposures

3.1. Target Animal

The xylanase enzyme is to be included in feed to deliver the standard minimum recommended dose of the product at 10,000 XU/Kg feed (10 XU/g feed) and a maximum of 50,000 XU/Kg feed (50 XU/g feed). The enzyme product is formulated to contain >150,000 XU/g product, which contains approximately 18% total organic solids (TOS). The nominal feed consumed per day by poultry (broiler) and swine are 232.5 and 2,400 grams, respectively. The average body weight of poultry (broiler) and swine are 2.5 kg and 60 kg, respectively.

Therefore, as a maximum:

For broilers,

If a bird is consuming 232.5 g per day and weigh 2.5 kg, then the EDI per kg BW is:

232.5 g/2.5 kg = 93 g feed /kg BW

Assuming 1 g of feed contains 20 XU then the EDI of xylanase/kg/day is:

93 x 50 XU/g feed = 4650 XU/kg/day

For swine,

If a pig is consuming 2,400 g per day and weigh 60 kg, then the EDI per kg BW is:

2,400 g/60 kg = 40 g feed /kg BW

Assuming 1 g of feed contains 20 XU then the EDI of xylanase/kg/day is:

40 x 50 XU/g feed = 2000 XU/kg/day

Poultry consume the highest amount of xylanase preparation per body weight per day among the food-producing target animals, thus providing a worst-case dietary intake for xylanase preparation for all food-producing target animals. Furthermore, a study was published by Flores et al. (2017) and listed in Appendix 14 in which broilers were fed doses higher than the recommended dose and there was no negative effect on live performance, while energy digestibility was improved by the xylanase product inclusion at 40,000 XU/kg, indirectly indicating that there were no safety concerns related to feeding xylanase in broilers.

3.2. Human Exposure and Safety

The xylanase is intended for use in animal feed only. It is not expected that humans would be exposed to xylanase through consumption of meat or eggs of animals that consumed xylanase. For safe handling, the SDS is provided (Appendix 23) and there are no adverse

effects on human health when xylanase is handled according to proper handling instructions.

To emphasize the safety of xylanase product in relation to human food; Xylanase enzyme is a protein, which will be handled similar to other proteins where it will undergo digestion and be broken down to amino acids before being absorbed and utilized in different tissue parts such as eggs (ovary) and muscles. Therefore, it will not be absorbed and deposited in its intact form rather individual amino acids as part of amino acids pool. Enzymes are not expected to exert enzymatic activity in the final food (meat and/or eggs) due to a variety of factors related to enzyme functionality in live animals. These factors include physiological conditions within the body and the substrate availability. Furthermore, the quality analysis of the xylanase product (Appendix 12) shows absence of heavy metals, microbial or chemical contaminants, meaning there is no exposure thus no increased risk for humans. Given these conditions, it is concluded that the xylanase enzyme will not be functional in the final product and will not pose any safety concerns in human food.

Part 4: Self-Limiting Levels of Use

Technological limitation on levels of xylanase enzyme inclusion in feed is only related to the economic feasibility of using the enzyme.

Part 5: Experience Based on Common Use in Food Before 1958

This does not apply.

Part 6: Narrative

The information provided in the following sections is the basis for our determination of the general recognition as safe for the xylanase enzyme preparation. The GRAS determination is based upon scientific procedures; the safety evaluation in Part 6 includes an evaluation of the production organism, the donor strain, the introduced DNA, the enzyme, and the manufacturing process. Data and information cited in this notification is not generally available and Part 6 contains information that is exempt from disclosure under the FOIA.

The identification and characterization of the inserted genetic material into the genetically modified organism is essential for the safety assessment. The methods used to develop the genetically modified production organism and the specific genetic modifications introduced into the production organism are described in Part 2.

6.1. Safety of the Production Organism

Production organism safety is the key consideration when assessing the probable degree of safety of an enzyme preparation intended for use in animal feed. Enzyme preparations that meet or surpass the criteria proposed by Pariza and Foster (1983) for human food should be safe for use in animal feed when utilized at the low levels normally employed for these catalysts. If the organism is nonpathogenic and nontoxigenic, then it is assumed that food or food ingredients produced from the organism, according to current Good Manufacturing Practices, is safe to consume (Pariza and Cook, 2010). A nontoxigenic organism is "one which does not produce injurious substances at levels that are detectable or demonstrably harmful under ordinary conditions of use or exposure" and nonpathogenic organism is "one that is very unlikely to produce disease under ordinary circumstances". *Komagataella phaffii (Pichia pastoris)* meet these criteria for non-toxigenicity and non-pathogenicity. Additionally, *Komagataella phaffii (Pichia pastoris)* has a Qualified Presumption of Safety (QPS) when used for production purposes according to the European Food Safety Authority (EFSA BIOHAZ Panel, 2020).

Komagataella phaffii (Pichia pastoris) has a history of safe use as an animal feed additive (CFR 21 Sec. 573.750 *Pichia pastoris* dried yeast) as well as an enzyme production strain (GRAS Notice 000204). *Komagataella phaffii (Pichia pastoris)* GS115 (ATCC® 20864™), as an example, is identified as a nontoxigenic, non-pathogenic, histidinol dehydrogenase (HIS4 gene) mutant derived from strain *Pichia pastoris* NRRL Y-11430 (ATCC® 76273™). The strain *Pichia pastoris* SMD 1168, derived from strain *Pichia pastoris* GS115, has been cited in GRAS Notice 000204 for safe use in enzyme production. In addition, no viable production strain has been observed in the final product as detailed in Part 2 of this document. Also, BRI has repeatedly used the decision tree procedures outlined by Pariza and Johnson and is the basis for our safety assessment (Appendix 13).

Furthermore, *Pichia pastoris* has been found to be safe by EFSA for enzyme products (EFSA Journal 2014; 12(5):3667; EFSA BIOHAZ Panel, 2013).

The production strain is a genetically modified strain of *Komagataella phaffii (Pichia pastoris)* expressing a xylanase from *Orpinomyces*. The host organism, *Komagataella phaffii*

(Pichia pastoris) has a long history of safe industrial use for the production of enzymes used in human and animal feed and has been routinely used as a host for recombinant enzymes. Based on the information presented here it is concluded that the *Komagataella phaffii (Pichia pastoris)* production strain is considered a safe strain for the production of endo-1,4-β-xylanase enzyme.

6.2. Safety of the Donor Organism

The organism from which endo-1,4-β-xylanase was isolated is *Orpinomyces sp.*, which belongs to the *Orpinomyces* genus. *Orpinomyces*, is an anaerobic gut fungi belong to the phylum Neocallimastigomycota, an early divergent basal fungal lineage. *Orpinomyces* are known to reside in the rumen, hindgut, and feces of ruminant and non-ruminant herbivorous mammals and reptilian herbivores.

As detailed in Part 2, the gene was synthesized to mimic the xylanase gene from *Orpinomyces sp.* By synthesizing the xylanase gene, it is ensured that no genetic material (target or other DNA) from the donor organism is found in the production strain. The introduced DNA does not code for any known harmful or toxic substances.

6.3. Safety of the endo-1,4-β-xylanase Enzyme

The subject of this GRAS conclusion is a xylanse enzyme (EC 3.2.1.8). Enzymes, including xylanase, have a long history of use in human food and animal feed. Xylanases are ubiquitous in nature and can be found in bacteria, fungi, and plants. They have been studied for over 60 years and used safely in feed applications for over 20 years. The xylanase enzyme has a long history of use in food processing as well. A wide range of enzymes are used in animal feed and can be found listed in the AAFCO Official Publication (Official Publication Association of American Feed Control Officials Inc, 2019: Feed Ingredient Definitions. publication. Chapter 6). Seven different xylanase source organisms are listed in the official publication (*Aspergillus niger,* var. *Aspergillus oryzae* expressing a *Thermomyces lanuginosus* xylanase gene*, Bacillus lentus, Bacillus subtilis,* var. *Humicola insolens, Paenibacillus lentus, Talaromyces funiculosus, Talaromyces versatilis, Trichoderma reesei*). Thorough searches of published literature yield several safety studies with various xylanase enzyme preparations. All studies concluded that xylanases were safe, and no research has any safety concerns regarding xylanases used as feed additives. There is no reasonable expectation that the xylanase preparation that is the subject of this document would behave differently than any other xylanase preparation regarding toxicity and pathogenicity.

The endo-1,4-β-xylanase enzyme preparation from *Komagataella phaffii (Pichia pastoris)*, expressing the recombinant gene (xylanase) from *Orpinomyces sp*. was evaluated according to the Pariza and Johnson Decision Tree.

According to Pariza and Johnson (2001), enzyme proteins do not generally raise safety concerns. Xylanase is a protein that breaks down in the animal's gut, just like any other protein, and is never released into the environment.

Because the genetic modifications are well characterized and specific, and the incorporated DNA does not encode and express any known harmful or toxic substances, the enzyme preparation derived from genetically modified *Komagataella phaffii (Pichia pastoris)* is considered safe.

Furthermore, BioResource International completed an extensive literature search using PubMed (including MEDLINE*), TOXLINE, and Google Scholar (details in Table below). Key words such as "xylanase", "toxicity", "animal consumption", "human consumption", "food", "feed" and others, was used for the search. The literature search produced no health or safety issues associated with the use of the xylanase enzyme from *Komagataella phaffii (Pichia pastoris)* for the intended uses listed in 2.5.2. use levels. Table below summarizes the outcomes of the Google Scholar search results for literature search.

Based on the publicly available, scientific data from the literature and additional supporting data generated by BRI, it has been concluded that xylanase enzyme produced by *Komagataella phaffii (Pichia pastoris)* is safe and suitable for the intended use.

Authors	Title	Publication	Volume	Number	Pages	Year	Publisher	Major outcomes
Search keywords: xylanase AND human consumption AND safety AND toxicity AND Pichia pastoris								
Kyzeková, Tamara; Krasňan, Vladimír; Rebroš, Martin;	Pichia pastoris—recombinant enzyme producent for environment treatment	Acta Chimica Slovaca	13	1	108-118	2020	Sciendo	Using P. pastoris for recombinant enzyme production to reduce environmental pollution
Baghban, Roghayyeh; Farajnia, Safar; Ghasemi, Younes; Mortazavi, Mojtaba; Zarghami, Nosratollah; Samadi, Naser;	New developments in Pichia pastoris expression system, review and update	Current pharmaceutical biotechnology	19	6	451-467	2018	Bentham Science Publishers	Potential for using P. pastoris for the production of complex eukaryotic proteins
Li, Cheng; Lin, Ying; Zheng, Xueyun; Yuan, Qingyan; Pang, Nuo; Liao, Xihao; Huang, Yuanyuan; Zhang, Xinying; Liang, Shuli;	Recycling of a selectable marker with a self-excisable plasmid in Pichia pastoris	Scientific reports	7	1	10-Jan	2017	Nature Publishing Group	The use of vectors that allow selectable marker recycling and as a tool to engineer P. pastoris for high heterologous protein productivity
Search keywords: xylanase AND animal consumption AND safety AND toxicity AND Pichia pastoris								
EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP); Rychen, Guido; Aquilina, Gabriele;	Safety and efficacy of ENZY CARBOPLUS®(endo-1, 4-beta-xylanase and endo-1, 3 (4)-beta-glucanase) as a feed additive for avian species, weaned piglets and minor weaned porcine species	EFSA Journal	15	12	e05097	2017	Wiley Online Library	EFSA opinion on the safety of an endo xylanase for avian and porcine species

Azimonti, Giovanna; Bampidis, Vasileios; Bastos, Maria de Lourdes; Bories, Georges; Chesson, Andrew; Flachowsky, Gerhard; Gropp, Jürgen;								
Liu, Taiyu; Zhao, Yixin; Zhang, Jianguo; Zhang, Jining;	Enhancement of xylanase expression by Komagataella phaffii through pexophagy inhibition	Biotechnology & Biotechnological Equipment	33	1	855-862	2019	Taylor & Francis	Increasing heterologous protein production by pexophagy inhibition in P. pastoris
Kyzeková, Tamara; Krasňan, Vladimír; Rebroš, Martin;	Pichia pastoris—recombinant enzyme producent for environment treatment	Acta Chimica Slovaca	13	1	108-118	2020	Sciendo	Using P. pastoris for recombinant enzyme production to reduce environmental pollution
Search keywords: xylanase AND Pichia pastoris AND safety OR toxicity OR animal								
Driss, Dorra; Soudani, Najla; Boudawara, Tahia; Zeghal, Najiba; Chaabouni, Semia Ellouze;	Toxicological study and oxidative stress evaluation for safety assessment of xylanase preparations in Wistar rats	Journal of Biochemical and Molecular Toxicology	28	11	490-500	2014	Wiley Online Library	Safety of expressed xylanase in P. pastoris and no presence of any mutagenic potential when tested in relevant genotoxicological assays
Pariza, Michael W; Cook, Mark;	Determining the safety of enzymes used in animal feed	Regulatory Toxicology and Pharmacology	56	3	332-342	2010	Elsevier	Consider production strain safety then using the decision tree mechanism for food processing enzymes
He, Jun; Yin, Jia; Wang, Li; Yu, Bing; Chen, Daiwen;	Functional characterisation of a recombinant xylanase from Pichia pastoris and effect of the enzyme on nutrient digestibility in weaned pigs	British journal of nutrition	103	10	1507-1513	2010	Cambridge University Press	The potential of xylanase in the improvement of digestibility in pigs
Birijlall, Natasha; Manimaran, Ayyachamy; Kumar, Kuttanpillai Santhosh; Permaul, Kugen; Singh, Suren;	High level expression of a recombinant xylanase by Pichia pastoris NC38 in a 5 L fermenter and its efficiency in biobleaching of bagasse pulp	Bioresource technology	102	20	9723-9729	2011	Elsevier	Efficiency of xylanase expressed in P. pastoris in biobleaching of bagasse pulp
Wang, Qian; Du, Wen; Weng, Xiao-Yan; Liu, Ming-Qi; Wang, Jia-Kun; Liu, Jian-Xin;	Recombination of thermo-alkalistable, high xylooligosaccharides producing endo-xylanase from Thermobifida fusca and expression in Pichia pastoris	Applied biochemistry and biotechnology	175	3	1318-1329	2015	Springer	Potential for G4SM1 in commercial XO production using P. pastoris as expression system
Shi, HongLing; Wang, JunQing; Wu, MinChen; Gao, ShuJuan;	Optimization of condition for fermentation of recombinant Pichia pastoris and enzymatic properties of xylanase produced.	Chinese Journal of Biologicals	25	10	1362-1365	2012	Editorial Office, Chinese Journal of Biologicals	Fermentation optimization for industrial xylanase production using P. pastoris

Govindarajulu, Natasha;	Cloning and expression of xylanase variants in Pichia pastoris	Thesis, Durban university of Technology				2017		The suitability of P. pastoris as an expression vector and the potential of xylanase for reducing environmental pollution

6.4. Safety of the Manufacturing Process

This section describes the safety of manufacturing process of xylanase, which follows standard industry practices and explained in detail in Part 2. The xylanase meets purity specifications for enzyme preparations as outlined in the monograph on Enzyme Preparations in the Food Chemicals Codex. The xylanase preparation is prepared in accordance with current good manufacturing practices, using ingredients that are acceptable for animal feed, and under conditions that ensure a controlled fermentation. These methods are based on generally available and accepted methods used for production of enzymes (See Part 2).

6.5. Safety Studies

This section describes the studies and analysis performed to evaluate the safety of the use of xylanase.

A broiler performance trial was performed in which xylanase enzyme produced by *Komagataella phaffii (Pichia pastoris)* was incorporated in the feed at concentrations up to 40,000 XU/kg feed (Flores et al., 2017). Results showed no adverse effect were observed on performance of broilers measured as feed intake, body weight, and mortality. Results indirectly show that the xylanase enzyme is safe and suitable for the intended use. Another trial was conducted on swine where xylanase was supplemented at 45,000 XU/kg in swine (Duarte et al., 2019). The published manuscript is listed in Appendix 15. Results showed the high inclusion level of the enzyme did not pose any safety concerns, therefore, the recommended use of xylanase in compound feed for poultry and swine is safe for consumers.

6.6. Summary and Conclusions

6.6.1. Poultry and Swine Safety Conclusion

The production strain is derived from a safe strain line, which has been used for a multitude of feed enzyme production. The enzyme is produced by methods and under

culture conditions that ensure controlled fermentation. The presence of toxic or undesirable substances as well as the introduction of contaminating microorganisms is prevented. In addition, raw materials of food or feed grades are used in manufacturing. Based on these facts and information listed in the dossier, it is concluded that the resulting enzyme product can be safely used in poultry and swine feed without showing any adverse effects on the performance of live animals.

6.6.2. Human Safety Conclusion

The enzyme is intended for use in animal feed, it is a protein that will be broken down into amino acids prior to utilization by body tissues, therefore, no residues are expected to enter the human food chain trough consumption of eggs and meat, and there are no safety concerns raised in humans due to consumption of eggs and meat from animals consuming feed containing the xylanase product. In addition, the product has been tested for heavy metals, mycotoxins, microbial contamination, and DNA of production strain in final product, all these tests showed that these do not cause a concern in xylanase product and subsequently human.

BRI has reviewed the available data and information. We are not aware of any data and/or information that is, or appears to be, inconsistent with our conclusion of GRAS. Based on this critical review and evaluation, a history of safe use of *Komagataella phaffii (Pichia pastoris)*, and the limited and well-defined nature of the genetic modifications, BRI concludes, through scientific procedures, that the subject of this notification; endo-1,4-β-xylanase enzyme preparation, meets the appropriate food grade specifications and is produced in accordance with current good manufacturing practices. Thus, it is generally recognized, among qualified experts, to be safe under the conditions of its intended use.

Part 7: Supporting data and Information

All information indicated in the List of Appendices from Appendix 1 to Appendix 11 are confidential. Appendices 12 to 23 and References are generally available.

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Appendices

Appendix 1. Whole genome sequencing -based taxonomic identification of *Komagataella phaffii* strain BG10 **(CONFIDENTIAL)**

(b) (4)

Report no: (b) (4)
Date: 7.12.2020
CONFIDENTIAL

Study Report

Whole genome sequencing -based taxonomic identification of *Komagataella phaffii* strain (b) (4)

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Review and approval
(b) (6) PhD, Bioinformatician

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Report Number
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This report contains scientific interpretation of the received data by the named scientists from (b) (4) It does not necessarily represent the official views of the competent authorities.

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Appendix 2. Whole genome sequencing -based taxonomic identification and description of genetic modifications in *Komagataella phaffi* (b) (4) **(CONFIDENTIAL)**

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Study Report

Whole genome sequencing -based taxonomic identification and description of genetic modifications in *Komagataella phaffii* (b) (4)

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Appendix 3. Open Reading Frame Analysis of the Complete Fusion DNA Sequence **(CONFIDENTIAL)**

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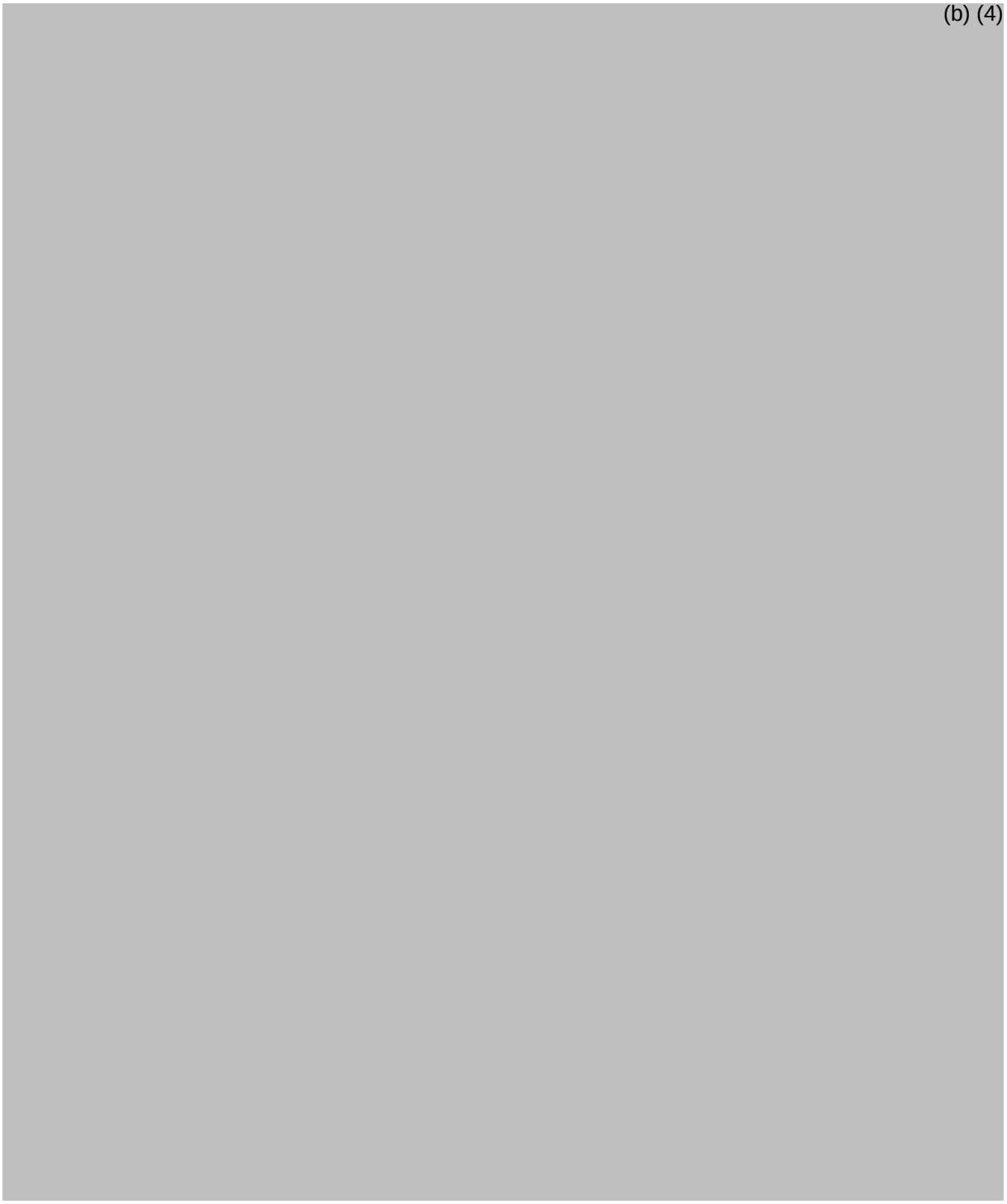

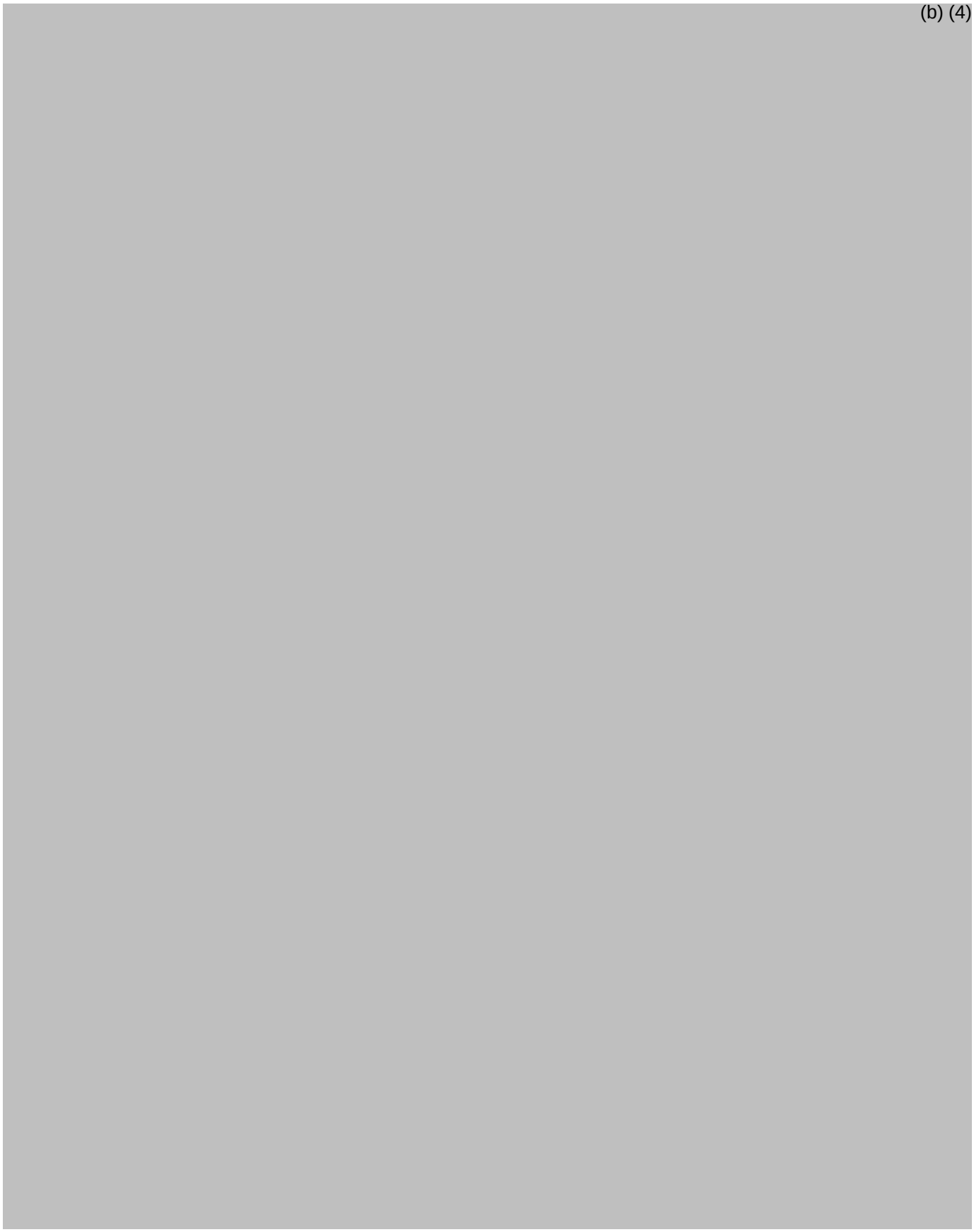
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Appendix 4. Absence of Transferable Antibiotic Resistance Gene DNA Elements in Xylanase Product

(CONFIDENTIAL)

A. Objective

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B. Background

(b) (4)

C. Required Materials and Equipment

(b) (4)

D. Strains and plasmids

(b) (4)

E. Method

(b)

Table 1. Assay Samples

Sample	Plasmid	Antibiotic Resistance	Plasmid Conc.	xylanase conc.
Plasmid ONLY	(b) (4)			
Plasmid + Xyl				
Xylanase ONLY				

F. Results

(b) (4)

G. Conclusion

(b) (4)

Appendix 5. Gene Stability Analysis of Genetically Modified *K. phaffii* Production Strain

(CONFIDENTIAL)

(b) (4)

Purpose:

(b) (4)

Methods:

Strains

(b) (4)

(b) (4)

Southern blot

(b) (4)


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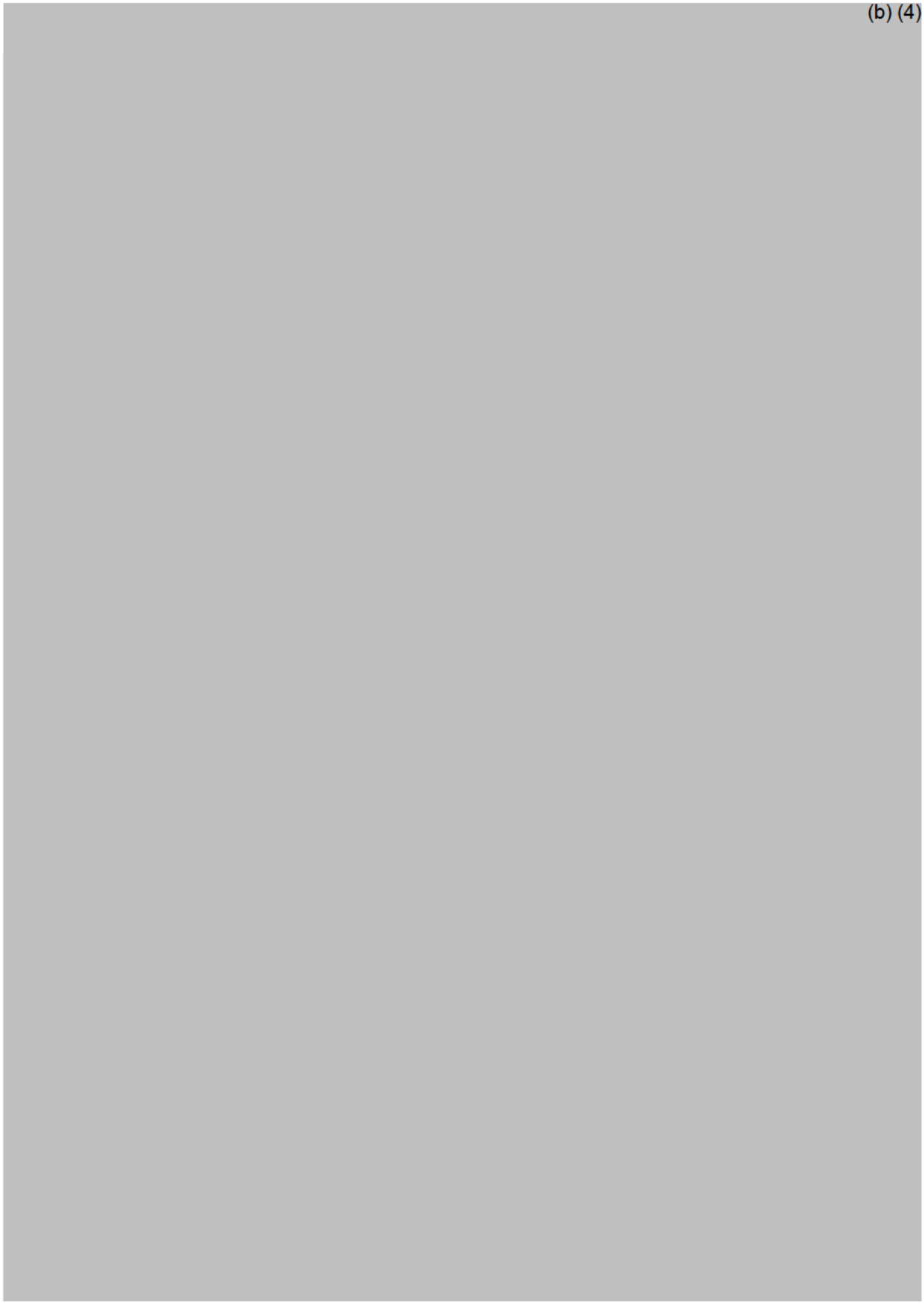
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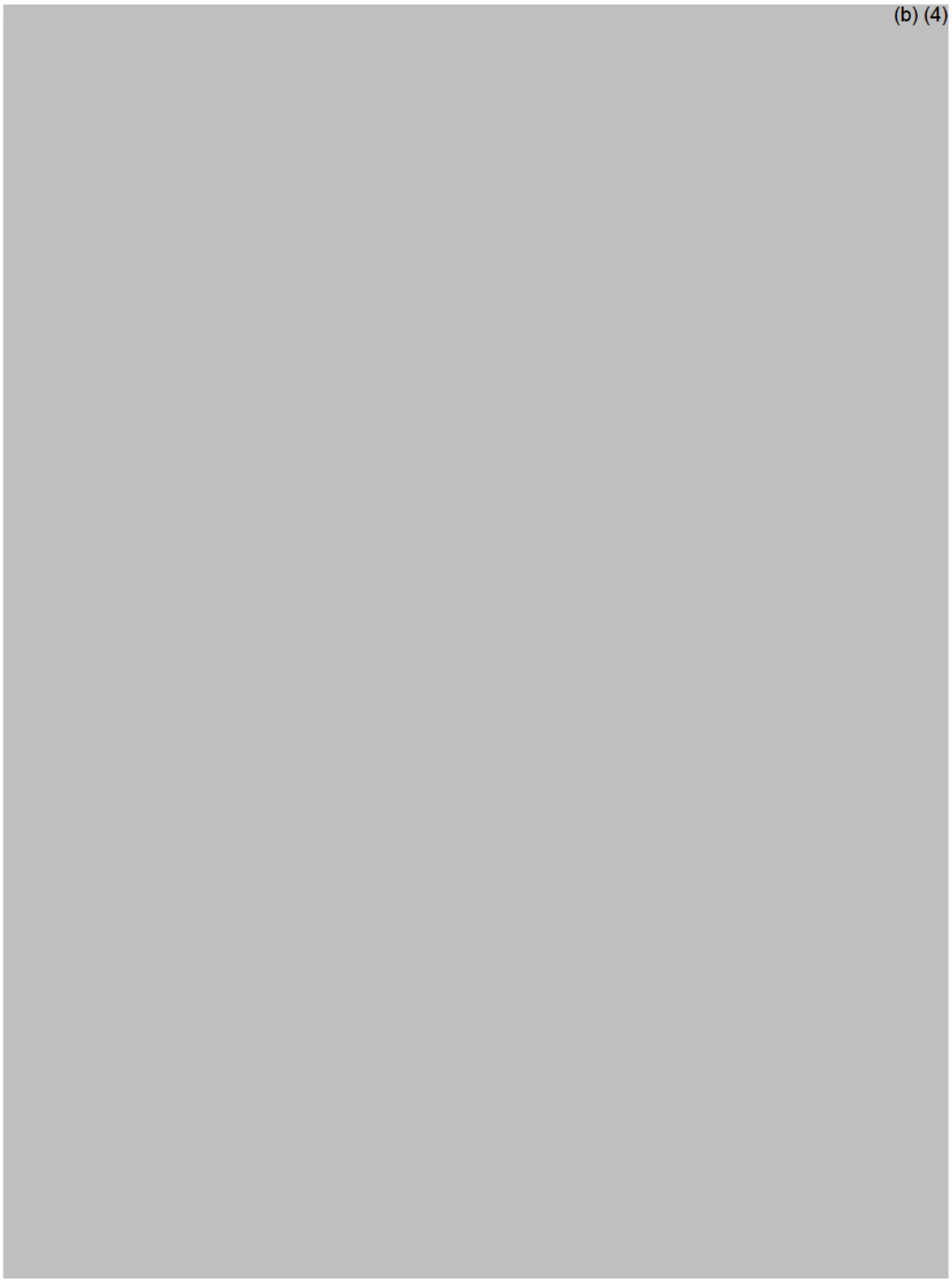
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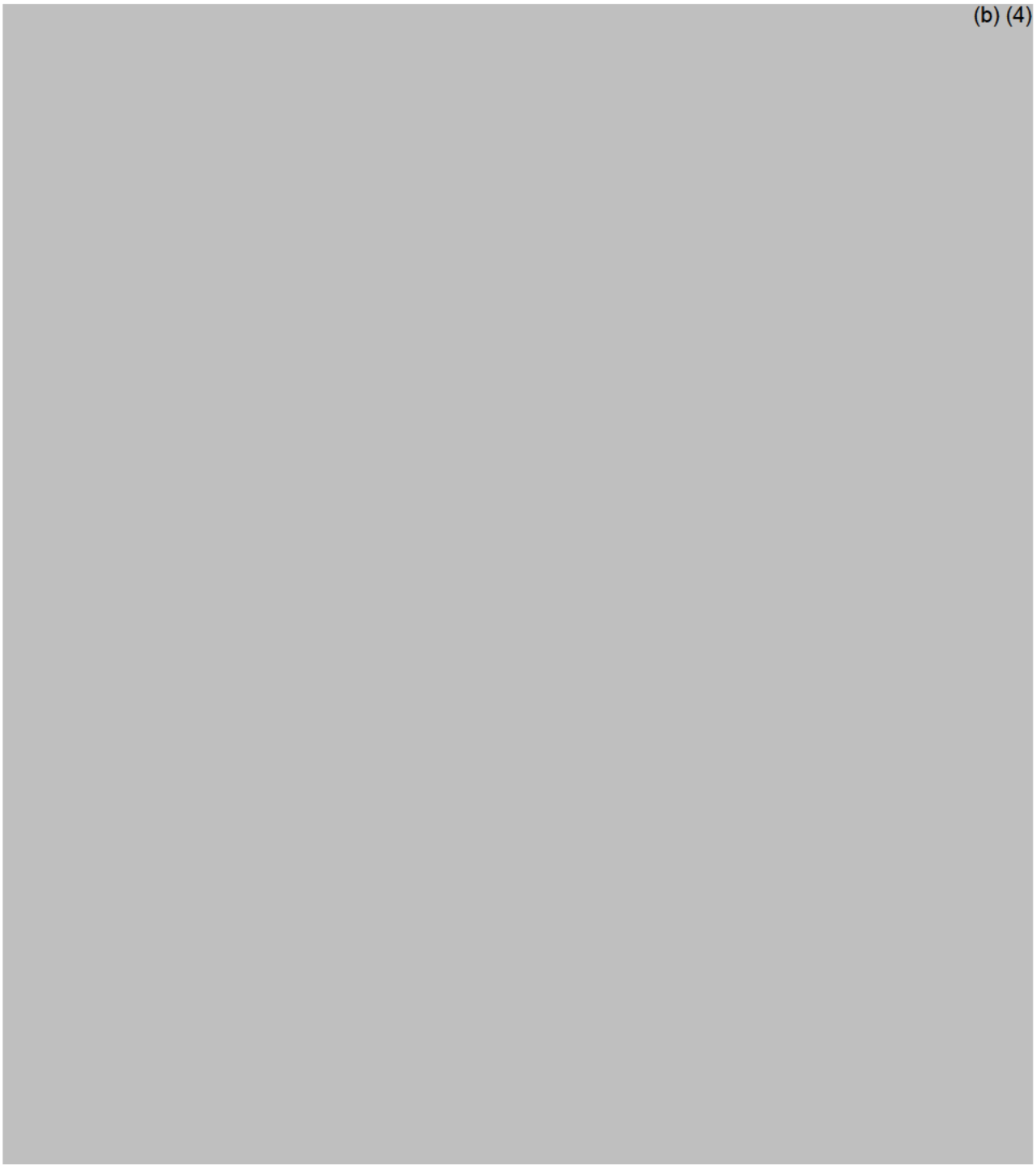
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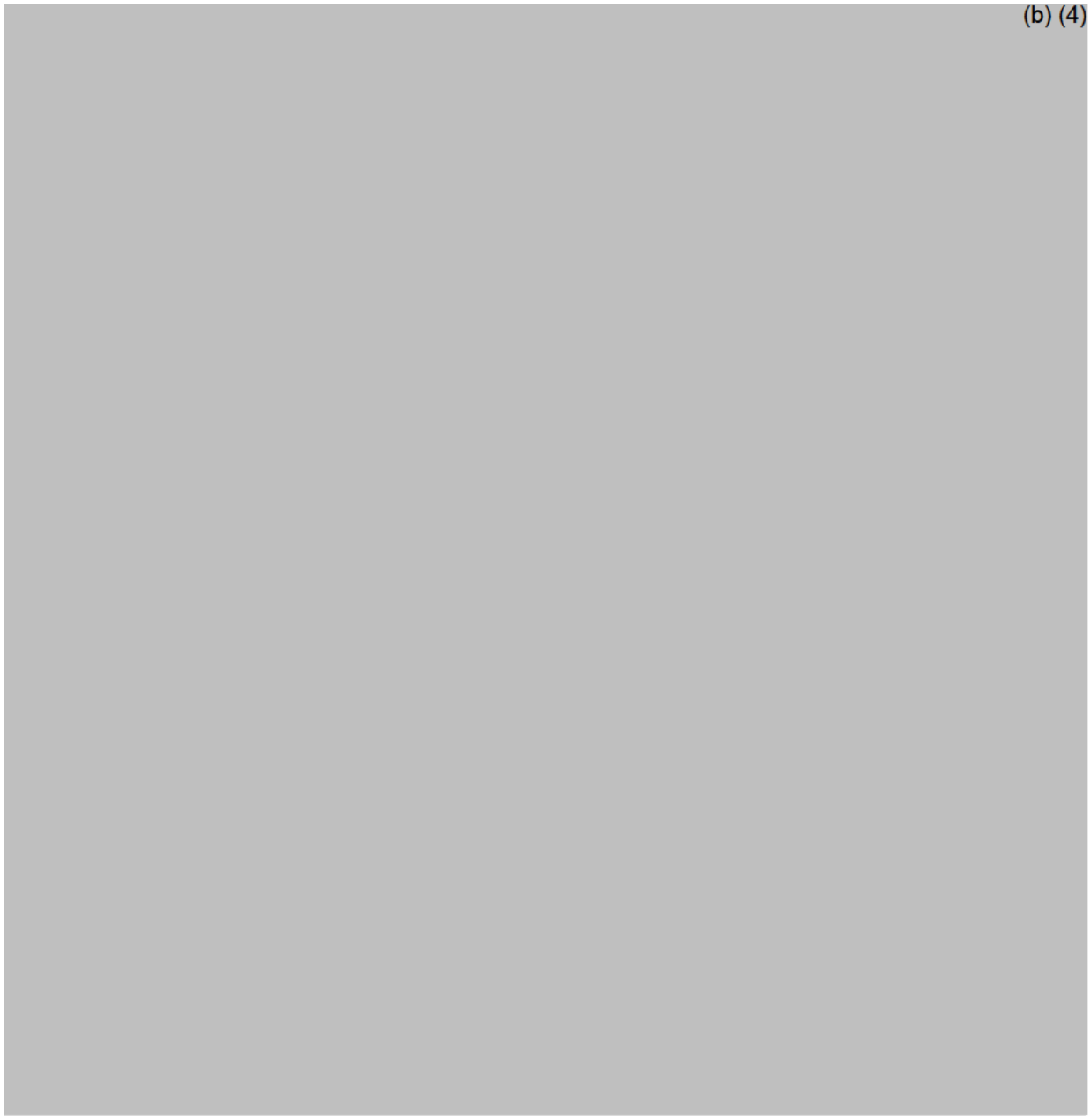
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Appendix 6. Presence of Production Strain DNA in Xylanase Product **(CONFIDENTIAL)**

(b) (4)

Report Number: (b) (4)
Date: 07.10.2020
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STUDY REPORT

(b) (4)

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3 MATERIALS AND METHODS

3.1 Test material

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3.2. Data

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4 RESULTS

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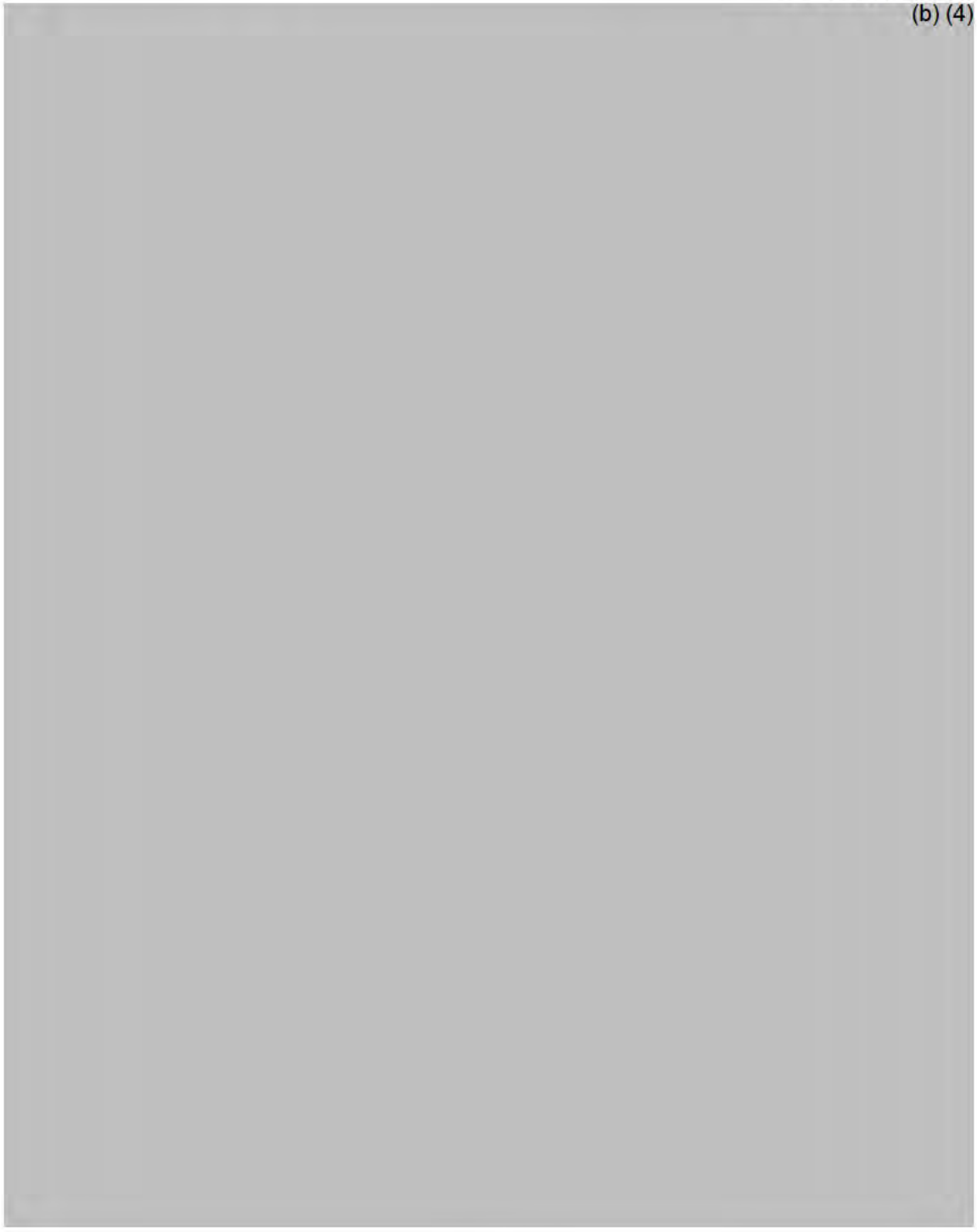

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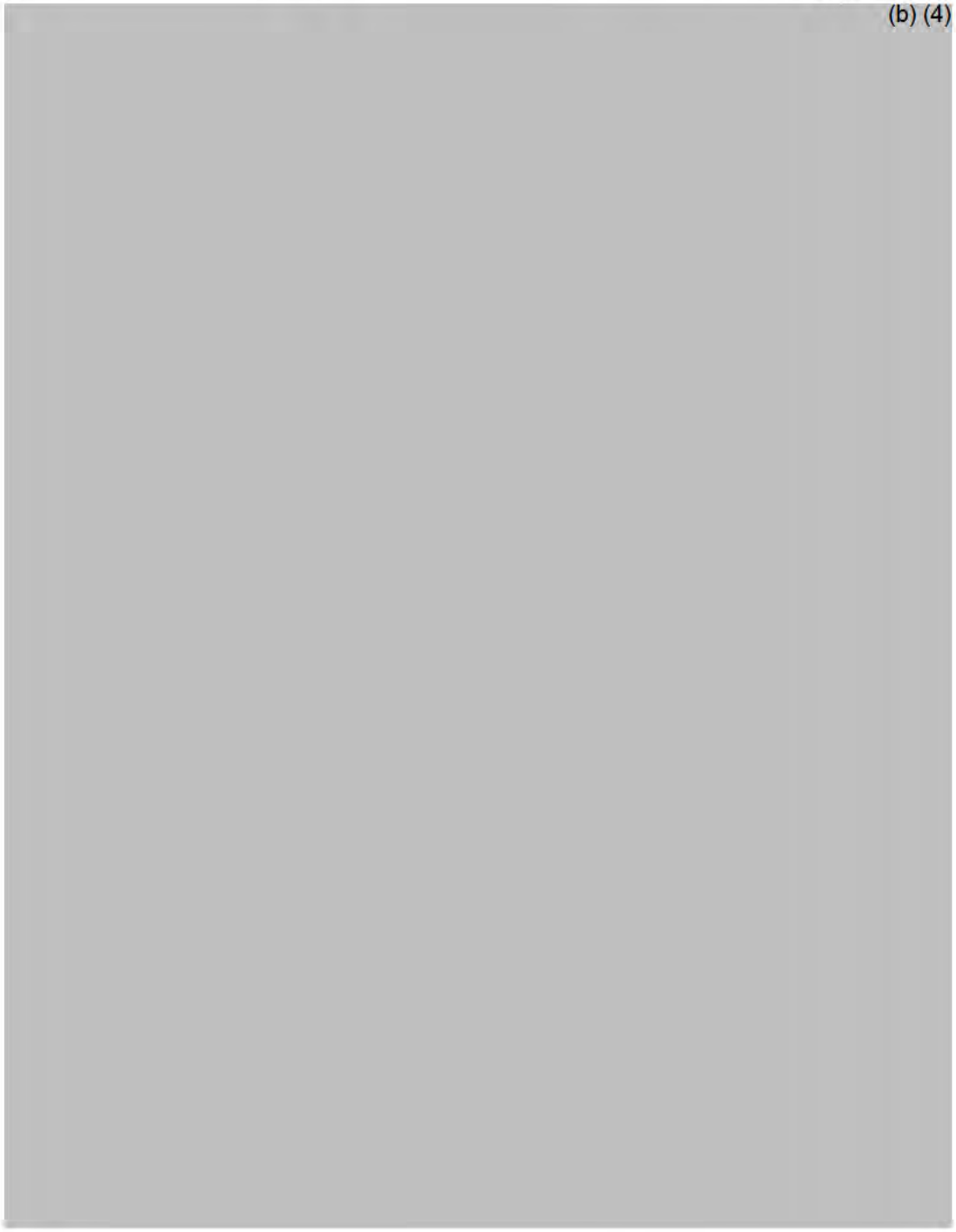

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Appendix 7. Raw Material Used in Fermentation and Downstream Processing **(CONFIDENTIAL)**

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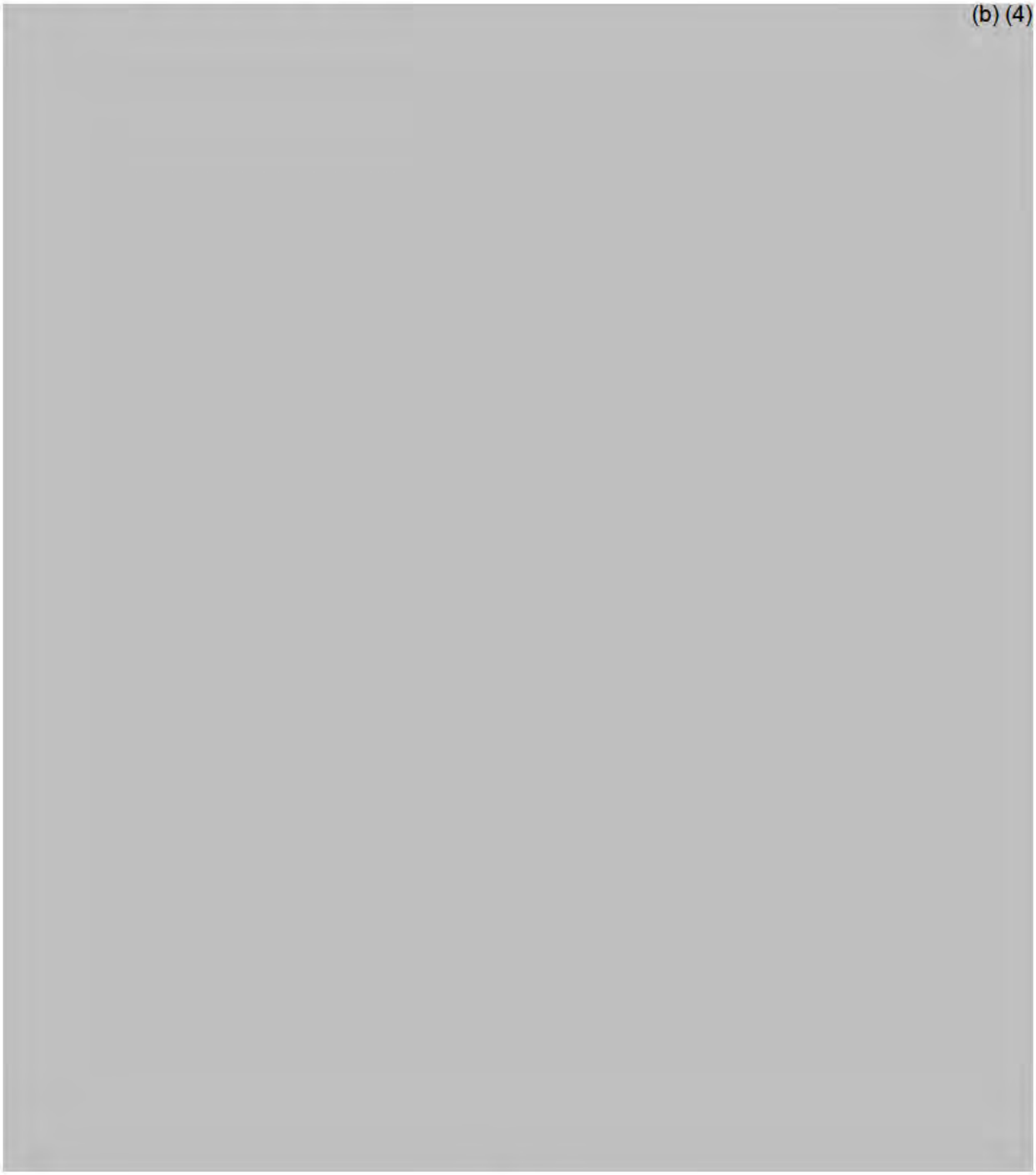
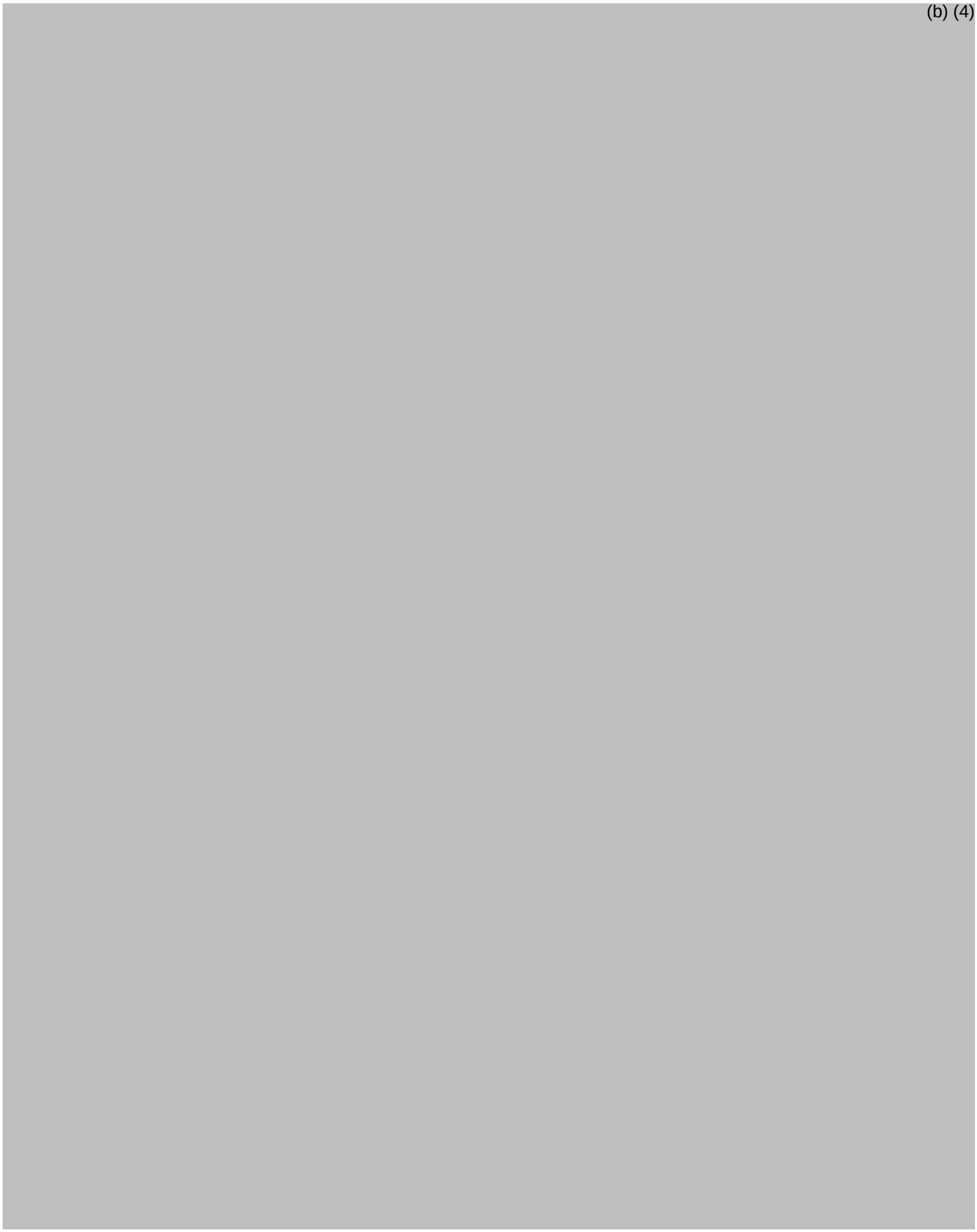
Raw materials used in the fermentation process

(b) (4)

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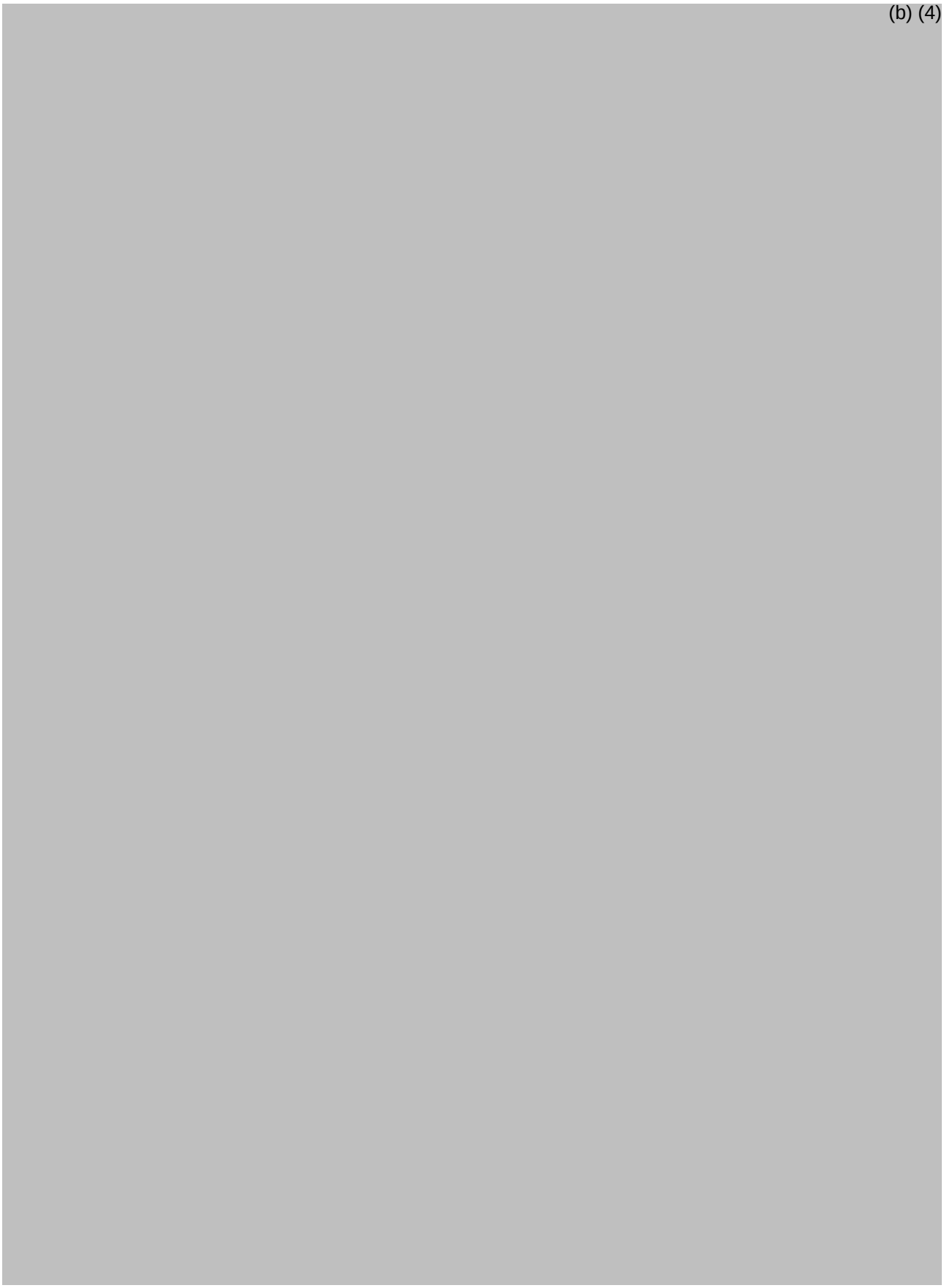
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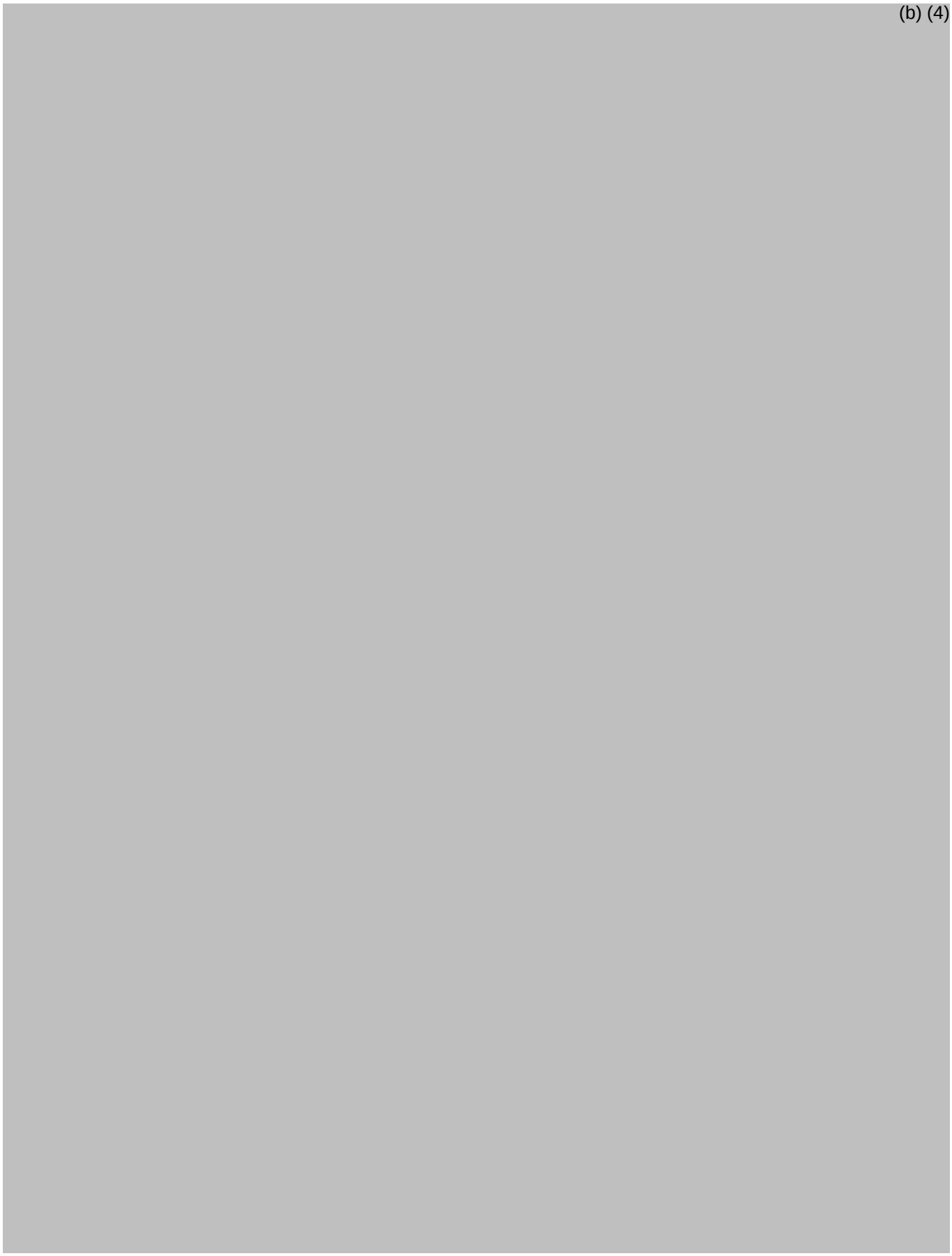

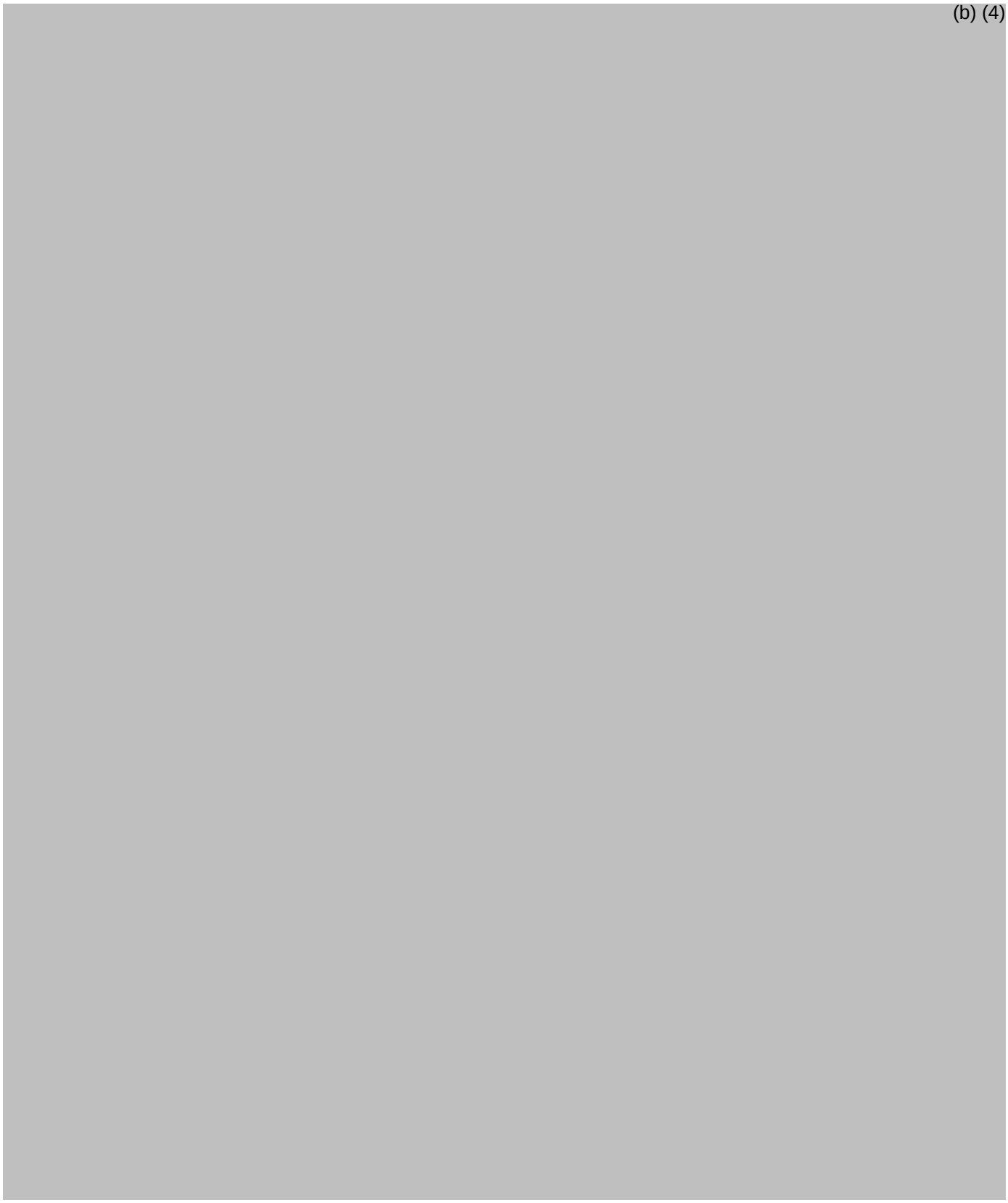
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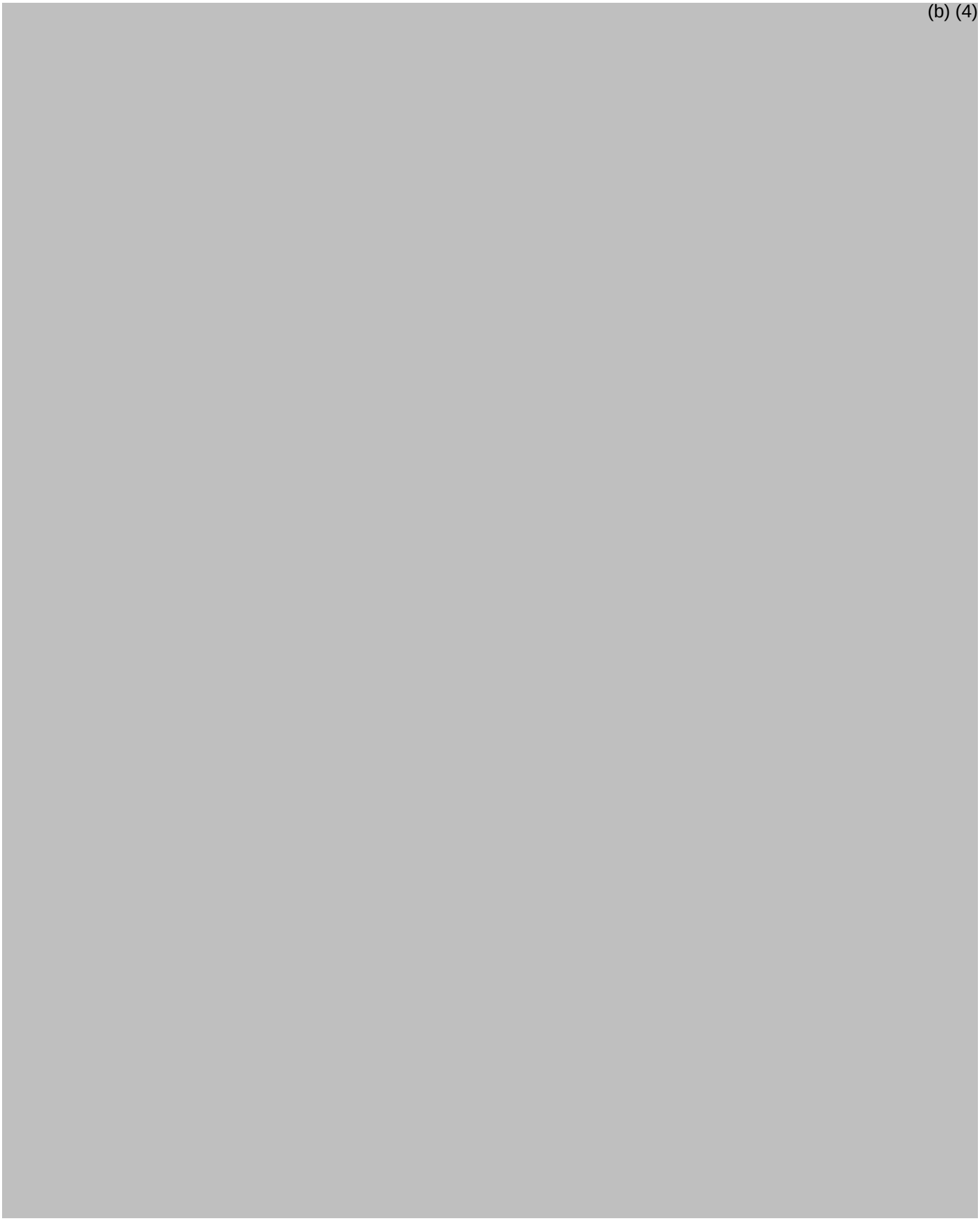
(b) (4)




(b) (4)

Appendix 8. Cell Bank Development and Maintenance **(CONFIDENTIAL)**

(b) (4)

Appendix 9. Growth Media and Reagents **(CONFIDENTIAL)**

(b) (4)

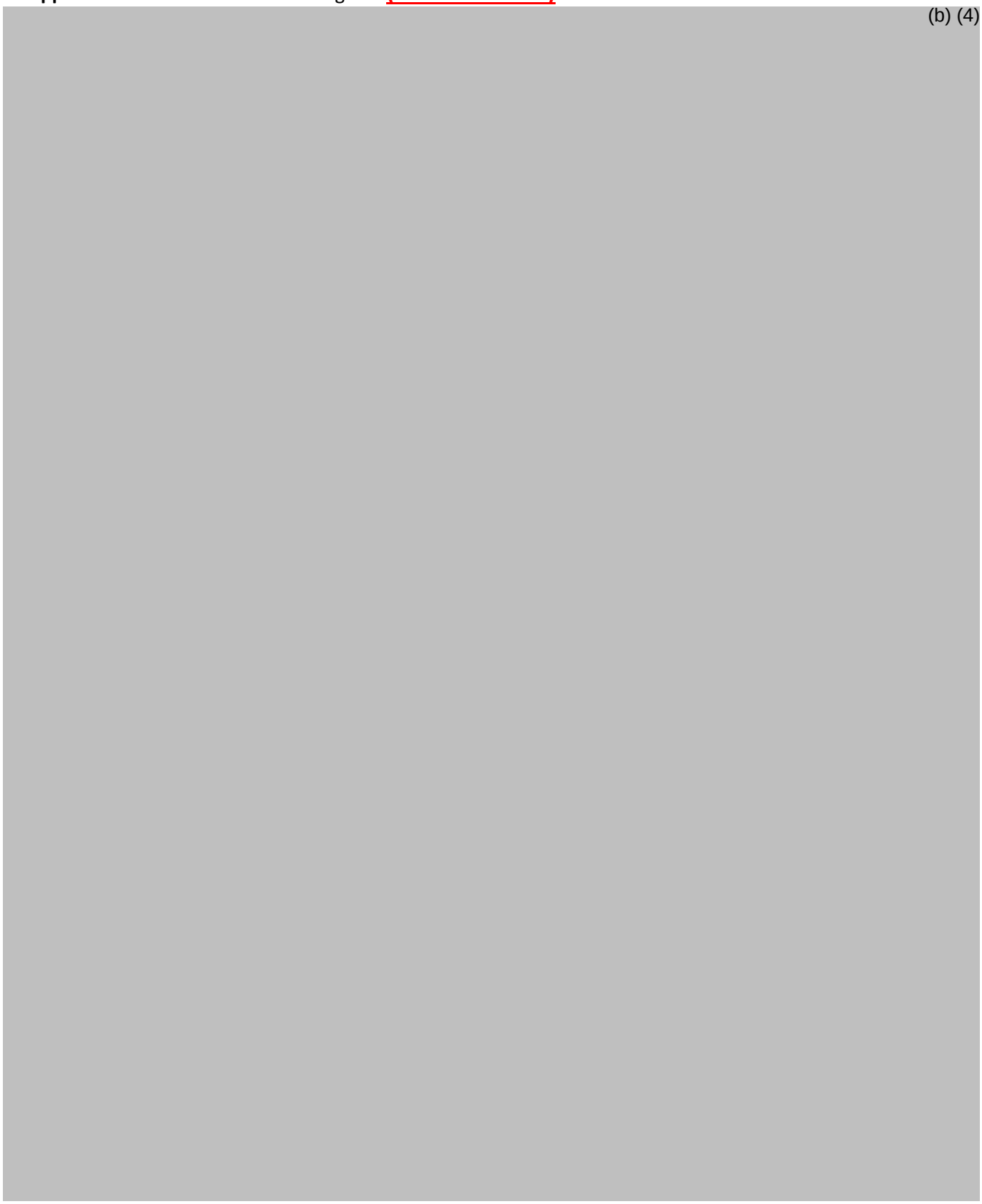


Table 4. Carrier

Name	Quantity (% range)
(b) (4)	

(b) (4)

Appendix 10. Fermentation and Downstream Processing **(CONFIDENTIAL)**

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

Table 1. (b) (4)

Ingredient	% Composition	Weight (kg)
(b) (4)		

(b) (4)

(b) (4)

(b) (4)

(b) (4)		

(b) (4)

(b) (4)

(b) (4)

(b) (4)

Table 3. (b) (4)

Ingredient	% Composition	Weight (kg)
(b) (4)		

(b) (4)

(b) (4)

(b) (4)

Table 4. (b) (4)

Ingredient	Quantity
(b) (4)	

(b) (4)

(b) (4)

(b) (4)

(b) (4)

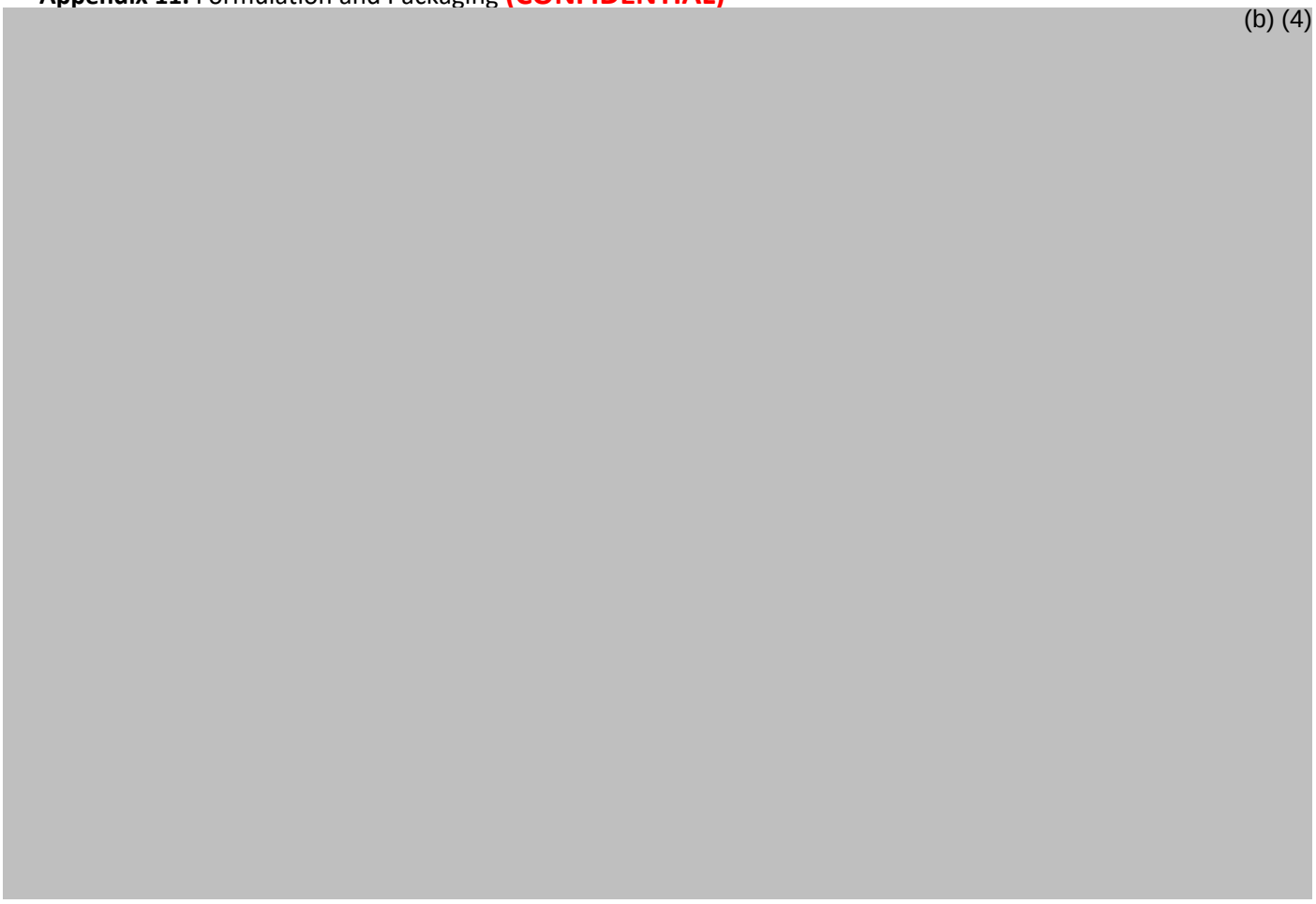
(b) (4)

(b) (4)

(b) (4)

Appendix 11. Formulation and Packaging **(CONFIDENTIAL)**

(b) (4)



Appendix 12. Specifications of Xylamax Product

Product name	Xylamax		
Raw Product Number	XY-C001	XY-C002	XY-C003
Production Date	April 2019	November 2019	January 2020
Best Before	April 2021	November 2021	January 2022
Property			
Appearance	Light grey powder	Light grey powder	Light grey powder
Moisture, %	1.5	1.4	1.5
Xylanase activity, XU/g	151,494	163,562	168,232
Mold, CFU/g	$< 10^3$	$< 10^3$	$< 10^3$
Coliforms, CFU/g	< 10	< 10	< 10
Escherichia coli, CFU/g	< 10	< 10	< 10
Salmonella	Not detected per 25 g	Not detected per 25 g	Not detected per 25 g
Heavy metals: Arsenic, mg/kg	0.306	0.292	0.373
Heavy metals: cadmium, mg/kg	0.388	0.360	0.381
Heavy metals: mercury, mg/kg	< 0.5	0.017	0.018
Heavy metals: lead, mg/kg	0.215	0.218	0.295
Mycotoxins: aflatoxin B1, B2, G1, G2, µg/kg	< 0.5	<0.5	< 0.5
Mycotoxins: fumonisin B1 and B2, µg/kg	< 25	< 25	< 25
Mycotoxins: zearalenone, µg/kg	< 30	< 30	< 30
Mycotoxins: deoxynivalenol, µg/kg	< 100	< 10	< 10
Mycotoxins: Ochratoxin, µg/kg	< 1	< 1	< 1
Dioxins, ng/kg TEQ	1	< 1	< 1
Dioxin & Dioxin-Like PCBs, ng/kg TEQ	< 1.5	< 1.5	< 1.5
Non-Dioxin-Like PCBs, µg/kg TEQ	< 10	< 10	< 10
Genetically modified organisms	Absent	Absent	Absent

Appendix 13. Decision tree for endo-1,4-β-xylanase enzyme preparation from *Komagataella phaffii* (b) (4)

To assess animal and human safety of this enzyme, the decision tree from Pariza and Johnson, 2001 is being used as follows:

1. *Is the production strain 'genetically modified'?*
   Yes, go to 2.

2. *Is the production strain modified using rDNA techniques?*
   Yes, go to 3.

3. *Issues relating to the introduced DNA (xylanase gene):*
   3a. Do the expressed enzyme product(s) which are encoded by the introduced DNA have a history for safe use in food or feed?
   Yes, go to 3c.
   Justification: As explained in Section 2.2., this product does not have any safety concerns. All parts of the product have been derived from sources that have a history of safe use in food or feed. In general, xylanases have a long history of safe use in food (Pariza and Johnson, 2001) and feed (Pariza and Cook, 2010).

   3c. Is the test article free of transferable antibiotic resistance gene DNA?
   Yes, go to 3e.
   Justification: Based on Section 2.2.11 and Appendix 4.

   3e. Is all the other introduced DNA well characterized and free of attributes that would render it unsafe for constructing microorganisms to be used to produce feed-grade products?
   Yes, go to 4.
   Justification: The starch binding domain gene and peptide linkages would not render the microorganism unsafe for production of feed-grade products, see Section 2.2.4.2.

4. *Is the introduced DNA randomly integrated into the chromosome?*
   No, go to 6.
   Justification: Integration events were targeted to the AOX1 loci of the *Komagataella phaffii* (*Pichia pastoris)* genome.

5. *Not applicable*

6. *Is the production strain derived from a safe lineage, as previously demonstrated by repeated assessment via the evaluation procedure?*

   Yes, the test article is ACCEPTED.

   Justification: *Komagataella phaffii (Pichia pastoris)* has a history of safe use as an animal feed additive (CFR 21 Sec. 573.750 *Pichia pastoris* dried yeast) as well as an enzyme production strain (GRAS Notice 000204). For example, *Pichia pastoris* GS115 (ATCC® 20864™) is identified as a nontoxigenic, non-pathogenic, histidinol dehydrogenase (HIS4 gene) mutant derived from strain *Pichia pastoris* NRRL Y-11430 (ATCC® 76273™, also known as CBS (b)). The strain *Pichia pastoris* SMD 1168, derived from strain *Pichia pastoris* GS115, has been cited in GRAS Notice 000204 (FDA, 2006) for safe use in enzyme production as well as GRN 764. *Pichia pastoris* is an FDA approved animal feed protein source and is approved for use in broiler feed up to 10% of the total feed (FDA, 1993).

Appendix 14. Published Utility Data in Broilers

Evaluation of a thermotolerant xylanase on broiler growth performance and dietary ileal digestible energy value

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Primary Audience: Live Production Personnel and Nutritionists

SUMMARY

Two experiments were conducted to evaluate the impact of a thermotolerant xylanase on male broiler performance and dietary ileal digestible energy (IDE). The first experiment consisted of 3 treatment groups with 12 replications per treatment each containing 35 Cobb 500 males for a total of 1,260 broilers placed in floor pens for a 42 d grow-out. The experiment treatments included a corn/soy diet with DDGS control formulated at a low energy level, and the control supplemented with one of 2 concentrations of xylanase (20,000 XU/kg [XYL20] and 40,000 XU/kg [XYL40]). No significant differences in body weight were observed with the inclusion of xylanase when compared to the control diet throughout the experiment. At d 28, the inclusion of XYL20 improved ($P < 0.05$) mortality corrected feed conversion ratio (FCR) compared to the control diet. Feed conversion ratio was also improved ($P < 0.01$) at d 42 for birds fed XYL20 when compared to the control. At d 42, inclusion of XYL20 and XYL40 significantly ($P < 0.05$) increased IDE compared to the control. Experiment 2 consisted of 4 treatment groups with 10 replications per treatment each containing 44 Cobb 500 males for a total of 1,760 broilers placed in floor pens for a 41 d grow-out. The dietary treatments included a positive control (PC) based on a corn/soy diet containing DDGS and phytase, a negative control (NC) diet (PC -150 kcal/kg in AME), NC + xylanase at 10,000 XU/kg (XYL10), and NC + xylanase at 20,000 XU/kg (XYL20). A significant increase ($P < 0.05$) in BW was observed in broilers fed the inclusion of XYL20 in the NC diet increased ($P < 0.05$) on d 14. A significant increase in cumulative body weight gain was observed on d 27 and d 41 with xylanase (XYL20) inclusion compared to the NC. These data demonstrate that xylanase inclusion increased energy utilization through improvements in IDE, which improved broiler performance.

Key words: xylanase, broiler, performance, digestible energy

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(b) (4)

Appendix 15. Published Utility Data in Swine

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Original Research Article

Dietary supplementation of xylanase and protease on growth performance, digesta viscosity, nutrient digestibility, immune and oxidative stress status, and gut health of newly weaned pigs

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Newly weaned pigs
Viscosity
Protease
Xylanase

ABSTRACT

This study was to investigate the effect of dietary supplementation with xylanase and protease on growth performance, digesta viscosity, apparent ileal digestibility (AID) of nutrients, and gut health in nursery pigs. Forty-eight pigs (24 barrows and 24 gilts at 21 d of age with 7.2 ± 0.4 kg BW) were randomly allotted to 4 dietary treatments (2 × 2 factorial arrangement) in a randomized complete block design and fed in 2 phases (phase 1 for 10 d and phase 2 for 14 d). Factors were xylanase (0 or 45,000 XU/kg) and protease (0 or 300,000 U/kg). Feed intake and BW gain were measured on d 10 and 24. Titanium dioxide (0.25%) was added to all diets as an indigestible external marker from d 20 to 24. On d 24, all pigs were euthanized to obtain jejunal and ileal digesta to measure viscosity and apparent ileal digestibility. The jejunal mucosa was collected to measure immune and oxidative stress status. Jejunal tissues were used to measure morphology and crypt cells proliferation. In phase 2, xylanase increased ($P < 0.05$) the average daily gain (ADG) which was further increased ($P < 0.05$) when combined with protease. Overall, combinational use of xylanase and protease increased ($P < 0.05$) ADG compared with the use of xylanase or protease alone, whereas protease improved ($P < 0.05$) feed efficiency. In jejunum, xylanase reduced ($P < 0.05$) viscosity of digesta, mucosal malondialdehyde (MDA), crypt depth and crypt cells proliferation, and protease increased ($P < 0.05$) villus height, and decreased ($P < 0.05$) crypt depth and crypt cells proliferation. Collectively, xylanase improved growth performance, digesta viscosity, and oxidative stress, whereas protease improved feed efficiency and gut morphology. The combinational use of xylanase and protease enhanced growth performance of newly weaned pigs.

(b) (4)

Appendix 16. Xylanase Shelf-Life Stability Study 1

Three different lots of xylanase were used to for this study (XLPe- I85-134, XLPe-I85-135, XLPe-I85-136). They were tested for a baseline activity at Month 0 using the Xylanase DNS assay method (Appendix 19). After baseline testing, samples of each lot were individually packaged for each time point of the study. Time points consisted of 1, 3, 4, and 6-month incubations.

To resemble storage condition and material, PBOM 3 ply bleached Kraft MW 80, metallicized with 0.4 micron Al / LDPE having a 2 mil HDPE liner bags were cut down to 6"x 6" squares and sealed on 3 sides to form smaller bags. Approximately 1 kg of xylanase was placed in each bag and sealed. Total of 5 bags per lot were prepared so that all three lots could be tested at each of the 4 time points with 1 spare bag.

Samples were placed in a 40°C and 75% humidity (b) (4) incubator. Incubator was equipped with thermometer and kept within ± 2°C of the target temperature.; humidity was controlled ± 5% from target. At each time point, 1 sample from each lot is pulled and tested via analytical method described in Appendix 19 for xylanase activity determination in product.

Table 1. Xylanase Storage at 40°C

	XLPe-I85-134		XLPe-I85-135		XLPe-I85-136	
Activity in XU/g; in % of initial activity	**XU/g**	**%**	**XU/g**	**%**	**XU/g**	**%**
Reference (25°C)	256,400	-	264,964	-	256,601	-
6 weeks (40°C)	(b) (4)	93%	(b) (4)	92%	(b) (4)	96%
16 weeks (40°C)		93%		95%		109%
21 weeks (40°C)		104%		92%		96%
27 weeks (40°C)		85%		78%		96%

Appendix 17. Xylanase Shelf-Life Stability Study 2

Three different lots of xylanase were used to for this study. They were tested for a baseline activity at Month 0 using the Xylanase DNS assay method (Appendix 19). After baseline testing, samples were individually packaged for each time point of the study. Time points consisted of 3, 9, 12, 15, 18, and 24 months.

To resemble storage condition and material, PBOM 3 ply bleached Kraft MW 80, metalized with 0.4 micron Al / LDPE having a 2 mil HDPE liner bags were cut down to 6"x 6" squares and sealed on 3 sides to form smaller bags. Approximately 500 g of xylanase was placed in each bag and sealed. Total of 8 bags per lot were prepared so that all three lots could be tested at each of the 8 time points.

Samples were placed in a 30°C incubator. Incubator was equipped with thermometer and kept within ± 2°C of the target temperature.

At each time point, a sample was pulled and tested for activity. Once samples have been pulled, they were not placed back in the incubator or retested after their original testing point. A new set of samples is tested at each time point. Each bag was opened with an I-cut and samples were pulled from the middle of the bag for testing.

Table 1. Average xylanase storage at 30°C

Activity in XU/g; in % of initial activity	U/g	%
Reference (25°C)	229,433	100%
3 Months (30°C)	255,281	111%
9 Months (30°C)	233,173	102%
12 Months (30°C)	242,245	106%
15 Months (30°C)	230,302	100%
18 Months (30°C)	212,523	93%
24 Months (30°C)	184,743	81%

Appendix 18. Xylanase in-Feed Stability and homogeneity: study 1 and 2

Study 1:

This study aimed to evaluate xylanase stability in a fully formulated poultry mash feed at room temperature over a period of 6 months.

Study Design

Number of Enzyme Product Lots: 3

Total Duration: 6 months

Sampling Frequency: Biweekly for three months (7 time points) and then at 5 and 6 months (2 time points)

Packaging Size: 8 lbs./bag

Sample Size: A composite sample of 250 grams from 5 locations of each bag (50 grams per location)

Replication: One bag per lot at each time point

Table 1. Number of samples vs. time

Enzyme Lot	Week							Month	
	0	2	4	6	8	10	12	5	6
1	3	1	1	1	1	1	1	1	1
2	3	1	1	1	1	1	1	1	1
3	3	1	1	1	1	1	1	1	1

Materials

1. Full formulated starter corn-soy based diet for poultry in mash form
2. Three production lots of xylanase with a minimum activity of 150,000 XU/g
3. Multi-wall paper with lines bags (50 lbs.) – bags usually used for feed storage
4. Reagents for standard xylanase in-feed assay detailed in Appendix 20

Methods

Feed Formulation

As shown in Table 2, a basal complete feed for starter broilers is formulated without xylanase as a control and a corresponding feed with 0.01% of xylanase is used for this in-feed stability study.

Table 2. Feed Formulation (Starter Diet)

	Diet Composition (%)	
Ingredient Name	Control	Xylanase
Corn	51.18	51.18
Soybean Meal	38.78	38.77
DICALCIUM PHOSPHATE	2.43	2.43
SALT, PLAIN (NaCl)	0.50	0.50
LIMESTONE FINE	0.52	0.52
L-LYSINE	0.06	0.06
POULTRY FAT	5.71	5.71
CHOLINE CHLORIDE 60	0.20	0.20
DL-METHIONINE	0.31	0.31
PX NCSU BR MINERAL	0.20	0.20
PX NCSU BR VITAMIN	0.05	0.05
SELENIUM PREMIX NCSU	0.05	0.05
Xylanase	0.00	0.01

Feed Mixing

Feed is mixed in a horizontal double ribbon mixer of a capacity of 300 lbs. A-180 lbs are mixed for each lot. A premix of xylanase is prepared by mixing xylanase with two pounds of either corn or wheat (depending on the diet) in a bench-top mixer for 2 minutes before being mixed with the rest of major and minor dry ingredients in the ribbon mixer. Dry ingredients (including major, minor, micro ingredients, and xylanase premix) are mixed for 2 minutes followed by adding wet ingredients and mixing for additional 3 minutes. This mixing time is selected to ensure uniformity and homogenous distribution of feed ingredients.

Packaging

Multi-wall paper with lines bags are used for packaging the finished feed. Original bag capacity is 50 lbs; however, bags were cut and modified to hold up to 8 lbs of finished feed. Modification of bags were achieved through trimming of sides and top of bags. Modified bags were used to store feed samples (8 lbs/bag). A total of 3 lots of feed were manufactured plus control; from each lot, a total of 11 feed samples were collected. Collected bags were randomly labelled from 1 to 11 per lot.

Storage

All feed samples were stored at room temperature in on a shelving unit in an indoor storage room. A digital thermometer recorded the temperature at least daily during the study.

Sampling

The feed samples are retrieved following randomized bags in Table 1. Each retrieved bag is opened completely by a razor to allow sampling of feed from 5 locations in each bag. Fifty grams of sample from

each location are mixed to create one composite sample per bag. The composite samples are used within the same day in the in-feed assay below.

In-feed Assay

The composite feed samples were tested following standard protocol for xylanase in-feed assay detailed in Appendix 20. Activity results were expressed as XU/g feed.

Statistical Analysis

For each lot, a linear regression was performed first using the average value at each time point.

The model is considered acceptable if it has a RSQ greater than 0.95. If the p-value for the slope (i.e. duration factor) is greater than 0.05, then it was concluded that there is no change in Xylanase activity during the study period. If the p-value is less than 0.05, the projected change of activity is calculated using the regression equation.

If the RSQ of the linear model is less than 0.95, a regression analysis using a quadratic or other nonlinear model was used. Additional sets of samples and time points may be tested to improve the model. Once the RSQ is greater than 0.95, the projected change of activity is calculated using the regression equation.

Results

Table 3. Xylanase Stability in Feed

Activity in XU/g; in % of initial activity	Lot 1		Lot 2		Lot 3	
Weeks	XU/g	%	XU/g	%	XU/g	%
0	17.25316	100%	17.19318	100%	18.6673	100%
2	(b) (4)	113%	(b) (4)	111%	(b) (4)	100%
4		126%		113%		105%
6		124%		113%		111%
8		118%		118%		108%
10		96%		98%		82%
12		109%		92%		86%
20		125%		105%		84%
24		108%		97%		86%

Conclusions

Xylanase enzyme is stable in complete feed for 6 months.

Study 2:

Summary and objectives

The objective of this study was to determine both the homogeneity and recovery of Xylamax® when incorporated into mash and pelleted feed manufactured using the same Xylamax® batch, feed formulation, and manufacturing specifications.

The homogeneity of Xylamax® in mash and pelleted feed was determined by calculating the coefficient of variation of xylanase activity in 10 independent samples of each feed form. The method used for determining the xylanase activity was SAP-2020 (xylanase in-feed assay, Appendix 20).

Percent recovery of xylanase in pelleted feed was determined through comparison of xylanase in mash and pelleted samples.

Test item used for homogeneity and in-feed recovery of the additive

Table 1. Details of Xylamax

TEST ITEM	LOT Nº, MANUFACTURE & EXPIRY DATE	ACTIVE SUBSTANCE	EXPECTED CONCENTRATION IN COMPLETE FEEDINGSTUFFS	SAFETY
Xylamax®	**Lot Nº:** XY20281 **Made:** Oct. 9th. 2020 **Expiry:** Oct. 8th, 2022 (Appendix 1)	endo-1,4-β-xylanase (≥150,000 XU/g)	10,000 XU/kg	See SDS (Appendix 23)

Xylamax® was added to a standard soy/corn-based mash feed for poultry and then pelleted at 85ºC. The composition of the feed is listed in Table below.

Ingredient Composition of the feed

Ingredient	%	Ingredient	%
Corn	67.43	Choline chloride, 60%	0.20
Soybean meal	26.55	Mineral premix	0.20
Poultry fat	2.00	Vitamin premix	0.05
Dicalcium Phosphate, 18.5%	1.77	L-lysine HCl	0.13
Calcium carbonate	0.86	Selenium premix, 0.06%	0.05
Sodium chloride	0.50	L-Threonine	0.09
DL-Methionine	0.17		

A total of 2000 lbs (907 kg) was mixed in a 2-ton mixer. Xylamax® was added at an inclusion rate of 70 g/MT. The enzyme was initially mixed with 11.04 lbs (5.00 kg) of ground corn (amount of corn used is included in the basal diet calculations) and then mixed in a bench top mixer for 3 minutes. The premix was added to a 2-ton mixer for blending into the feed. The complete feed was then dispatched to a 150hp pellet mill.

Pelleting Parameters

The diet was pelleted in a 150hp (b) (4) pellet mill (b) (4). The target conditioning parameters was 85˚C for 30 seconds, with a production rate of ~ 4 tons per hour and a die size of 11/64" with a 1 3/8" effective thickness.

Experimental design

HOMOGENEITY

Sampling plan: Homogeneity of Xylamax® was determined by calculating the coefficient of variation of xylanase enzymatic activity (expressed in XU/kg) in 10 independent samples collected from a single batch of mash feed and 10 independent samples collected from a single batch of pelleted feed. Each sample was analysed in triplicate for xylanase.

Samples (500g each) of feed was collected during manufacturing. The feed mill design allows for collection of mash samples as feed is dispatched from the mixer to the conditioner, thus allowing for a real-time estimate of homogeneity. The feed mill design also allows for collection of pelleted samples in real-time when the pelleted feed is dispatched from the pellet mill to the storage bins. Samples were collected in sterile plastic bags labelled with study ID, sample ID, and collection date.

Once all samples were collected, they were transported to the BRI laboratory for xylanase activity analysis. Xylanase activity was measured as described in SAP-2020 (Appendix 20).

Data analysis

Homogeneity

For homogeneity, the coefficient of variation (CV) was calculated:

$$CV = \text{standard deviation}/\text{mean} \times 100$$

Pelleted percent recovery

To determine thermostability of xylanase at a conditioning temperature of 85 °C, percent recovery of xylanase was calculated:

$$\text{Percent recovery (\%)}=(1-(\frac{mash\ enzyme\ activity-pellet\ activity}{mash\ enzyme\ activity}))x\ 100$$

Results

Homogeneity

Homogeneity of xylanase in mash and pelleted feed samples		
Sample ID	**Mash Xylanase activity (XU/kg)**	**Pelleted Xylanase activity (XU/kg)**
1	(b) (4)	(b) (4)
2		
3		
4		
5		
6		
7		
8		
9		
10		
Mean	**11,630**	**8,152**
SD	**0.69**	**0.16**
%CV	**6.00%**	**1.93%**

Pelleted xylanase recovery

The percent xylanase recovery of pelleted feed, when compared to mash, was 70% (Appendix 3).

Discussion and conclusion

Homogeneity & Pelleted xylanase recovery

Homogeneity was estimated from a 2000 lb (907 kg) batch of mash and pelleted feed by quantifying xylanase activity in 10 samples per feed form. Table 2 summarizes the homogeneity xylanase activity results. The 10 mash samples had an average xylanase activity of 11,630 XU/kg and a coefficient of variation value of 6.0%. The 10 pelleted samples had an average xylanase activity of 8,152 XU/kg and a coefficient of variation of 1.93%. The recovery of xylanase in pelleted feed was 70%. Thus, it can be concluded that:

- Xylamax® can be homogenously mixed in mash and pelleted feed

Appendix 19. Standard Analytical Procedure for xylanase in product activity

Xylanase Product Activity Assay

Principle

One unit of Xylanase activity is defined as the amount of enzyme needed for the release of 1 nanomole of reducing sugars per second from 0.5% xylan at 50°C in 50 mM Trisodium Citrate pH 6.0.

Chemicals

(b) (4)

Equipment and Supplies

(a) Circulating water bath -Thermostatically controlled to 50°C ± 0.5°C
(b) Circulating water bath - Thermostatically controlled to 95°C ± 10°C
(c) Manual single channel pipets
(d) Repeater Pipettor
(e) Spectrophotometer
(f) Vortex mixer
(g) 16 x 150 mm Glass tubes
(h) 16 x 100 mm Glass tubes
(i) 360° Rocker
(j) 50 mL centrifuge tubes
(k) 15 mL centrifuge tubes
(l) Digital Timer
(m) Insulated Ice bucket
(n) Heat resistant 16 mm diameter test tube rack
(o) Heat and moisture protective gloves

Safety

Always use general laboratory safety precautions. Consult the MSDS for each substance used for full information. The notes below are meant to highlight certain risks but may not be comprehensive.

(a) Good laboratory practice must be followed while handling and preparing all reagents.
(b) Safety glasses and gloves must be worn.
(c) Wear a laboratory coat when working in the laboratory.
(d) Always add concentrated acid or base to water.
(e) Always work with strong acids and alkalis under a suitable hood.
(f) Always wear approved respirator when working with powdered product.

Reagents

(b) (4)

Sample Preparation

(b) (4)

(b) (4)

Standard Curve Preparation

(b) (4)

Assay

(b) (4)

(b) (4)

Xylanase Activity Calculation

(b) (4)

Acceptance Criteria

Standard Curve

RSQ: ≥ 0.99

Sample

Technical Replicate RSD: ≤ 10%

Sample Replicate RSD: ≤ 10%

Appendix 20. Standard Analytical Procedure for xylanase in-feed activity

Xylanase In-Feed Assay – XylX6

Principle

This assay is used for quantitation of *endo*-1,4-β-D-xylanase activity in feed products. Enzymatic cleavage of XylX6 colorimetric substrate by endo-xylanase removes the blocked end of the oligosaccharide attached and allows the ß-xylosidase within the substrate solution to hydrolyze the remaining oligosaccharide resulting in the release of the colorimetric group, 4-nitrophenol. Xylanase enzymatic activity is calculated relative to a reference standard added to 50 mM Trisodium Citrate pH 6.0 buffer measured at A_{400}.

One unit of Xylanase activity is defined as the amount of enzyme needed for the release of 1 nanomole of reducing sugars per second from 0.5% xylan at 50°C in 50 mM Trisodium Citrate pH 6.0.

Chemicals

(b) (4)

[redacted]

Equipment and Supplies

(a) Dry Bath - Thermostatically controlled to 50°C ± 0.5°C
(b) Micropipettes
(c) Macropipettes
(d) Repeater Pipette
(e) Spectrophotometer
(f) Centrifuge capable of spinning 50 mL conical tubes at ≥ 7,000 *g*
(g) Vortex mixer
(h) 2 mL microcentrifuge tubes
(i) Floor Shaker
(j) 100 mL graduated cylinder
(k) 15 mL centrifuge tubes
(l) High Velocity 50 mL centrifuge tubes
(m) 250 mL bottles
(n) 16 x 150 mm Glass test tubes
(o) Digital Timer
(p) pH Meter with 0.01 pH resolution
(q) Analytical Balance with 0.01 g resolution

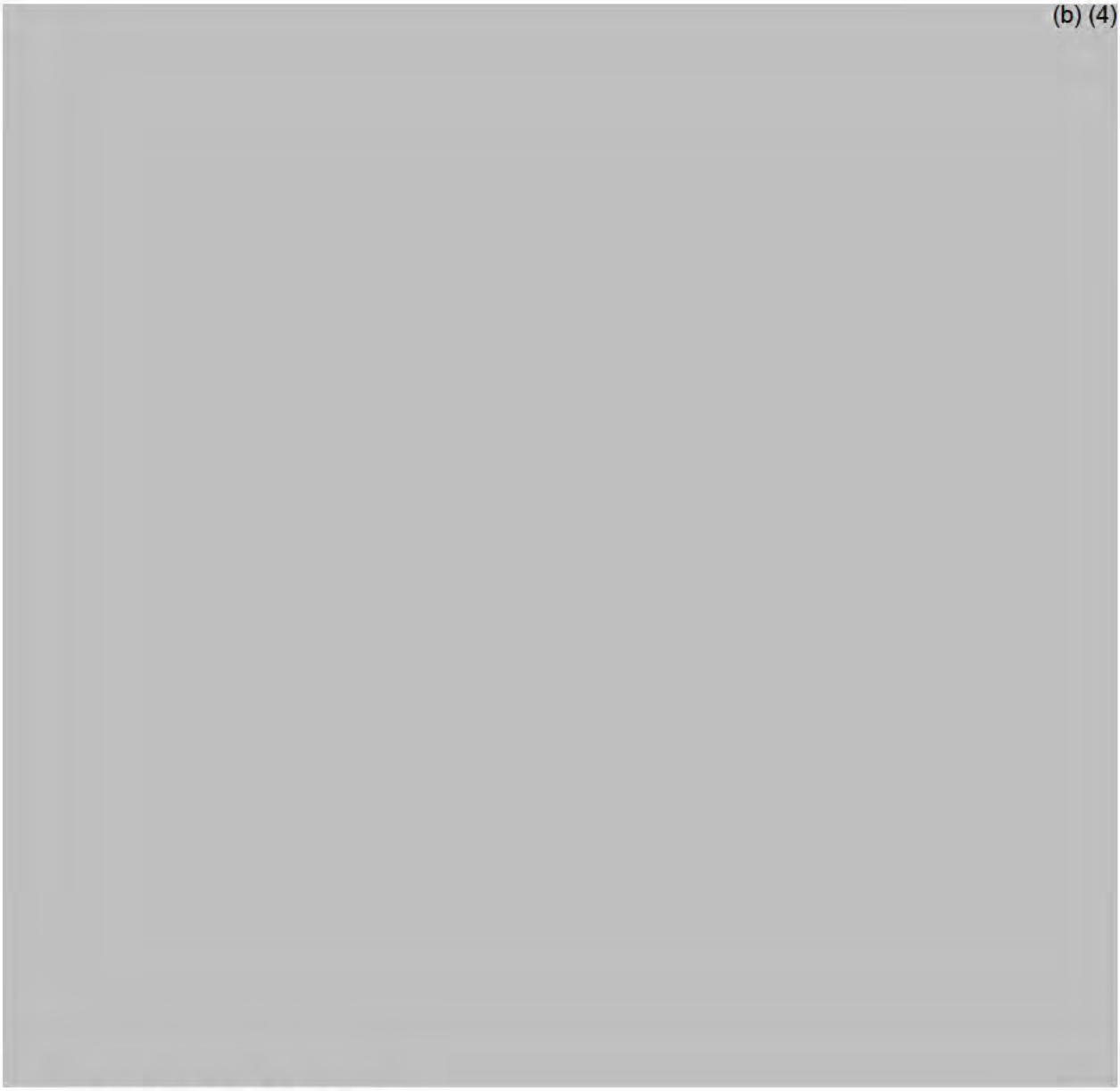
Safety

Always use general laboratory safety precautions. Consult the MSDS for each substance used for full information. The notes below are meant to highlight certain risks but may not be comprehensive.

(a) Good laboratory practice must be followed while handling and preparing all reagents.
(b) Safety glasses and gloves must be worn.
(c) Wear a laboratory coat when working in the laboratory.
(d) Always add concentrated acid or base to water.
(e) Always work with strong acids and alkalis under a suitable hood.
(f) Always wear approved respirator when working with powdered product.

(b) (4)

(b) (4)



Xylanase Activity Calculation

(b) (4)

(b) (4)

Assay Validation

Table 2. Assay Validation parameters

Assay Validation	XU/g
Minimum Activity Detection	4
Maximum Activity Detection	28
Linear Range	5 - 28
Accuracy (±)	10%

Acceptance Criteria

Standard Curve

RSQ: ≥ 0.99

Sample

Technical Replicate RSD: ≤ 5%

Sample Replicate RSD: ≤ 10%

Appendix 21. Assay validation for xylanase in product activity assay

Assay Validation Report for Xylamax® Xylanase Activity: Xylanase Activity Assay - DNS Reducing Sugar Method

SAP-2000

PURPOSE AND SCOPE

The purpose of this document is to report validation of acceptable performance characteristics for Bioresource International (BRI) analytical method for measurement of xylanase activity in Xylamax® feed additive (Standard Analytical Procedure SAP-2000). Method validation is performed according to Regulation (EC) No 882/20042. Validation of the analytical method serves to set acceptable standard criteria for testing xylanase in feed additive matrices containing xylanase.

OVERVIEW

The xylanase enzyme in Xylamax® catalyzes the hydrolysis of xylosidic linkages in an arabinoxylan backbone (and other β-1,4-linked xylans) depolymerizing arabinoxylan into smaller oligosaccharides therefore yielding xylose as end-product. The Standard Analytical Procedure SAP-2000 is carried out to determine the enzyme activity of the xylanase described here which is given in X unit (one XU unit is defined as the amount of enzyme needed for the release of 1 nanomole of reducing sugars (xylose equivalents) per second from 0.5% xylan from beechwood at 50°C in 50 mM trisodium citrate buffer pH 6.0. As international units (IU), one XU unit is 0.06 micromole/minute. Xylanase reacts with wheat arabinoxylan and releases xylose as a reducing sugar. This sugar reacts with dinitrosalicylic acid (DNS) to form a red complex, which is measured spectrophotometrically at a wavelength of 540 nm. This method is used as the standard method for product release of xylanase formulation at BRI.

Table below summarizes the outcomes of characterization studies performed. Analytical method validation was performed with comprehensive experiments to document performance, including accuracy, precision, repeatability, intermediate precision, specificity, detection limit, quantification limit, linearity, and range. Relative standard deviation (RSD) is the standard deviation divided by the average, represented as a percentage. Details related to identification of method type and validation approach, test method applications and validation protocol, the intended use of each test method application, and the analytical performance characteristics for each test method application are illustrated under each examined performance characteristic.

Type of analytical procedure characteristics	Assay Acceptance Criteria	Assay Validation
Accuracy	80 – 120%	98 - 102%
Precision		
Repeatability	≤ 10% RSD_r	5% RSD_r
Intermediate Precision	≤ 15% RSD_R	6% RSD_R
Specificity	No interference of target analysis	No interference of target analysis
Detection Limit	Signal-to-noise ratio 3:1	60 nmole xylose (9,920 XU/g)
Quantification Limit	Signal-to-noise ratio 10:1	196 nmole xylose (32,712 XU/g)

Linearity	Record	2 – 8 µg of Xylamax (40% - 160% target concentration)
Range	Record	2 – 8 µg of Xylamax (40% - 160% target concentration)

Relative standard deviation (RSD) is the standard deviation divided by the average, represented as a percentage.

RESOURCES

The SAP-2000 is company specific; method was developed and validated in-house. Equipment, software, and materials are listed under each performance characteristics tested.

REQUIREMENTS

Analytical method validation requires the evaluation of a method with comprehensive experiments to document performance, including sensitivity, specificity, accuracy, precision, detection limit, range, and limit of quantification.

DETAILED DATA

ACCURACY

Accuracy is the agreement between the value found and an accepted reference value. The accuracy was demonstrated by measuring triplicate samples at 3 different concentrations between 80 and 120% of the target concentration. Mean, standard deviation, relative standard deviation (RSD), and percent recovery were calculated. Percent recovery calculated with an enzyme standard theoretical value.

EXPERIMENT DESIGN

SAP-2000 was used to test 3 Xylamax® samples prepared at 3 different concentrations (80%, 100%, 120%, of the standard concentration). Xylanase concentration in Xylamax® product was expected to be ~160,000 XU/g standard concentration.

Statistical Analysis

Standard linear regression analysis was used to determine activity. The activity was reported as the percent recovery of the known activity value. Results illustrated below.

RESULTS

Target Concentration	Average XU/g	Standard Deviation	Relative Standard Deviation	95% Confidence Interval	Mean Recovery %
120%	161,891	2,532	2%	2,351	101%
100%	158,400	4,750	3%	7,472	99%
80%	159,785	1,569	1%	3,446	100%

LIMIT OF DETECTION / QUANTIFICATION

The detection limit of an individual analytical procedure is determined based on the lowest amount of target in a sample which can be detected but not necessarily quantitated as an exact value. The detection limit was determined by calculating the standard deviation of a number of blank samples and then multiply by three to estimate the signal at the limit of detection. The quantification limit of an analytical procedure is the lowest amount of target in a sample which can be quantitatively determined with suitable precision and accuracy. The recommended signal-to-noise ratio is 10:1. The quantification limit is calculated as 10*standard deviation of blank samples determined in 'Detection Limit' experiment.

EXPERIMENT DESIGN

Methods

SAP-2000 was used to test 3 three blank samples that were individually prepared (Xylamax®) and measured in quadruplicate. All samples were assayed for activity.

Statistical Analysis

Standard linear regression analysis was used to determine activity. The standard deviation, relative standard deviation (coefficient of variation) and confidence interval were reported. The detection limit was calculated as 3*standard deviation of blank samples. The quantification limit was calculated as 10*standard deviation of blank samples.

RESULTS

Measure	Value
A540-B Standard Deviation	0.0026
Relative Standard Deviation	1.13%
95% Confidence Interval	0.0015

Measure	Limit of Detection	Limit of Quantification
A540-B	0.0079	0.0264
Nanomoles Xylose	60	196
XU/g	9,920	32,712

PRECISION (REPEATABILITY & INTERMEDIATE PRECISION)

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements from multiple samples of a homogenous sample under the assay conditions. Precision was estimated with 3 analysts, on 2 different days, evaluating 3 samples. Collected data were analyzed using ANOVA to calculate repeatability standard deviation, contribution to total variation, and intermediate precision.

EXPERIMENT DESIGN: REPEATABILITY AND INTERMEDIATE PRECISION

Methods

SAP-2000 was used to test 3 Xylamax® samples that were prepared (Xylamax®) and measured in triplicate. All samples were assayed for activity. Xylanase concentration in Xylamax® product was expected to be ~155,000 XU/g standard concentration.

Statistical Analysis

Analysis of variance (ANOVA) was performed, activity mean, repeatability Standard deviation, contribution to total variation, and intermediate precision were calculated

Results

Measure	Xylamax®
Average XU/g	157,371
Repeatability Standard Deviation (S_r)	2,963
Contribution to total variation (S_b)	628
Intermediate Precision (S_I)	3,029
Expected Activity XU/g	155,000
Percent Recovery	101.5%
Relative Standard Deviation (RSD)	2.00%

RECOVERY

Recovery has been previously been determined to be ± 5% of the theoretical value based on the accuracy and repeatability data represented above.

SPECIFICITY/SELECTIVITY

Specificity is the ability to assess unequivocally the target in the presence of components which might be expected to be present. The specificity/selectivity of an assay method can be determined by adding a 'placebo' (non-product compound, limestone in this case as it is the major carrier in formulation and demonstrating the compound does not interfere with the analysis of the target.

EXPERIMENT DESIGN

Methods

SAP-2000 was used to test 3 Xylamax® samples and 3 limestone samples. All samples were assayed for activity. Xylanase concentration in Xylamax® product was expected to be ~150,000 XU/g standard concentration.

Statistical Analysis

Standard linear regression analysis was used to determine activity. The specificity was demonstrated as the absence of activity in limestone samples compared to Product samples, this was represented as a percent recovery of the known activity value for Product.

Metrics	Xylamax®	Limestone
Average XU/g	145,851	15,942
Standard Deviation	3,710	2,665
Standard Error of the Means	2,142	1,538
Relative Standard Deviation	3%	17%

LINEARITY

Linearity is the ability of the assay to return values that are directly proportional to the concentration of the target. The linearity was demonstrated by preparing standard solutions at various concentrations (≥6, 40 – 160 % of target concentration), with 3 individually prepared repeats at each concentration, and performing the standard assay procedure. Mean, standard deviation, and relative standard deviation (RSD) were calculated. Target concentration versus activity (absorbance or calculated activity) and a regression equation were calculated to determine if the acceptance criteria is met.

Methods

SAP-2000 was used to test Xylamax® samples prepared at 6 different concentrations (40 – 160% standard concentration) and assayed for activity. All samples were assayed for activity. Xylanase concentration in Xylamax® product was expected to be ~155,000 XU/g standard concentration.

Statistical Analysis

Standard linear regression analysis was used to determine activity. The standard deviation, relative standard deviation (coefficient of variation) and confidence interval were reported for each concentration. The linearity is defined as the concentrations of product which return values that are proportional to the target concentration, with an acceptable RSD value (<10%). Calculated nanomoles based on 155,000 XU/g and 20 minute incubation (1 unit of activity is 1 nanomole of xylose released per second).

Results

Concentration Xylamax® µg/mL	% Xylamax® relative to target	Average nanomole	Calculated nanomole	nanomole relative to target	RSD
2	40%	354	372	105%	1%
3	60%	584	558	95%	3%
4	80%	716	744	104%	4%

5	100%	913	930	102%	3%
6	120%	1095	1116	102%	3%
8	160%	1419	1488	105%	4%

(b) (4)

The SAP-2000 is linear from 2 – 8 µg of Xylamax® added to the reaction. The linear regression analysis shows an R^2 of 99.70%.

Slope	175,641,549
Intercept	27.3181
R^2	99.70%

RANGE

Range is the concentrations of target molecule that are below the low and high limits of quantification. The data for the linearity and accuracy experiment can be used to determine the range (3 replicates at each level). An acceptable range is defined as the concentration interval over which precision, accuracy, and linearity are obtained. This will be determined from analyzing the combined precision, accuracy, and linearity data. The range is defined as the low and high end of the linearity experiment. The range and the linearity are interchangeable in this experiment; therefore, linearity data can be used to indicate the range for this SAP.

APPLICABILITY

Xylanase is the active component in Xylamax®. Method SAP 2000 has been validated as the appropriate analytical procedure used to quantify the amount of xylanase in Xylamax® based on xylanase reaction with xylan from beechwood and the release of xylose as a reducing sugar. This sugar reacts with dinitrosalicylic acid (DNS) to form a red complex, which is measured spectrophotometrically at a wavelength of 540 nm. Validation studies were conducted testing between 40% to 160% of the standard xylanase target concentration. This SAP was developed for testing the feed additive therefore, the matrix tested was the feed additive product. The detailed SAP includes details related to equipment, software, reagents, safety precautions, and equipment needed.

Appendix 22. Assay validation for xylanase in-feed activity assay

Xylanase In-Feed Activity Assay Validation

SAP-2020 Rev 000 Xylanase In-Feed Assay - XylX6

Objective

Testing was completed using freshly prepared feed to determine acceptable assay validation parameters. Six assays were completed over the course of three days. Due to perceived substrate degradation, the results from all data collected during assay five have been removed from calculations. Associated data is located in P1150 Validation, the results are as follows.

Findings

I. Accuracy

U/g	Average	Expected Activity	Percent Recovery
50% Dosed Control Feed	8.0	7.51	107%
100% Dosed Control Feed	14.5	14.94	97%
150% Dosed Control Feed	22.4	22.45	100%

Accuracy was determined by comparing averaged U/g calculations taken from multiple runs of 50%, 100% and 150% dosed feed with expected activities calculated based on dry weight Xylamax addition to basal feed.

II. Precision

Precision - Repeatability		
Samples	A_{400-5}	U/g
Run 1	0.4060	14.81
Run 2	0.4343	14.78
Run 3	0.4143	14.71
Run 4-1	0.4067	13.79
Run 4-2	0.4347	14.74
Run 4-3	0.4400	14.92
Run 6-1	0.4437	14.67
Run 6-2	0.4240	14.00
Run 6-3	0.4190	13.83
Run 6-4	0.4407	14.56
Average	0.4263	14.48
StDev	0.013526	0.410199
%RSD	3.17%	2.83%

Repeatability data was collected from five runs performed over three days. Multiple weigh outs of 100% dosed feed were tested in two of these runs.

III. Detection / Quantitation Limit

	Average	St. Dev	Variance	RSD
XU/g	0.745	0.428	0.183	57%

XU/g	
Detection Limit	1.3
Quantification Limit	4.3

Detection and Quantitation Limits were determined using the average results from all 0 value curve data.

IV. Linearity / Range

A400 (Background Corrected)	Average	RSD
0% Dosed Control Feed	0.034	-
25% Dosed Control Feed	0.149	9%
50% Dosed Control Feed	0.241	7%
100% Dosed Control Feed	0.436	6%
150% Dosed Control Feed	0.645	6%
200% Dosed Control Feed	0.834	7%
U/g	Average	RSD
0% Dosed Control Feed	0.75	-
25% Dosed Control Feed	4.88	9%
50% Dosed Control Feed	8.02	9%
100% Dosed Control Feed	14.48	3%
150% Dosed Control Feed	22.36	5%
200% Dosed Control Feed	29.12	7%

Slope	35.36433
Intercept	-0.52531
R^2	0.999603

Linearity is demonstrated to be strong based on the 0.9996 r-squared value achieved from the averages of the calculated run's corrected A_{400} data plotted against the XU/g values averaged from the calculated runs.

V. Specificity

Limestone Control	Average	St. Dev	Variance	RSD
A_{400}	-0.0065	0.0025	0.00000625	-38%
XU/g	-0.89	0.1566	0.02452360	-18%
100% Dosed Control Feed				
A_{400}	0.422	0.00878	0.00007704	2%
XU/g	14.80	0.30143	0.09086159	2%

Specificity testing was tested using heat treated pulverized limestone and control feed.

Results

Based on the findings of five runs, the following are the established limits of the assay. Specifications for outlier determination have also been established to limit the variability of data evaluation between lab personnel.

Assay Validation

Assay Validation	XU/g
Minimum Activity Detection	4
Maximum Activity Detection	28
Linear Range	5 - 28
Accuracy (±)	10%

Acceptance Criteria

Standard Curve

RSQ: ≥ 0.99

Sample

Technical Replicate RSD: ≤ 5%

Sample Replicate RSD: ≤ 10%

Outlier Determination

For the purposes of this assay, an outlier is defined as:

- an individual technical replicate that when removed brings the sample reaction set %RSD into acceptable range.

- a sample reaction set that when removed from sample average calculation brings the sample %RSD into acceptable range.

No more than one technical replicate and one sample reaction set may be removed from calculation to achieve an acceptable %RSD range. If the removal of an outlier does not bring the %RSD into range, the sample must be retested.

Appendix 23. Safety Data Sheet of Xylamax

Safety Data Sheet

1. Identification

Product identifier	**Xylamax ®**
Other means of identification	None
Recommended use	Enzyme Feed Additive
Recommended restrictions	None known

Manufacturer/Importer/Supplier/Distributor Information

Company name	BioResource International, Inc.
Address	4222 Emperor Blvd, Suite 460 Durham, NC 27703 United States
Telephone	+1 (919)993-3389
Email	info@briworldwide.com
Contact person	Not available.
Emergency phone number	+1 (919)993-3389

2. Hazard(s) identification

Physical hazards	Not classified		
Health hazards	Sensitization, respiratory	Category 1	H334 – May cause allergy or asthma symptoms or breathing difficulties if inhaled
OSHA defined hazards	Not classified		
Label elements			
Signal word	Danger		
Hazard statement	**H334** – May cause allergy or asthma symptoms or breathing difficulties if inhaled.		

Precautionary statements

Prevention	**P261** – Avoid breathing dust
Response	**P304 + P340** – If inhaled: If breathing is difficult, remove person to fresh air and keep comfortable for breathing
Storage	**P402** – Store in a dry place
Disposal	**P501** – Dispose of contents/ Container in accordance with local/regional/notional/international regulations

Hazard(s) not otherwise classified (HNOC)	None known.
Supplemental information	None.

3. Composition/information on ingredients

Substances

Chemical name	Common name and synonyms	CAS number	%
Pulverized limestone (CaCO3)		1317-65-3	70-90
Xylanase, endo-1, 4-		9025-57-4	10-30

Composition comments

4.First-aid Measures

Inhalation	Move to fresh air. Get medical attention if irritation, allergic symptoms, or other symptoms develop and persist
Skin Contact	No first aid should be required. Wash with soap and water after handling. Seek medical attention if irritation or rash develops and persists.
Eye contact	In case of eye contact, flush eyes immediately with plenty of water. Remove contact lenses, if present and easy to do after the first 5 minutes. Continue rinsing. Seek medical attention if irritation persists
Ingestion	No first aid should be needed. Not intended for human consumption but no adverse effects are expected from ingestion.
Most important symptoms/effects, acute and delayed	Mild eye and skin irritation. Inhalation of dust from dried product may cause allergic respiratory reaction in sensitized individuals with symptoms of wheezing and difficulty breathing.
Indication of immediate medical attention and special treatment needed	Immediate medical attention is required for inhalation allergic reactions
General Information	Ensure that medical personnel are aware of the material(s) involved, and use precautions to protect themselves

5. Fire-fighting measures

Suitable extinguishing media	Use water spray, water fog, carbon dioxide, foam, or dry chemical. Water is most effective.
Specific hazards arising from the chemical	Dust generated in handling this material may present an inhalation hazard if suspended in air at high concentrations. Minimize the generation and accumulation of dust.
Special Protective equipment and precautions for firefighters	Firefighters should wear positive pressure self-contained breathing apparatus and protective gear.
Fire fighting equipment/instructions	In case of fire and/or explosion do not breath fumes. Move containers from fire area if you can do so without risk clothing to avoid contact
Specific methods	Use standards firefighting procedures and consider the hazards of other involved materials
General fire hazards	Avoid generating dust; fine dust dispersed in air in sufficient concentration, and in the presence of an ignition source is a potential dust explosion hazard

6. Accidental release measures

Personal precautions, protective equipment and emergency procedures	Keep unnecessary personnel away. Keep people away from and upwind of spill/leak. Use only non-sparking tools. Dust deposits should not be allowed to accumulate on surfaces, as these may form an explosive mixture if they are released into the atmosphere in sufficient concentration. Wear appropriate protective equipment and clothing during clean-up. Avoid inhalation of dust. Use a NIOSH/MSHA approved respirator if there is a risk of exposure to dust/fume at levels exceeding the exposure limits. Do not touch damaged containers or spill material unless wearing appropriate protective clothing. Ensure adequate ventilation. Local authorities should be advised if significant spillages cannot be contained. For personal protection, see section 8 of the SDS
Methods and materials for containment and cleaning up	Eliminate all ignition sources (no smoking, flares, sparks, or flames in immediate area). Take precautionary measures against static discharge. Use only non-sparking tools. Avoid dispersal of dust in the air (i.e. cleaning dust surfaces with compressed air). Minimize dust generation and accumulation. Collect dust using a vacuum cleaner equipped with HEPA filter. Stop the flow of material if this is without risk. Large spills: Wet down with water and dike for later disposal. Shovel the material into waste container. Absorb in vermiculite, dry sand or earth and place into containers. Following product recovery flush area with water. Small spills: Sweep up or vacuum up spillage and collect in suitable container for disposal. Wipe up with absorbent material (e.g. cloth, fleece). Clean surface thoroughly to remove residual contamination.
Environmental precaution	Never return spills to original containers for re-use. For waste disposal, see section 13 of the SDS. Avoid discharge into drains, water courses or onto the ground.

8. Exposure controls/Personal protection

Occupational exposure limits

US. OSHA Table Z-1 Limits for Air Contaminants (29 DFR 1910.1000)

Components	Type	Value	Form
Limestone (CAS 1317-65-3)	PEL/TWA as PNOC	5 mg/m3 to 15 mg/m3	Respirable fraction to Total dust
Xylanase, endo-1, 4- (CAS 9025-57-4)	PEL/TWA as PNOC	5 mg/m3 to 15 mg/m3	Respirable fraction to Total dust

Biological limit values	No biological exposure limits noted for the ingredient(s).
Exposure guidelines	If exposure limits have not been established, maintain airborne levels to an acceptable level.
Appropriate engineering controls	Explosion-proof general and local exhaust ventilation. Good general ventilation should be used. Ventilation rates should be matched to conditions. If applicable, use process enclosure, local exhaust ventilation, or other engineering controls to maintain airborne levels below recommended exposure limits. Eye wash facilities and emergency shower must be available when handling this product.

Individual protection measures, such as personal protective equipment	
Eye/face protection	Follow facility requirements. Safety glasses or dust goggles recommended to avoid eye contact.
Skin Protection	
Hand protection	Wear appropriate chemical resistant gloves. Suitable gloves can be recommended by the glove supplier.
Skin Protection	
Other	Wear appropriate chemical resistant clothing
Respiratory protection	Chemical respirator with organic vapor cartridge, full facepiece, dust and mist filter
Thermal hazards	Wear appropriate thermal protective clothing, when necessary
General hygiene considerations	When using, do not eat, drink, or smoke. Always observe good personal hygiene measures, such as washing after handling the material and before eating, drinking, and/or smoking. Routinely wash work clothing and protective equipment to remove contaminants.

9. Physical and chemical properties

Appearance	
Physical state	Solid.
Form	Powder.
Color	Grey
Odor	Neutral.
Odor Threshold	Not Available.
pH	Not Available.
Melting point/freezing point	Not Available.
Initial boiling point and boiling range	
Flash point	Not Available.
Evaporation rate	Not Available.
Flammability (solid, gas)	Not Available.
Upper/lower flammability or explosive limits	
Flammability Limit – lower (%)	Not Available.
Flammability Limit – upper (%)	Not Available.
Explosive limit – lower (%)	~100 g/m3 (fine dust
Explosive limit – upper (%)	Not Available.
Vapor pressure	Not Available.
Vapor density	Not Available.
Relative density	Not Available.
Solubility(ies)	
Solubility (water)	Not Available.
Partition coefficient (n-octanol/water)	Not Available.

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Auto-ignition temperature	Not Available.
Decomposition temperature	Not Available.
Viscosity	Not Available.
Other information	
Explosive properties	No-data- Available.
Oxidizing properties	No-data- Available.

10. Stability and reactivity

Reactivity	The product is stable and non-reactive under normal condition of use, storage, and transport.
Chemical stability	Stable under normal storage and handling conditions.
Possibility of hazardous reactions	No dangerous reaction known under conditions of normal use.
Conditions to avoid	Keep away from heat, sparks, and open flame. Minimize dust generation and accumulation. Contact with incompatible materials.
Incompatible materials	Strong oxidizing agents. Sensitive to moisture.
Hazardous decomposition products	Thermal decomposition will release oxides of carbon and nitrogen.

11. Toxicological information

Information on likely routes of exposure

Inhalation	May cause allergy or asthma symptoms or breathing difficulties in inhaled.
Skin contact	No adverse effects due to skin contact are expected.
Eye Contact	May cause mild irritation.
Ingestion	Not intended for human consumption but no adverse effects are expected from ingestion.
Symptoms related to the physical, chemical and toxicological characteristics	Coughing. Difficulty in breathing.

Information on toxicological effects

Acute toxicity	Not available.
Skin corrosion/irritation	Not classified.
Serious eye damage/eye irritation	Mild irritation possible.

Respiratory or skin sensitization

Respiratory sensitization	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
Skin sensitization	Not classified. Not classified.
Germ cell mutagenicity	May cause allergy or asthma symptoms or breathing difficulties if inhaled.

Carcinogenicity

IARC Monographs. Overall Evaluation of Carcinogenicity

Not listed.

NTP Report of Carcinogens

Not listed.

OSHA Specifically Regulated Substances (29 CFR 1910.1001-1050)

Not listed.

Reproductive toxicity	Not classified.
Specific target organ toxicity – single exposure	Not classified.
Specific target organ toxicity – repeat exposure	Not classified.

Aspiration hazard	Not classified.

12. Ecological information

Ecotoxicity	The product is not classified as environmentally hazardous. However, this does not exclude the possibility that large or frequent spills can have a harmful or damaging effect on the environment.
Persistence and degradability	No data is available on the degradability of this product.
Bioaccumulative Potential	No data available.
Mobility in soil	No data available.
Other adverse effects	None known.

13. Disposal considerations

Disposal Instructions	This product, if disposed as purchased would not meet the criteria of a RCRA hazardous waste.
Local disposal regulations	Dispose in accordance with all applicable regulations.
Hazardous waste code	The waste code should be assigned in discussion between the user, the producer, and the waste disposal company.
Waste from residues/unused products	Dispose of in accordance with local regulations. Empty containers or liners may retain some product residues. This material and its container must be disposed of in a safe manner (see: Disposal instructions).
Contaminated packaging	Since emptied containers may retain product residue, follow label warnings even after container is emptied. Empty containers should be taken to an approved waste handling site for recycling or disposal.

14. Transport information

DOT	Not regulated as dangerous goods.
IATA	Not regulated as dangerous goods.
IMDG	Not regulated as dangerous goods.
Transport in bulk according to Annex II of MARPOL 73/78 and the IBC code	Not applicable.

15. Regulatory information

US federal regulations	The product is not hazardous under the criteria of the Federal OSHA Hazard Communication Standard (29 CFR 1910.1200).

TSCA Section 12(b) Export Notification (40 CFR 707, Subpt.D)
Not regulated
OSHA Specifically Regulated Substances (29 CFR 1910.1001-1050)
Not regulated
CERCLA Hazardous Substance List (40 CFR 302.4)
Not listed
Superfund Amendments and Reauthorization Act of 1986 (SARA)

Hazard Categories	Immediate Hazard – No Delayed Hazard – No Fire Hazard – No Pressure Hazard – No Reactivity – No

SARA 302 Extremely hazardous substance
Not listed.

SARA 313 (TRI reporting)
Not regulated
Other federal regulations
Clean Air Act (CAA) Section 112 Hazardous Air Pollutants (HAPs) List
Not Regulated.
Clean Air Act (CAA) Section 112(r) Accidental Release Prevention (40 CFR 68.130)
Not regulated.
Safe Drinking Water Act (SDWA) Not regulated.
US State regulations
US. Massachusetts RTK – substance List
Limestone (CAS 1317-65-3)
US. New Jersey Worker and Community Right-to-Know act
Limestone (CAS 1317-65-3)
US. Pennsylvania Worker and Community Right-to-Know Law
Limestone (CAS 1317-65-3)
US. Rhode Island RTK
Not regulated.
US. California Proposition 65
California Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65): This material is not known to contain any chemical currently listed as carcinogens or reproductive toxins.

16. Other information, including date of preparation or last revision

Issue date 12-March-2015
Revision date 06-July-2020
Version # 03
NFPA ratings

Disclaimer BioResource International, Inc. cannot anticipate all conditions under which this information and its product, or the products of other manufacturers in combination with its product, may be used. It is the user's responsibility to ensure safe conditions for handling, storage and disposal of the product, and to assume liability for loss, injury, damage or expense due to improper use. The information in the sheet was written based on the best knowledge and experience currently available.
Xylamax ® is a trademark of BioResource International, Inc. and are registered in the United States and other countries.

Cerrito, Chelsea

From: Kristi Smedley <smedley@cfr-services.com>
Sent: Thursday, February 18, 2021 12:35 PM
To: Animalfood-premarket
Subject: RE: [EXTERNAL] RE: BioResource International, Inc.'s GRAS Submission for Xylanase Enzyme in Swine and Poultry Feed
Attachments: 16.Rij 1986 Yeast Book-reduced.pdf

Good Idea, I have reduced the file, I hope it works!

Kristi O. Smedley, Ph.D.

Center for Regulatory Services, Inc.
5200 Wolf Run Shoals Rd.
Woodbridge, VA 22192

Ph. 703-590-7337
Cell (b) (6)
Fax 703-580-8637

From: Animalfood-premarket [mailto:Animalfood-premarket@fda.hhs.gov]
Sent: Thursday, February 18, 2021 11:50 AM
To: Kristi Smedley
Cc: Animalfood-premarket
Subject: RE: [EXTERNAL] RE: BioResource International, Inc.'s GRAS Submission for Xylanase Enzyme in Swine and Poultry Feed

Dear Kristi,

Thank you for #22 and 26. Can you send #16 by e-mail in a zip folder or as a reduced size PDF? That should help make the size more manageable to provide by e-mail.

Thanks!
Chelsea

From: Kristi Smedley <smedley@cfr-services.com>
Sent: Thursday, February 18, 2021 11:10 AM
To: Animalfood-premarket <Animalfood-premarket@fda.hhs.gov>
Subject: [EXTERNAL] RE: BioResource International, Inc.'s GRAS Submission for Xylanase Enzyme in Swine and Poultry Feed

Chelsea:

I have attached reference number 22 and 26.

I note that number 16 is a book (The yeasts - a taxonomic study, 3rd ed.) . I have a full electronic copy but it is 99 mb (too large to email). I am thinking this may be a resource currently in the DAF library. Under the GRAS regulations, we do not need to provide a copy, unless it is requested. If this is a request, I can burn it to a DVD and FEDEX it to you. Or do you have access to a drop box I could send it to?

Kristi O. Smedley, Ph.D.

Center for Regulatory Services, Inc.
5200 Wolf Run Shoals Rd.
Woodbridge, VA 22192

Ph. 703-590-7337
Cell (b) (6)
Fax 703-580-8637

From: Animalfood-premarket [mailto:Animalfood-premarket@fda.hhs.gov]
Sent: Wednesday, February 17, 2021 9:43 AM
To: Kristi Smedley (smedley@cfr-services.com)
Cc: Animalfood-premarket
Subject: BioResource International, Inc.'s GRAS Submission for Xylanase Enzyme in Swine and Poultry Feed

Dear Dr. Smedley,

We are currently determining if BioResource International, Inc.'s GRAS submission, dated December 29, 2020, regarding a xylanase enzyme to be used in swine and poultry feed is acceptable for filing. On the compact disc (CD) provided as part of the submission, the following references were not provided (the references are numbered as they appear in Part 7):

16. Kreger-van Rij, N.J.W., 1984. The yeasts - a taxonomic study, 3rd ed. Elsevier Sci. Publ., Amsterdam.
22. United States Pharmacopeial Convention. Food Chemical Codex. Edition 10. Monograph: Enzyme Preparations. United States Pharmacopeial Convention, Board of Trustees, 2016. Pg 445-450.
26. Baghban, R., Farajnia, S., Ghasemi, Y., Mortazavi, M., Zarghami, N., & Samadi, N. (2018). New developments in pichia pastoris expression system, review and update. Current Pharmaceutical Biotechnology, 19(6), 451-467. doi:http://dx.doi.org.prox.lib.ncsu.edu/10.2174/1389201019666180718093037

We are requesting a copy of these references, which can be sent to this e-mail address. Please let me know if you have any questions.

Kind regards,
Chelsea

Chelsea Cerrito, MAS
Animal Scientist, Division of Animal Feeds (DAF)

Center for Veterinary Medicine
Office of Surveillance and Compliance
U.S. Food and Drug Administration
Tel: 240-402-6729
Personal e-mail address: Chelsea.Cerrito@fda.hhs.gov
To schedule a meeting with DAF, please e-mail: animalfood-premarket@fda.hhs.gov

The yeasts

a taxonomic study

third revised and enlarged edition

edited by

N.J.W. Kreger-van Rij

Elsevier

T-0005

Cerrito, Chelsea

From: Kristi Smedley <smedley@cfr-services.com>
Sent: Thursday, October 07, 2021 12:54 PM
To: Animalfood-premarket
Cc: 'Rasha Qudsieh'; Conway, Charlotte
Subject: RE: [EXTERNAL] RE: GRAS Notice No. AGRN 44 - Call Regarding Xylanase enzyme to be used in swine and poultry feeds
Attachments: CFR Services Cover Letter BRI AGRN 44 amendment Oct 7 2021.pdf; Attachment A. Chemistry Manufacturing --BRI Xylanase GRAS - Response to FDA.pdf; Attachment A-Revised Appendix 7.pdf; Attachment A. Addendum to Appendix 21.pdf; Attachment A. Addendum to Appendix 22.pdf; Attachment A. New Appendix 24. GRAS XY purity (b) (4)df; Attachment A-Revised Appendix 23.pdf; Attachment B.Utility- BRI Xylanase GRAS - Response to FDA.pdf; Attachment C. TAS-BRI Xylanase GRAS - Response to FDA.pdf; Attachment D. Molecular Biology- BRI Xylanase GRAS - Response to FDA.pdf; Attachment D. Molecular Biology- BRI Xylanase GRAS - Response to FDA.pdf; Attachment E. Microbial Safety-BRI Xylanase GRAS - Response to FDA.pdf

Please see attachments to this email that provides the response to concerns raised by the Division specific to AGRN 44.

Should have any problems receiving these attachments or on the provided information, please contact me.

Kristi O. Smedley, Ph.D.

Center for Regulatory Services, Inc.
5200 Wolf Run Shoals Rd.
Woodbridge, VA 22192

Ph. 703-590-7337
Cell (b) (6)
Fax 703-580-8637

From: Animalfood-premarket [mailto:Animalfood-premarket@fda.hhs.gov]
Sent: Wednesday, September 22, 2021 12:38 PM
To: Kristi Smedley
Cc: Rasha Qudsieh; Animalfood-premarket; Conway, Charlotte
Subject: RE: [EXTERNAL] RE: GRAS Notice No. AGRN 44 - Call Regarding Xylanase enzyme to be used in swine and poultry feeds

Dear Dr. Smedley,

With regards to GRAS Notice No. AGRN 44, please find attached our meeting minutes from the September 15, 2021 teleconference and response to your request for the meeting minutes. Please let us know if you have any questions.

Kind regards,
Chelsea

From: Animalfood-premarket <Animalfood-premarket@fda.hhs.gov>
Sent: Wednesday, September 15, 2021 12:44 PM
To: Kristi Smedley <smedley@cfr-services.com>
Cc: Animalfood-premarket <Animalfood-premarket@fda.hhs.gov>; Rasha Qudsieh <rQudsieh@briworldwide.com>
Subject: RE: [EXTERNAL] RE: GRAS Notice No. AGRN 44 - Call Regarding Xylanase enzyme to be used in swine and poultry feeds

Dear Kristi,

Thank you for your request for minutes. A copy will be e-mailed to you and Dr. Qudsieh as soon they are available.

Kind regards,
Chelsea

From: Kristi Smedley <smedley@cfr-services.com>
Sent: Wednesday, September 15, 2021 12:10 PM
To: Animalfood-premarket <Animalfood-premarket@fda.hhs.gov>
Cc: Rasha Qudsieh <rQudsieh@briworldwide.com>
Subject: RE: [EXTERNAL] RE: GRAS Notice No. AGRN 44 - Call Regarding Xylanase enzyme to be used in swine and poultry feeds

Chelsea:

Thank you for organizing this meeting. It was very beneficial, and we will be working on our amendment.

We are requesting the notes of this meeting. I am requesting that they be sent by email to both Rasha (Rasha Qudsieh (rQudsieh@briworldwide.com)) and I.

Kristi O. Smedley, Ph.D.

Center for Regulatory Services, Inc.
5200 Wolf Run Shoals Rd.
Woodbridge, VA 22192

Ph. 703-590-7337
Cell (b) (6)
Fax 703-580-8637

From: Animalfood-premarket [mailto:Animalfood-premarket@fda.hhs.gov]
Sent: Wednesday, September 08, 2021 9:08 AM
To: Kristi Smedley
Cc: Animalfood-premarket
Subject: RE: [EXTERNAL] RE: GRAS Notice No. AGRN 44 - Call Regarding Xylanase enzyme to be used in swine and poultry feeds

Dear Dr. Smedley,

I have scheduled the call for Wednesday, September 15 from 10-11 am US Eastern Time.

Below are the Zoom details for the call. Once you click on the hyperlink "Join Zoom Meeting", you will be prompted to connect your audio either by using your computer audio or dialing in by phone (will require entering the meeting ID and passcode (b) (6).

Please let me know if you have any questions.

Kind regards,
Chelsea

Join Zoom Meeting

One tap mobile: (b) (6)

Meeting URL:
Meeting ID:
Passcode:

Join by Telephone

For higher quality, dial a number based on your current location.
Dial: (b) (6)

Meeting ID:

Passcode:

International numbers

From: Kristi Smedley <smedley@cfr-services.com>
Sent: Tuesday, September 07, 2021 11:51 AM
To: Animalfood-premarket <Animalfood-premarket@fda.hhs.gov>
Subject: [EXTERNAL] RE: GRAS Notice No. AGRN 44 - Call Regarding Xylanase enzyme to be used in swine and poultry feeds

Thank you, we prefer Wednesday, September 15 from 10 – 11 am.

Kristi O. Smedley, Ph.D.

Center for Regulatory Services, Inc.
5200 Wolf Run Shoals Rd.
Woodbridge, VA 22192

Ph. 703-590-7337
Cell (b) (6)
Fax 703-580-8637

From: Animalfood-premarket [mailto:Animalfood-premarket@fda.hhs.gov]
Sent: Tuesday, September 07, 2021 9:01 AM
To: Kristi Smedley (smedley@cfr-services.com)
Cc: Animalfood-premarket
Subject: GRAS Notice No. AGRN 44 - Call Regarding Xylanase enzyme to be used in swine and poultry feeds

Dear Dr. Smedley,

I hope this e-mail finds you well. We would like to schedule a call with you, as well as any others from BioResource International, Inc., to discuss the GRAS notice. We are available during the following dates and times (US Eastern):

1. Wednesday, September 15 from 10 – 11 am
2. Thursday, September 16 from 12 – 1 pm

Please let me know if one of these options works or if I should look for more options. I will send Zoom information for the call once it has been scheduled.

Kind regards,
Chelsea

Chelsea Cerrito, MAS
Animal Scientist, Division of Animal Feeds (DAF)

Center for Veterinary Medicine
Office of Surveillance and Compliance
U.S. Food and Drug Administration
Tel: 240-402-6729
Personal e-mail address: Chelsea.Cerrito@fda.hhs.gov
To schedule a meeting with DAF, please e-mail: animalfood-premarket@fda.hhs.gov

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CFR@c-fr-services.com

Since 1973
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October 7, 2021

David Edwards, Director
Division of Animal Feeds (HFV- 220)
Center for Veterinary Medicine
Food and Drug Administration
7519 Standish Pl.
Rockville, MD 20855

Subject: Response to Division of Animal Feeds Concerns—September 22 2021
Xylanase preparation for the use in swine and poultry feed
AGRN 44

Notifier: BioResource International, Inc.
4222 Emperor Blvd., Suite 460
Durham, NC USA 27703

Dear Dr. Edwards:

On behalf of BioResource International, Inc., I am providing responses to the clarification issues as raised by animal GRAS notice for the use of Xylanase prepared from *Komagataella phaffii* expressing the gene encoding xylanase from *Orpinomyces sp.* for use in poultry and swine diets. These issues were raised in a teleconference of September 15, 2021 and the letter issued on September 22, 2021.

We have provided 6 sections of attachments (some include revised or new appendices) to this letter each one covering one area of the provided concerns as covered in the September 22, 2021 memo:

A. Chemistry and Manufacturing Control Issues
B. Utility
C. Target Animal Safety
D. Molecular Biology
E. Microbial Safety
F. New references

Please note that we have revised the maximum suggested use level of the Xylanase preparation to 40,000 XU/Kg feed.

Should you have any questions on the filing, please contact me directly. We are providing by email.

Sincerely,

(b) (4)

Kristi O, Smedley
Consultant to BioResource International, Inc.

Cc: Rasha Qudsieh, BRI

ATTACHMENTS:

As described in the letter

ATTACHMENT A- AGRN 44—October 6, 2021

Chemistry, Manufacturing, and Controls (CMC)

Specifications

CVM: CVM requested the notifier to address and clarify the presence of other potential contaminants[3], as appropriate, in the amendment. CVM requested the notifier to clarify the specification limits for some of the parameters, including clarifying how those limits were established. For example, for lead, where the limits are higher than the values supported by the batch analyses or for mold where the specification is set at ≤1000 colony forming units per gram (CFU/g) but it is not clear how the data submitted for mold relates to any batches notified substance. The notifier should also explain why it is not necessary to analyze for the presence of formaldehyde, formic acid, or other potential contaminants. The notifier should provide calculation for total organic solids (TOS) referred to in the submission and clearly state if the TOS value is for the enzyme or its marketed form(s).

[3]: As a follow-up to the September 15, 2021 teleconference, CVM clarifies that it requests the method used and results of three representative batches of each market formulation be provided for these specifications.

Response:
Information listed in Table 2 and Appendix 12 have been revised and an updated Table 2 and Appendix 12 are provided in the amendment to explain the levels of potential contaminants in 3 batches of the final product Xylamax. Specification limits are provided in the revised Table 2. Based on the nature of the substances and raw material specifications used in the entire process of Xylamax manufacturing, it is not expected to have any contaminants in the final market product. To ensure that the assessment is correct, several additional contaminants (not part of the final product specification testing) were tested in multiple Xylamax final product batches as a confirmation, the results are listed as part of Appendix 12 table and in Table A. In addition, these additional contaminants will be re-evaluated on a yearly basis to make sure the assessment remains correct and final product is not contaminated. For the heavy metals tested, the detected levels are very low therefore are not considered a safety concern. Furthermore, the pre-established quality control standards are in place to ensure quality of material going into final product are safe and comply with standards. As indicated in ICCF Guidance # 4, the specifications should be based on analysis of multiple batches and should reflect the identity, safety, quality (including the purity) and intended effect of the feed ingredient. In addition this guidance suggests that the specification for fermentation products should be most specific on the microbial and mycotoxin contamination. As demonstrated in our multiple batch analysis, mycotoxin and microbial assessment was below the LOQ of the assay. As such, BRI has chosen specific mycotoxins, heavy metals, and microbial speciation's, to assure their product is safe and manufactured under controlled process. The limits set are based on the results of the three batch analysis as well as the safety of the contaminant (importantly this product will be incorporated in the feed at very low levels, a maximum of 0.025 grams xylanase prepation/Kg body weight. Hence there is no safety concern related to the established specifications.

For the analyses on presence of formaldehyde, formic acid, or other potential contaminant. The analysis of *Enterobacteriaceae*, ethanol, isopropanol, and methanol was performed on an additional 3 samples of Xylamax final products, and for Glycols (diethylene glycol, ethylene glycol, and propylene) on an additional final Xylamax product, results are summarized in Table A below. Results illustrate that for these analyses, the values were below the limit of quantification (LOQ) of each analytical procedure, therefore, none of these contaminants were detected in the final product which indicates that none were present in any of the raw material used in manufacturing, furthermore, the absence of methanol contamination indirectly illustrates that formaldehyde is not a contamination concern neither in raw material nor in final manufactured product. Certificates of Analysis from the analytical labs are provided as a supplement in Attachment A (Attachment A. New Appendix 24. GRAS XY purity (b) (4)) providing the method used for determining each analyte. We do not believe a specification for these contaminants is warranted.

Table A. Additional Xylamax purity analyses

	Batch No XY20281	Batch No XY20288	Batch No XY20295
	cfu/g		
Enterobacteriaceae	< 10	< 10	< 10
	ppm		
Ethanol	< 10	< 10	< 10
Isopropanol	< 10	< 10	< 10
Methanol	< 10	< 10	< 10
Glycols	Batch No XY18138		
Diethylene glycol	< 0.01%		
Ethylene glycol	< 0.01%		
Propylene glycol	< 0.01%		

The Total Organic Solids (TOS) parameter is calculated as a mean for standardizing the quantity of material derived from the enzyme source in order to assess its toxicological significance. It is defined as the sum of the organic compounds excluding diluents, and it was calculated for the final product formulation (Xylamax), the calculation was performed according to the following equation:
% TOS = 100 - (A + W + D), where
A = ash; W = Water; D = diluent and carrier
Ash was determined according to analytical method of AOAC Official Method 942.05; while moisture (water) was determined according to AOAC Official Method 934.01, 2006, vacuum oven. Since this was performed on a final product formulation, no further dilution via carrier was performed.

Sections to be replaced/updated in the dossier:
For section 2.4.2. specifications
Update text to reflect Xylamax product formulation instead of endo-1,4-β-xylanase:

"Three different batches of Xylamax product formulation were assessed…."

Please replace existing Table (Table 2. Enzyme production specification and frequency of testing) with Table 2 below (Table 2. Enzyme production specification), and replace existing table in Appendix 12 (Appendix 12. Specifications of Xylamax Product) with Appendix below (Appendix 12. Specifications and contaminant testing of Xylamax Product).

Revised Table 2. Xylamax specification

Property	Specification	Test method
Appearance	Light Grey powde	Visual inspection
Moisture, %	< 3 %	Loss on drying assay (BRI SAP)
Xylanase activity, XU/	≥ 150,000 XU/g	DNS reducing sugar assay (BRI SAP)
Mycotoxins, ppb		
Aflatoxin B1	<0.5	LC-MS/MS: detection in ppb (1 ppb = 1 μg/kg)
Aflatoxin B2	<0.5	
Aflatoxin G1	<0.5	
Aflatoxin G2	<0.5	
Heavy Metals, mg/kg		
Arsenic	<3	ICP-MS AOAC 2013.06
Lead	<3	
Microbial contaminants, CFU/g		
Coliforms	<10	E-Cultural (Non-chromogenic Media) / VRB+MUG FDA BAM Chapter
E. Coli	<10	
Salmonella	ABSENCE in 25 g	RT-PCR AOAC-RI 121501
Molds	<10	E-Cultural (Chromogenic Media) / FDA BAM Chapter 18

Revised Appendix 12. Three Batch analysis testing of Xylamax Product

Analysis	Batch No XY-C001	Batch No XY-C002	Batch No XY-C003
Production Date	April 2019	November 2019	January 2020
Best Before	April 2021	November 2021	January 2022
Appearance	Light grey powde	Light grey powde	Light grey powde
Moisture, %	1.5	1.4	1.5
Xylanase activity, XU/g	151,494	163,562	168,232
Mycotoxins	Ppb (LOQ)		
Ochratoxin	nq(<1)	nq(<1)	nq(<1)
Aflatoxin B1	nq(<0.5)	nq(<0.5)	nq(<0.5)
Aflatoxin B2	nq(<0.5)	nq(<0.5)	nq(<0.5)
Aflatoxin G1	nq(<0.5)	nq(<0.5)	nq(<0.5)
Aflatoxin G2	nq(<0.5)	nq(<0.5)	nq(<0.5)
Deoxynivalenol	nq(<100)	nq(<100)	nq(<100)
Fumonisin B1	nq(<25)	nq(<25)	nq(<25)
Fumonisin B2	nq(<25)	nq(<25)	nq(<25)
Zearalenone	nq(<30)	nq(<30)	nq(<30)
Heavy Metals	mg/kg (LOQ)		
Arsenic	0.306	0.292	0.373
Lead	0.215	0.218	0.295
Cadmium	0.388	0.360	0.381
Mercury	nq (<0.018)	nq (<0.018)	nq (<0.018)
Dioxins,/Furans and PCBs	ng/kg (LOQ)		
Dioxins and furans: WHO(2005)-PCDD/F TEQ (upper-bound)	0.0443	0.032	0.198
WHO(2005)- PCB TEQ (upper-bound)	0.0011	0.00137	0.00126
Microbial contaminants	CFU/g		
Coliforms	nq (<10)	nq (<10)	nq (<10)
E. Coli	nq (<10)	nq (<10)	nq (<10)
Salmonella	ABSENCE in 25 g	ABSENCE in 25 g	ABSENCE in 25 g
Molds	nq (<10)	nq (<10)	nq (<10)

nq: not quantifiable (< limit of quantification)

Manufacturing

CVM: CVM noted that some raw materials (for example, (b) (4) are mentioned in the narrative of the notice (titled "20201229_Xylanase FDA GRAS Dossier final Dec 29 2020") but are not listed as raw materials in the table provided. The notifier should provide a table listing all the raw ingredients along with applicable regulatory status of each ingredient categorized by each manufacturing process stage, such as production tank, recovery, formulation, etc., and describe which ingredient is used for which part of the manufacturing process such that CVM can understand at which stage of the manufacturing process these ingredients are added (for example, in fermenter or market formulation). The notifier should clearly state that no other raw materials are used for the manufacturing process. The notifier provided a composition table for the enzyme and should clarify if this table is for the marketed formulation of the enzyme. The notifier should clarify if Xylamax is their market formulation and provide its composition, including all ingredients added to the formulation.

Response:
Appendices 7 to 10 contain detailed information about raw material used at each step of the production. Appendix 7 (Raw Material Used in Fermentation and Downstream Processing) provides an overview of each raw material used in the process of fermentation and manufacturing, in which media the raw material was used, the regulatory status, and a detailed description and specification of each raw material component. Appendix 7 has been updated to include the missing raw materials used during the fermentation/manufacturing process (b) (4) and which stage of manufacturing each raw material was used at. An updated Appendix 7 is provided below to be used in the amendment with changes highlighted. Appendix 9 (Growth Media and Reagents) provides information on the raw materials including percentages/quantities included in each growth media preparation in details. Appendix 10 (Fermentation and Downstream Processing) provides further details about the raw materials used in fermentation and downstream processing with quantities and steps further explained, in a step by step approach, how each media is mixed and prepared.

For composition Table (Table 1. Product composition), the submitted text, table (and title) were amended, and an updated/amended version is provided below (section 2.4.1). The text preceding the Table have been updated to make it clear that this composition represents the market formulation of Xylamax. To clarify, Xylamax is the only formulation product containing xylanase as the active agent, with a minimum enzyme activity of 150,000 XU/g, is a subject of this notice.

Sections to be replaced/updated in the dossier:

For Appendix 7. Raw Material Used in Fermentation and Downstream Processing (CONFIDENTIAL). Please use updated tables provided in Attachment A-Revised Appendix 7

For Appendix 23. Safety Data Sheet of Xylamax, please use amended SDS provided in Attachment A. Revised Appendix 23

For section 2.3.1. Raw Materials (CONFIDENTIAL)
Please replace the existing text under this section with the following: (b) (4)

For section 2.4.1. please replace existing text and Table 1 with the text and Table 1 below:

2.4.1. Quantitative Composition
The composition of the Xylamax market formulation is detailed in Table 1. Xylamax is the formulated product containing xylanase as the active agent, with a minimum enzyme activity of 150,000 XU/g as covered by this notice.

(b) (4)

Table 1. Composition of Xylamax market formulation

Component	CAS Number	Percent (w/w)
Active agent: Endo-1,4-β-xylanase	9025-57-4	10-30
Excipient: Limestone CaCO3	1317-65-3	70-90
Excipient: Starch	9005-25-8	5-10

Stability and Homogeneity

CVM: CVM stated that the notifier should clarify if the xylanase shelf-life stability study was performed on the market formulation of the enzyme. The notifier stated for stability of the substance that the storage temperature does not exceed 25 °C. The notifier provided a stability study at 30 °C and used a reference at 25 °C. The notifier should provide a justification for performing the study at 30 °C and clarify if the reference sample is for the 0-month/initial time point. The notifier has referred to the activity of the enzyme in XU/g and U/g; the notifier should clarify if units per gram (U/g) is same as xylanase units per gram (XU/g). The notifier provided a table for average xylanase storage and should clarify if the enzyme activity values shown are average of two or more replicates per time point. The notifier should provide an explanation for the variation in the percent activity of enzyme stability in feed.

CVM noted that stability of enzyme in premix was not provided in the notice. CVM stated that if a premix for the enzyme is used then information on the stability and homogeneity, if applicable, of enzyme in the premix may be required. The notifier stated that their enzyme market formulation will be added directly to finished feed and not through a premix.[4]

[4] As a follow-up to the September 15, 2021 teleconference, CVM notes that in the homogeneity study the notifier refers to a premix before adding the enzyme to the feed.

The notifier stated that the samples in the homogeneity study were analyzed in triplicates and they should clarify if the results are average of triplicates. The notifier stated that the enzyme is added at an inclusion rate of 70 grams per metric ton (g/MT) for the homogeneity study and they should clarify if this is a representative inclusion rate in the feed. Also, the notifier should address the effect of pelleting on the stability of the enzyme in the feed ingredient, as appropriate. Typically, CVM requires pelleted feed stability for three batches of the substance and data from before and after pelleting (pelleting conditions should be representative of United States conditions) to demonstrate pelleted feed stability.

Response:

The xylanase shelf-life stability was performed on Xylamax market formulation. It is guaranteed that the market product formulation will meet its minimum enzyme activity of 150,000 XU/g product if stored at the recommended conditions (25°C for 24 months). However, the shelf-life stability data is provided under the simulation of temperature abuse conditions (to show additional guarantee), reference sample indicated in Appendix 17 is for the 0-month/initial time point activity measurement. Expression of xylanase activity units per gram (U/g) is same as xylanase units per gram (XU/g) so both terms were used interchangeably. In both shelf-life stability trials, each sample bag (representing

one time point) was analyzed in triplicate. The values in summary tables each represent the average of 3 (triplicate) samples.

With regards to the variation in the percent activity of enzyme stability in feed, data in Appendix 18 study 1 presents in-feed stability data in mash feed prepared from 3 different lots of Xylamax final product, the feed was stored for 24 weeks (6 months) which is in practical industry sitting not expected, because in reality, complete feed would not be stored for more than 4 weeks (1 month) due to multiple reasons including stability of other more susceptible feed ingredients such as fat/oil which will cause rancidity if stored for prolonged period of time. Therefore, the important time period to emphasize for Xylamax stability in feed would be for the first 4 weeks as that is the industry representative timeframe of feed storage (at max). Data show that there was no large variation in enzyme recovery in the mash feed during the first 4 weeks, minimum enzyme recovery was at 100% and max recovery was an average of 115% which is acceptable, these variations could be due to having variation among different analyst perform the assay. Data beyond 4 weeks does not represent a real-life situation in relation to in-feed stability therefore will not be considered as part of making the conclusion on the stability of Xylamax in-feed. Variation after that time period is more likely an anlysis/analyst issue, as the data as a whole does not suggest xylanase degradation in activity.

Enzyme market product is not intended to be used in a premix, the product will be directly mixed into feed. The note about the product being added to a premix in the homogeneity study is referring to mixing all micro-ingredients (vitamins, minerals, synthetic amino acids, and enzyme) as a feed manufacturing practice rather than a product specification requirement. The term premix used in the homogeneity study was referring to the marketed product .

For the homogeneity study, each collected sample was analyzed in triplicate, the data provided in table is an average of 3 (triplicate) samples for both mash and pelleted feed homogeneity. The enzyme is added at an inclusion rate of 70 grams per metric ton (g/MT) which is a representative minimum inclusion rate in the feed.

For the effect of pelleting on the stability of Xylamax in feed, was not included in the original notice submission because data was not complete when dossier was first submitted, however, both mash and pellet samples were collected from study 2 in Appendix 18 (Appendix 18. Xylanase in-Feed Stability and homogeneity: study 1 and 2) and stored for 3 months (each timepoint had a designated pre-labelled bag), each sample was analyzed in triplicates and average values are recorded in summary table for both mash and pellet. Text added below to be included as an addition to Appendix 18.

Feed manufacturing performed in this study followed the United States manufacturing conditions by pelleting at 85°C and conditioning the feed for 30 sec retention time. The analyzed activity in mash feed was above the minimum activity and was maintained for 3 months of feed storage, while in the pelleted feed, the activity was slightly reduced but maintained the activity during the 3 month storage.

BioResource has concluded that the studies were robust and satisfy their marketing requirements to assure that the product is stable in the marketed container, stable once added to feed, stable in pelleted feed, and can be homogenously mixed. These data support the utility of the enzyme preparation.

Sections to be replaced/updated in the dossier:

***** The text/Tables below are to be added to the end of Appendix 18. Xylanase in-Feed Stability and homogeneity: study 1, 2, and 3 *****

In addition to determination of homogeneity and recovery of Xylamax, study 2 aimed to determine the effect of time on Xylamax stability in feedstuffs based on testing the stability of processing from mash to pellet at 85 °C, as well as monthly testing for Xylamax active ingredient (xylanase) in feed samples (mash and pellet) stored for 3 months at ambient conditions (25° C ± 2°C and 60% RH) packed in labelled paper bags to mimic storage in feed bags. Samples were tested at time zero (homogeneity data) and after 1, 2, and 3 months and the active substances determined (xylanase activity). Feed is not expected to be stored for more than one month in a practical industry situation therefore, 3 month in-feed stability data should be more than sufficient to demonstrate xylanase stability in feed over time. Each sample was analyzed in triplicate and average values were reported. The methods used for determination of

xylanase activity xylanase in-feed assay in mash and pelleted feed. Xylamax enzyme activity was reduced by 29.9% after pelleting at 85 °C. Stability results are summarized in tables below:

Stability of Xylamax in mash and pelleted feed (study 2)-Expressed as XU/Kg

Sample	Expected (XU/kg)	0 months	1 months	2 months	3 months
Mash	10,000	11,633	10,802	10,830	11,052
Pellet	10,000	8,152	8,072	7,825	7,782
Enzyme loss % compared to time 0 (mash)			5.0%		
Enzyme loss % compared to time 0 (pellets)			4.5%		

BRI has concluded that Xylanase activity is not impacted by storage at ambient conditions, in mash or pelleted feeds.

Study 3:

General objective:
To determine xylanase enzyme recovery after pelleting at 85 °C when xylamax is added at 2 different rates to feed (70 g/MT and 140 g/MT).

Ingredient Composition of the feed:

2 different lots of Xylamax (XY20355 and XY20356) final product were tested in a 2000-lb batch, Table below illustrates the corn-SBM-based feed formula, feed was conditioned to 85°C with 30 sec retention time, then pelleted using (b) (4) pellet mill.

Composition of basal diet.

Ingredient	%	Ingredient	%
Corn	64.28	Choline chloride, 60%	0.20
Soybean meal	30.40	Mineral premix	0.20
Poultry fat	2.00	Vitamin premix	0.05
Defluorinated Phosphate	1.91	L-lysine	0.11
Calcium carbonate	0.25	Selenium premix	0.05
Sodium chloride	0.28	L-Threonine	0.09
DL-Methionine	0.18		

Pelleting Parameters
Treatments were pelleted in a (b) (4) pellet mill (model: (b) (4). The target conditioning temperature is 85 °C. The target conditioning time was 30 seconds, with a production rate of (b) (4) per hour and a die size of (b) (4) with a (b) (4) effective thickness

Sampling Procedure
1. Mash Feed

3 samples (~ 500 g each) per feed batch of the mash feed were collected at 20 sec intervals as mash feed is dispatched from the mixer to the conditioner. Samples were collected in pre-labelled zip top bags.

2. Hot Pellet Samples

3 (~1000 g each) hot pellet samples were collected at 30 sec intervals in mesh-lined trays at the exit chute of the pellet mill before they reach the cooler. The samples with trays were placed immediately into a portable air cooler, collected samples were numbered according to their collection sequence and labelled with the hot pellet temperature measured at time of collection. The cooler draws air from underneath the box to the top of the container. The mesh-lined trays

allow the air to be drawn around and through the pellets to facilitate cooling of the samples. Samples were cooled for approximately 10 minutes to reach a temperature within +/- 5 °C of ambient. After the samples were cooled, a 500 g sample of each was collected in pre-labelled zip top bags and stored in cooler until delivering samples to R&D lab.

All 3 samples from mash and pellets were analyzed in triplicates (total 9 replicates per feed batch) using the xylanase in-feed assay listed in Appendix 20. Average recovery of xylanase enzyme from the feed batches are summarized in Table below.
Based on the analyzed recoveries of xylanase enzyme in pelleted feed (from studies 2 and 3), it can be concluded that xylanase enzyme loss on pelleting had an average of 27.8% loss, therefore, it can be recovered with an approximate average of 72% when subjected to conditioning temperature of 85 °C during pelleted feed manufacturing process.

Stability of Xylamax under processing at 85° C (Compilation of both study 2 and study 3)

Xylamax lot #	XY20281	XY20355	XY20356
	XU/Kg feed		
Activity in mash prior pelleting	11,633	10,239	21,261
Activity in pellets	8,151	6,907	16,789
Activity loss	-29.9%	-32.5%	-21.0%

BRI has concluded that the pelleting process decreased the Xylanase activity approximately 30%, and the label will reflect that information.

***** END OF ADDED SECTION TO APPENDIX 18 *****

Analytical Method

CVM:CVM noted that "beechwood xylan" is identified in the definition of the enzyme activity. "Xylan" is also mentioned in the analytical methods. The notifier should revise the analytical procedures and validations to specify beechwood xylan or provide a statement clarifying that the xylan referred to in the analytical methods and validations is beechwood xylan. CVM requested the notifier to explain the analytical methods and the purpose of the materials (for example, α-amylase, used in the analytical methods). The notifier should provide explanation and information for the analytical method validations.

The notifier should clarify if pages 145 and 171 of 171 in the notice were intentionally left blank.

Response:
The active substance in Xylamax is endo-1,4-β-xylanase. There is currently no available standard method for quantifying xylanase activity. Thus BRI has validated and further verified (at a third party lab) the internal methods for xylanase determination in product and feed.

There are 2 standard analytical procedures (SAPs) to quantify xylanase activity: one in product (Appendix 19. Standard Analytical Procedure for xylanase in product activity) and one in feed (Appendix 20. Standard Analytical Procedure for xylanase in-feed activity), both were validated, then verified in a second laboratory. Based on the performance characteristics, these methods are considered valid for the proposed use. Thus, BRI suggests the use of DNS Reducing Sugar Method for official control of xylanase in product and the XylX6 method for official control of xylanase activity in feed.

For both SAPs, one unit of Endo-1,4- β- Xylanase activity (XU) is defined as the amount of enzyme needed for the release of 1 nanomole of reducing sugars (xylose equivalents) per second from 0.5% xylan at 50°C in 50 mM trisodium citrate buffer pH 6.0. The xylan referred to in the definition, analytical methods and validations is beechwood xylan.

The purpose of using α-amylase in the assay is because xylanase has a starch binding that is removed by the α-amylase freeing it up to interact with the substrate (beechwood xylan). (We note that this is simulating the natural addition of amylase (as provided in the saliva and pancreatic secretion of poultry and swine, to assist with the digestion of starch)).

Beechwood xylan is the substrate with which xylanase will interact in the product assay, the purpose of using beechwood xylan is to provide a controlled quantity of the substrate upon which xylanase will work yielding the release of xylose (reducing sugar) that interacts with Dinitrosalicylic acid (DNS) to form a red complex that can be spectrophotometrically measured at wavelength 540 nm, and therefore, the quantification of xylanase activity.

XylX6 is the substrate with which xylanase will interact in the in-feed assay, the activity that needs to be detected for xylanase activity in feed is at a lower level compared to the activity in the product, furthermore, the matrix within which the enzyme is existing is different between product and feed therefore, different substrates were used for each assay (product and in-feed).

Pages 145 and 171 of 171 in the notice were intentionally left blank.

Sections to be replaced/updated in the dossier:

Please refer to "Attachment A. Revised section to add to Appendix 21" and "Attachment A. Revised section to add to Appendix 22" for revised sections to be added to each Appendix.

LIST OF REVISED AND NEW APPENDECIES RELATED TO CMC

NEW: Attachment A Appendix 24

REVISED: Attachment A-Revised Appendix 7 (CONFIDENTIAL)

REVISED: Attachment A-Revised Attachment 23

REVISED: Attachment A. Addendum to Appendix 21 (CONFIDENTIAL)

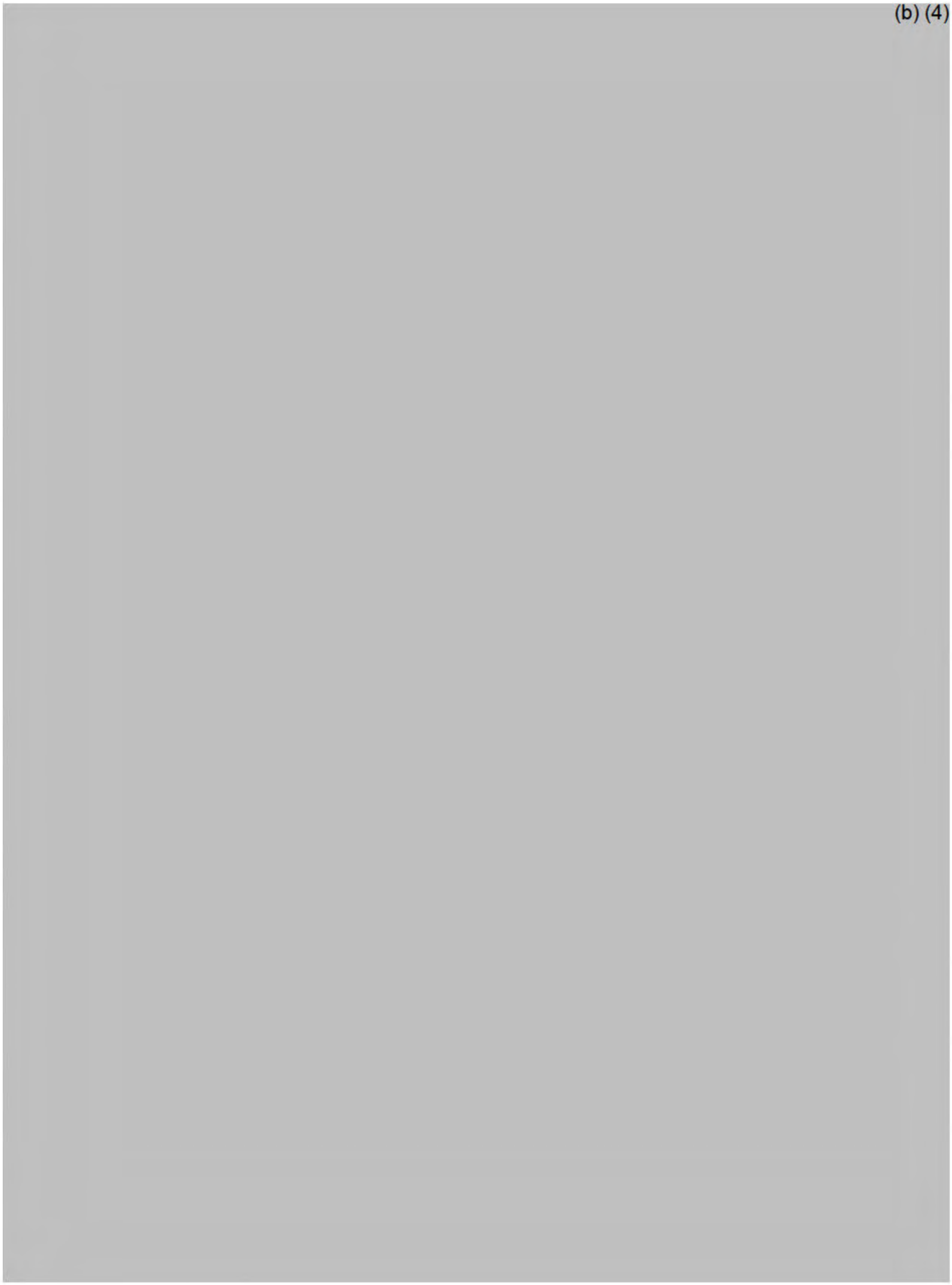
REVISED: Attachment A. Addendum to Appendix 22 (CONFIDENTIAL)

Attachment A. Revised Appendix 7

Revised Appendix 7. Raw Material Used in Fermentation and Downstream Processing (CONFIDENTIAL)

(b) (4)

[redacted]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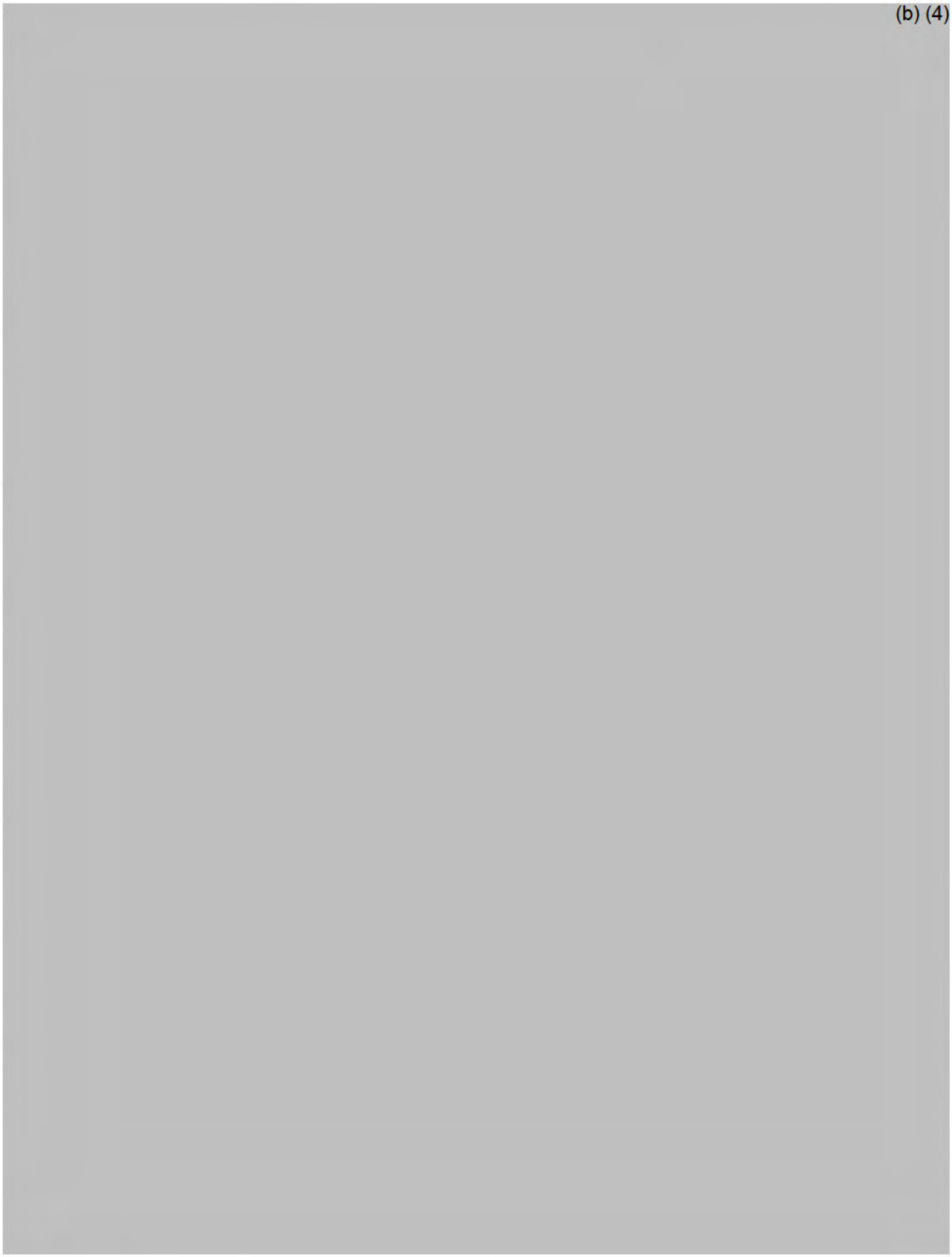
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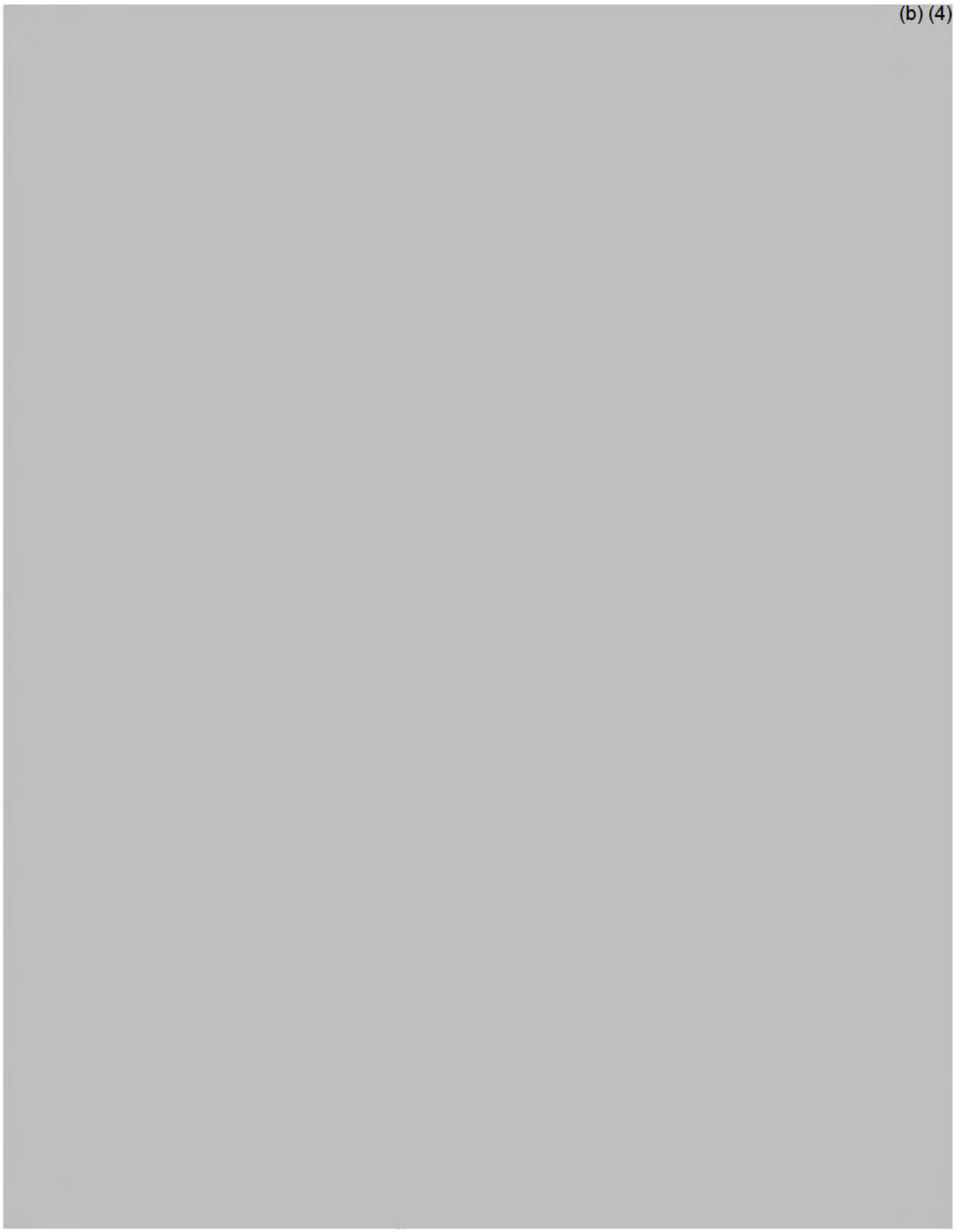
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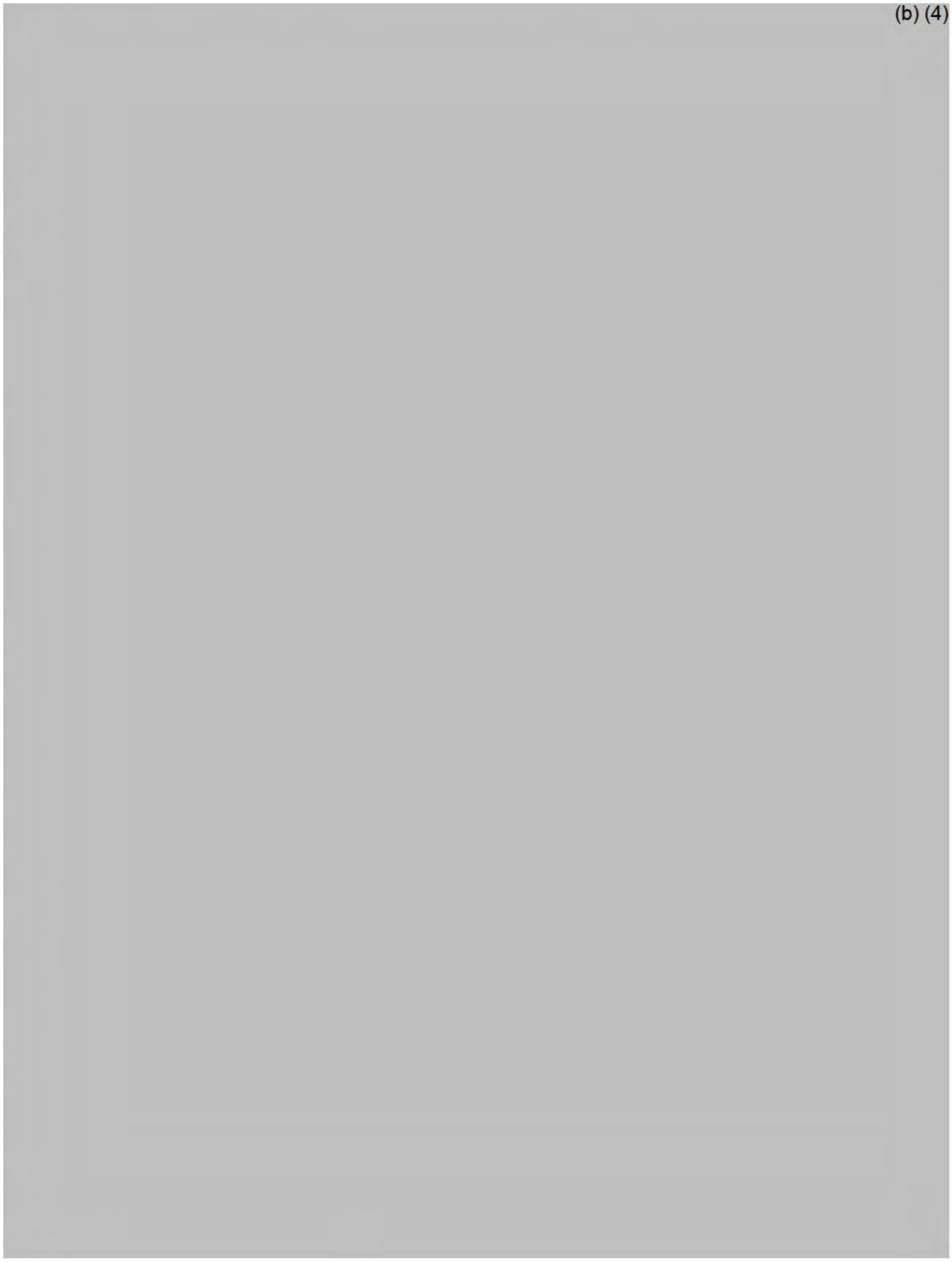
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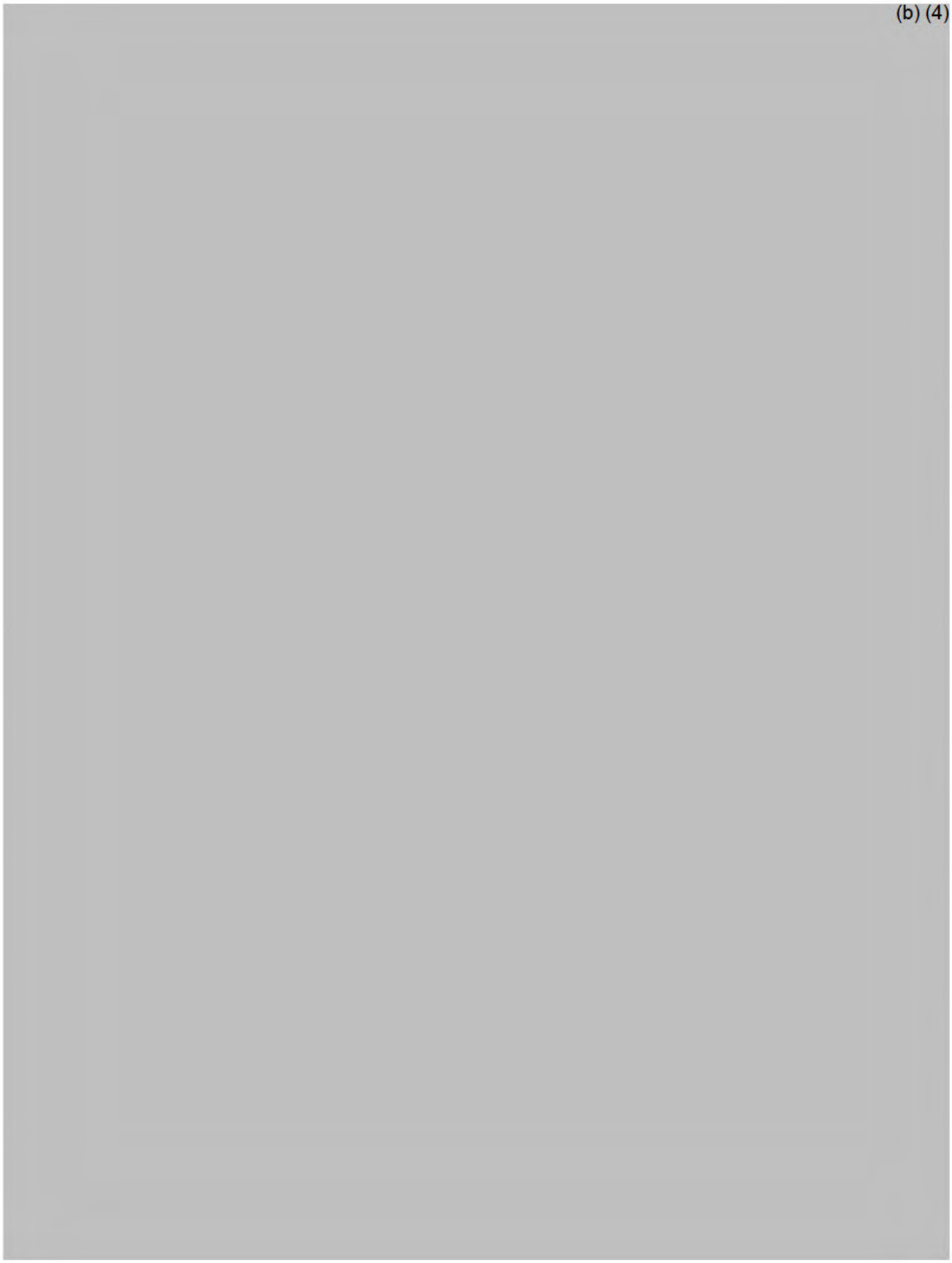
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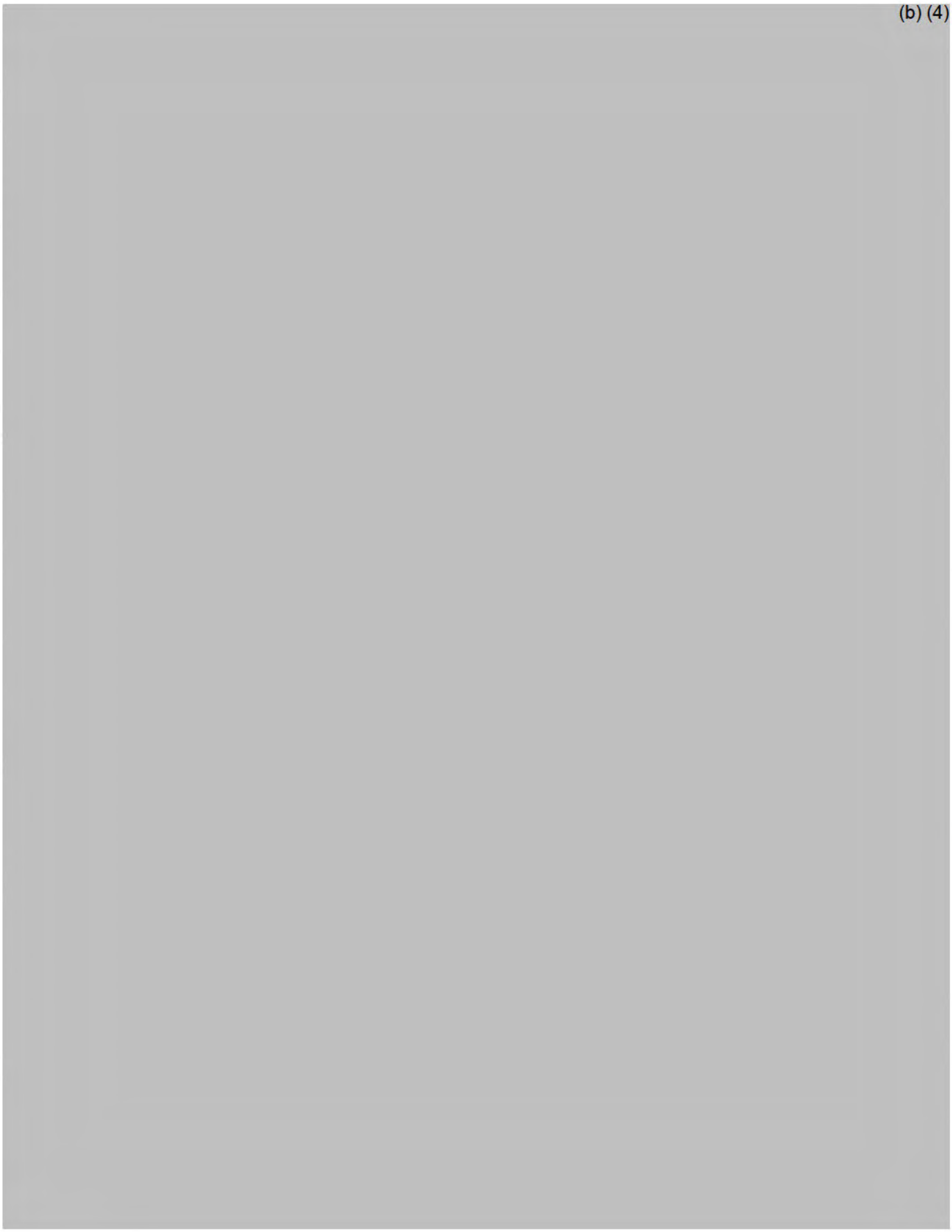
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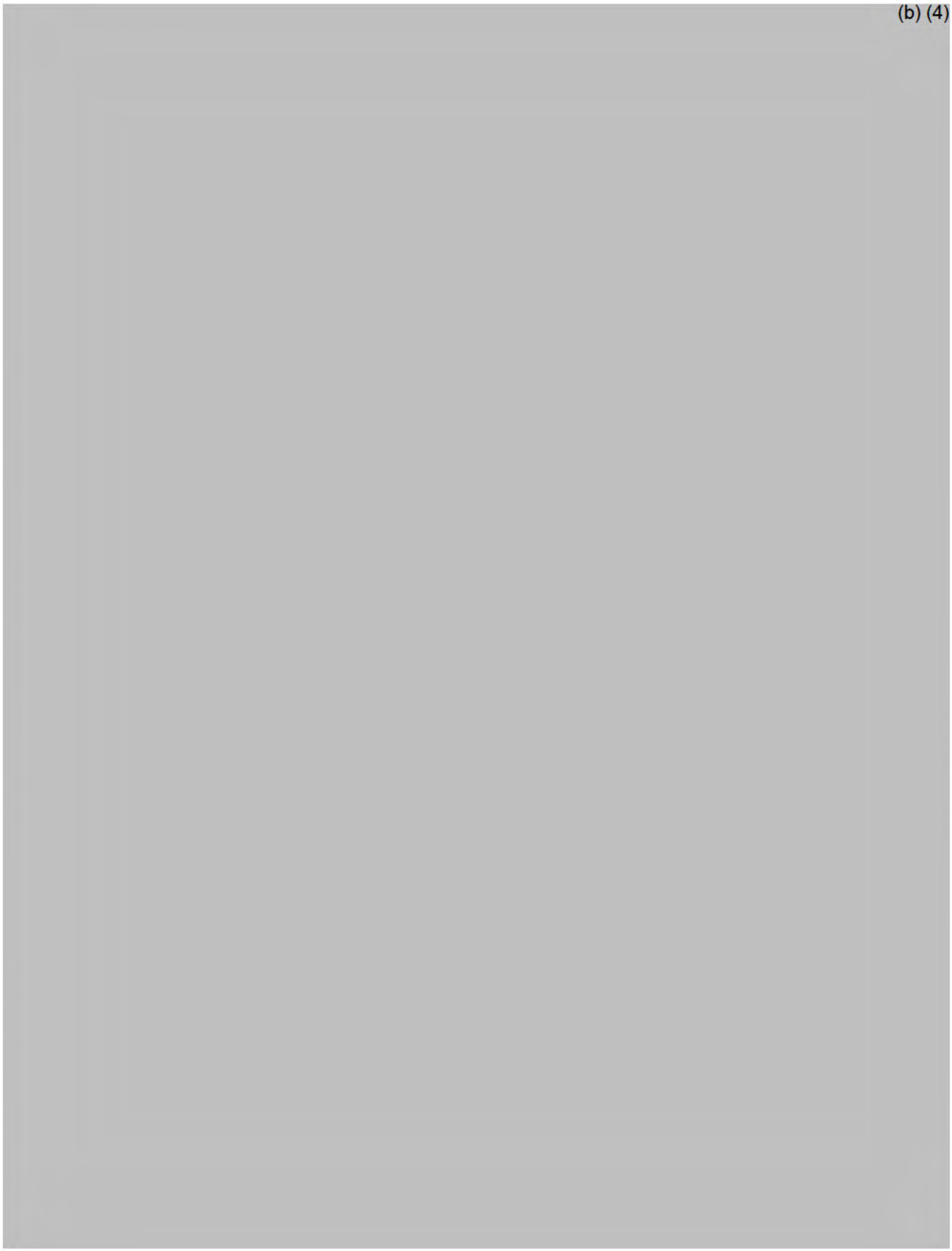
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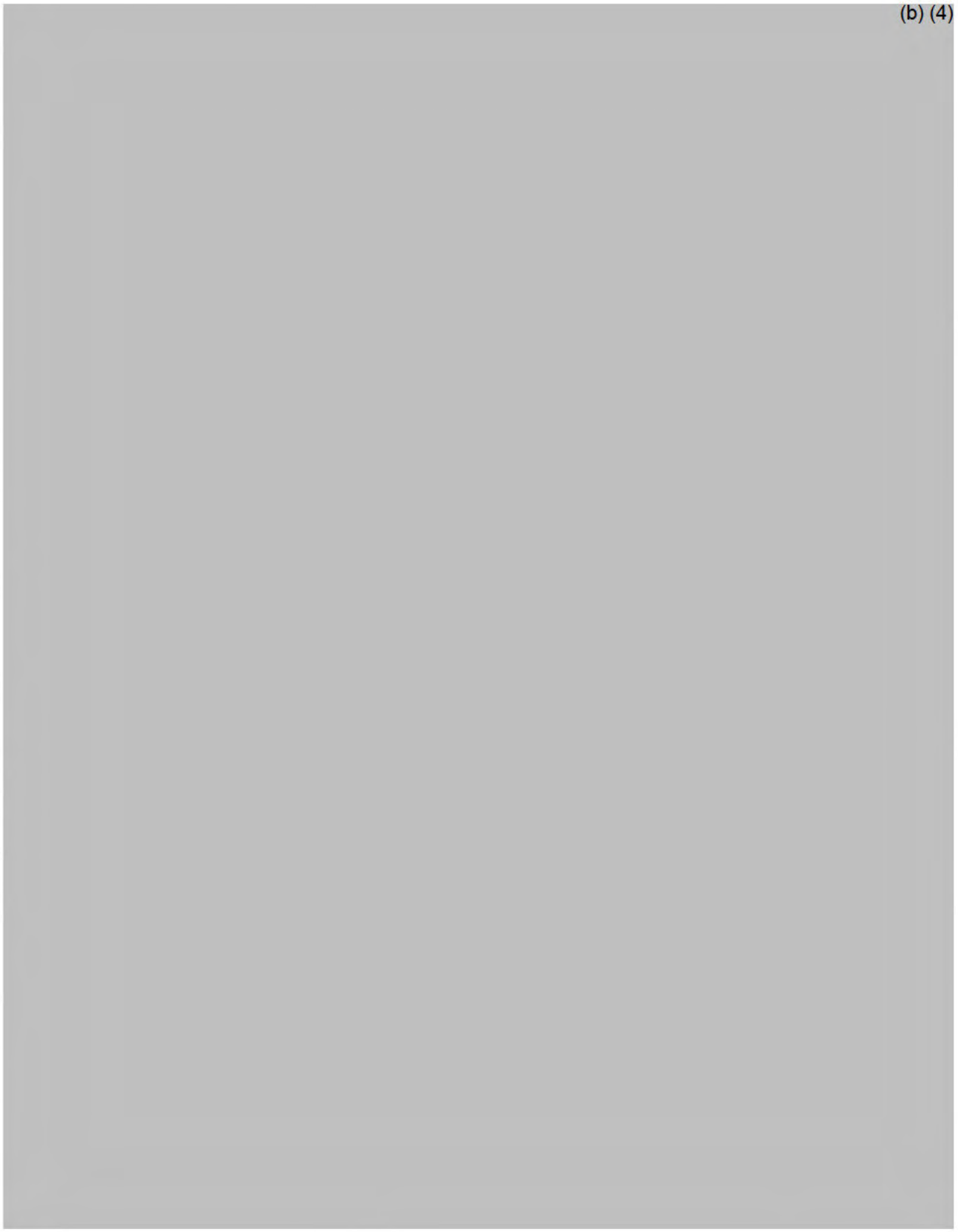
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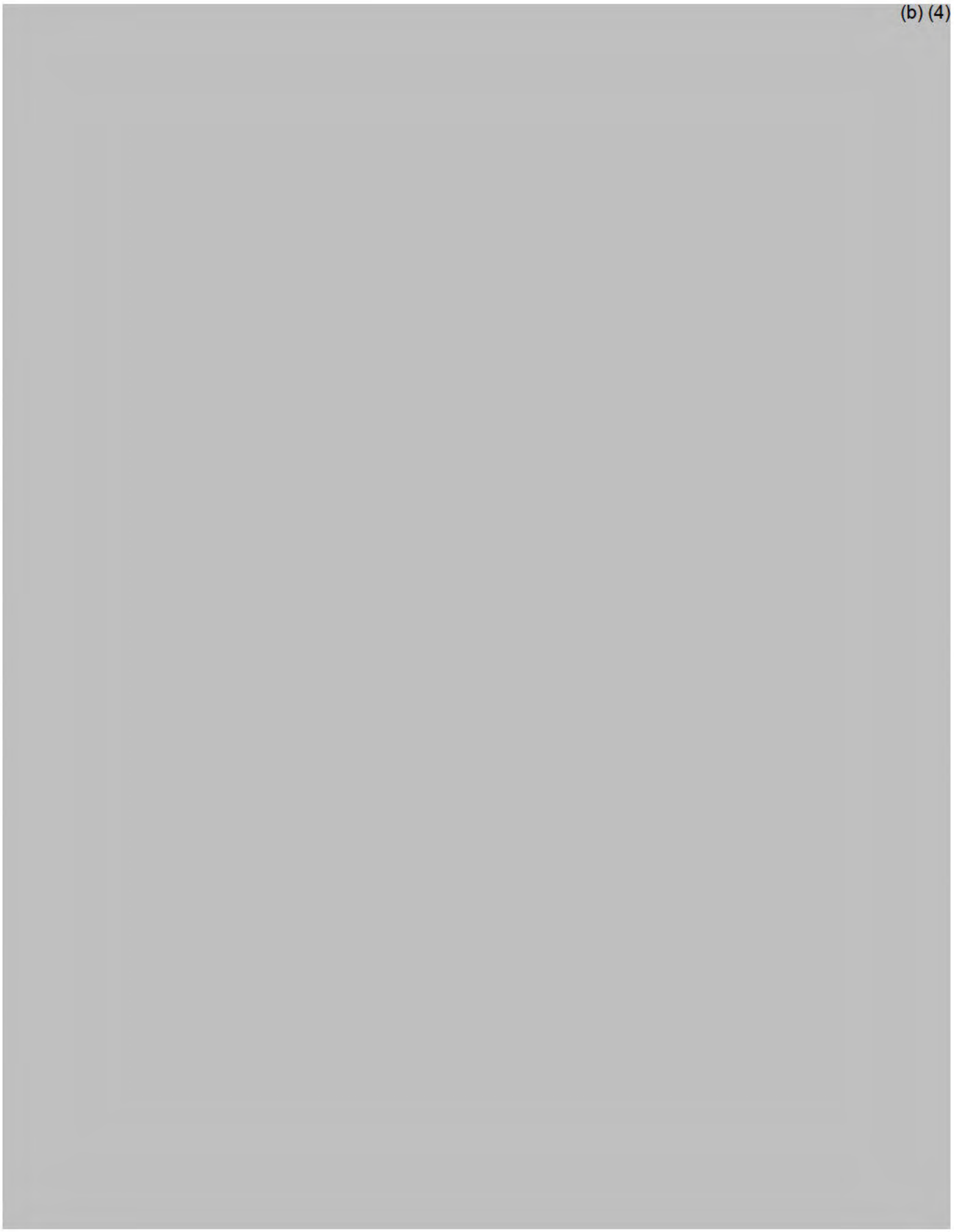
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Appendix 21. Assay validation and verification for xylanase in product activity assay. **CONFIDENTIAL**

*****Also add the verification report below at end of Appendix 21******

(b) (4)

(b) (4)

VERIFICATION OF ANALYTICAL METHOD FOR MEASURING XYLANASE ACTIVITY IN XYLAMAX FEED ADDITIVE

Experiment number: (b) (4)

FINAL REPORT

Revision number: 0

Date: 15th March 2021

RESEARCH ACTIVITY CONTRACTED WITH:

BioResources International
4222 Emperor Boulevard, Suite 460,
Durham NC 27703 (USA)

Study monitors: Rasha Qudsieh and Ching-Sung Tsai

Study director: (B)(6)

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(b) (4)

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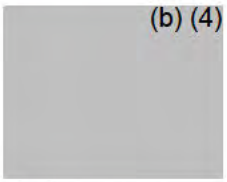
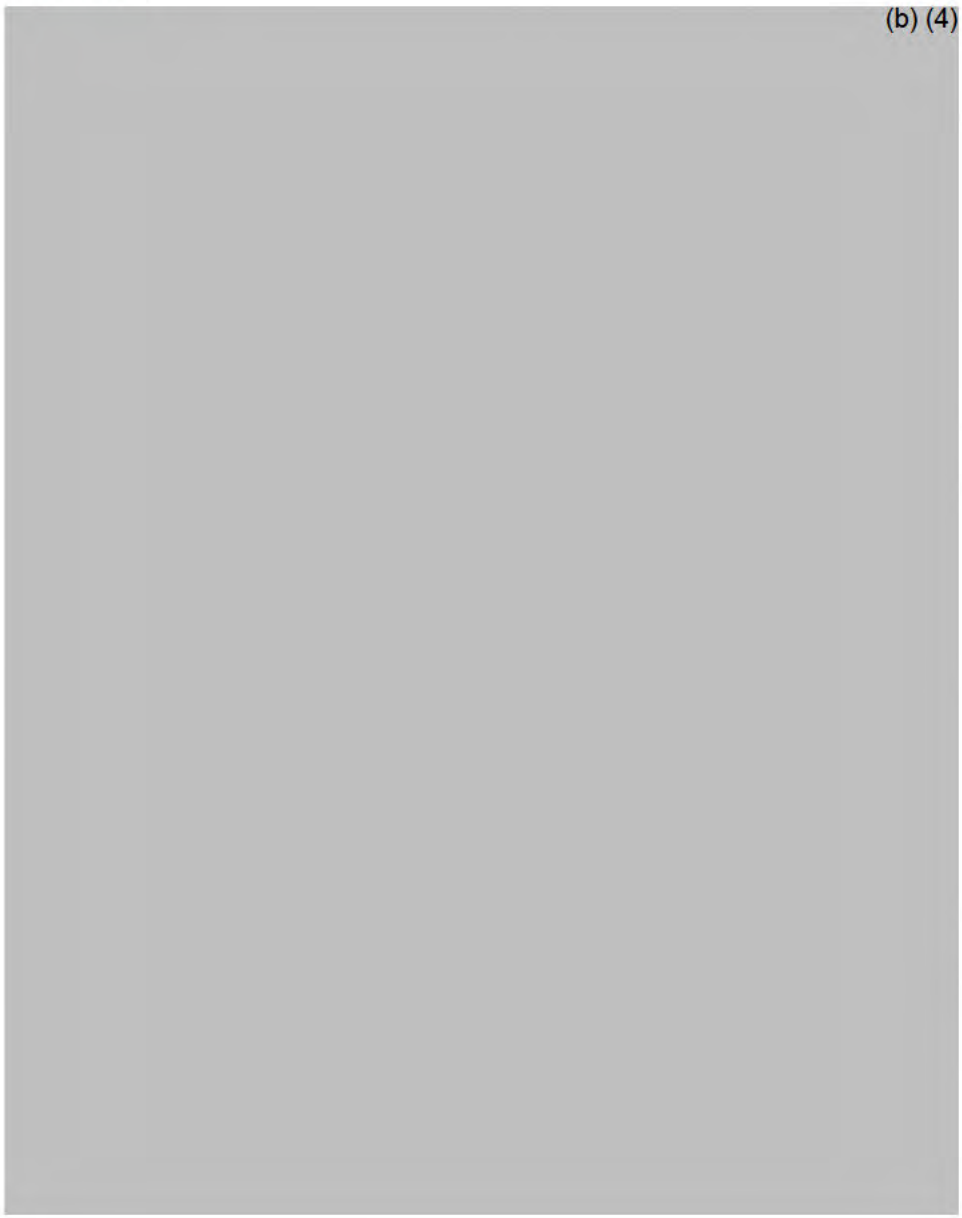


SUMMARY



(b) (4)

RESPONSIBILITIES

R&D

Study Director

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Study monitors

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Analytical Laboratory

(b) (4), (b) (6)

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(b) (4)

OBJECTIVE

(b) (4)

QUALITY ASSURANCE

(b) (4)

(b) (4)

(b) (4)

DEFINITIONS

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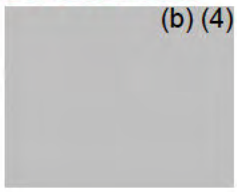

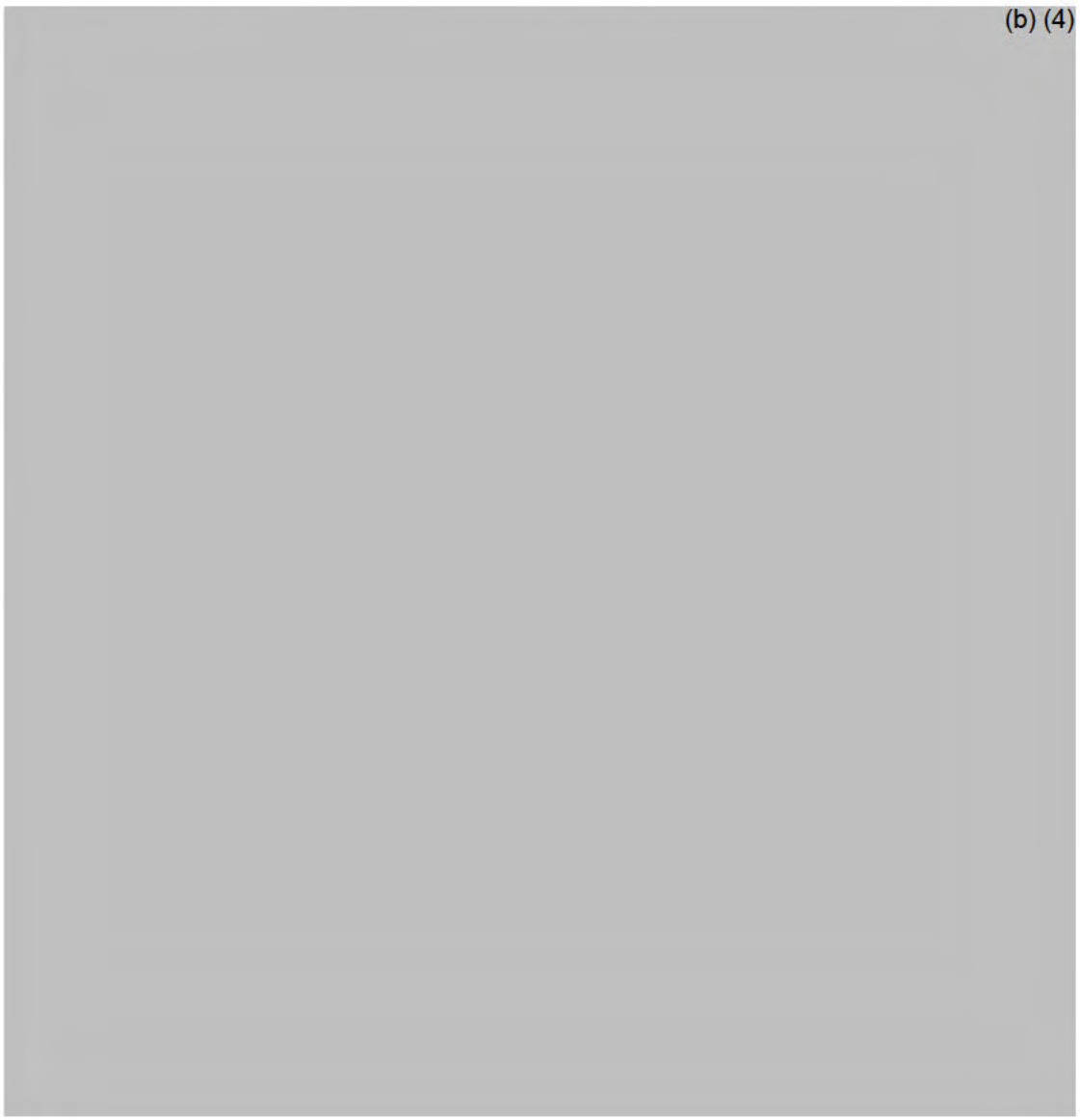

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RESULTS AND DISCUSSION

(b) (4)

Parameter	Results of verification test
Repeatability (known sample)	(b) (4)
Intermediate precision (known sample)	
Reproducibility (unknown sample)	
Quadratic curve regression coefficient	
Range of applicability	
Recovery (%)	
LOD (limit of detection)	
LOQ (limit of quantification)	

Repeatability, intermediate precision, and reproducibility

(b) (4)

(b) (4)

(b) (4)

Table 2: Results of xylanase activity found in the evaluation of repeatability and intermediate precision of the analytical method under study.

Sample:	(b) (4)	
Curve:	(b) (4)	(b) (4)
Subsample	Results day 1 (XU/g)	Results day 2 (XU/g)
1	(b) (4)	(b) (4)
2		
3		
4		
5		
6		
Mean		
Std.dev.		
RSD_r (%)		
RSD_R (%)		

Table 3: Results of xylanase activity found in the blind sample of feed additive for evaluation of reproducibility.

Sample	(b) (4)
Curve:	(b) (4)
Subsample	Results (XU/g)
1	(b) (4)
2	
3	
Mean	
Std.dev.	
RSD_r (%)	

(b) (4)

(b) (4)

(b) (4)

(b) (4)

Range, linearity, and recovery

(b) (4)

Limits of detection (LOD) and quantification (LOQ)

(b) (4)

(b) (4)

Table 4: Results of xylanase activity found in the blank sample of feed additive for LOD and LOQ calculation.

Sample	(b) (4)	
Curve:	(b) (4)	(b) (4)
Subsample	Results day 1 (XU/g)	Results day 2 (XU/g)
1	(b) (4)	(b) (4)
2		
3		
Mean		
Std.dev.		
Average Std.dev.	(b) (4)	
LOD (XU/g)		
LOQ (XU/g)		

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(b) (4)

CONCLUSION

(b) (4)

REFERENCES

(b) (4)

Date: Date:

Appendix 22. Assay validation and verification for xylanase in-feed activity assay **CONFIDENTIAL**

***** Add the verification report below at end of Appendix 22*****

(b) (4)

VERIFICATION OF ANALYTICAL METHOD FOR MEASURING XYLANASE ACTIVITY IN FEEDS

Experiment number: (b) (4)

FINAL REPORT
Revision number: 0
Date: 19th March 2021

RESEARCH ACTIVITY CONTRACTED WITH:

BioResources International
4222 Emperor Boulevard, Suite 460,
Durham NC 27703 (USA)

Study monitors: Rasha Qudsieh and Ching-Sung Tsai

Study director: (b)(6)

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(b) (4)

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(b) (4)

SUMMARY

(b) (4)

(b) (4)

(b) (4)

(b) (4)

RESPONSIBILITIES

R&D

Study Director

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Study monitors

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(b) (4)

Analytical Laboratory

(b) (4), (b) (6)

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(b) (4)

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RESULTS AND DISCUSSION

(b) (4)

Parameter	Results of verification test
Repeatability (known sample)	(b) (4)
Intermediate precision (known sample)	
Reproducibility (unknown sample)	
Quadratic curve regression coefficient	
Range of applicability	
Recovery (%)	
LOD (limit of detection)	
LOQ (limit of quantification)	

(b) (4)

Repeatability, intermediate precision, and reproducibility

(b) (4)

(b) (4)

Table 2. Results of xylanase activity found in the evaluation of repeatability and intermediate precision of the analytical method under study.

Sample:	(b) (4))	
Curve:	(b) (4)	(b) (4)
Subsample	Results day 1 (XU/g)	Results day 2 (XU/g)
1		(b) (4)
2		
3		
4		
5		
6		
Mean		
Std.dev.		
RSD_r (%)		
RSD_R (%)		

(b) (4)

Table 3. Results of xylanase activity found in the blind sample of feed for evaluation of reproducibility.

(b) (4)		
Curve:	(b) (4)	
Subsample	Results (XU/g)	
1	(b) (4)	
2		
3		
Mean		
Std.dev.		
RSD$_r$ (%)		

(b) (4)

(b) (4)

(b) (4)

(b) (4)

Table 4. Results of xylanase activity found in the blank sample of feed sample for LOD and LOQ calculation.

Sample:	(b) (4)	
Curve:	(b) (4)	(b) (4)
Subsample	Results day 1 (XU/g)	Results day 2 (XU/g)
1		(b) (4)
2		
3		
Mean		
Std.dev.		
Average Std.dev.		
LOD (XU/g)		
LOQ (XU/g)		

CONCLUSION

(b) (4)

REFERENCES

(b) (4)

CONFIDENTIAL

(b) (4)

(b) (4)

Signatures:

(b) (4), (b) (6)

(b) (4)

BioResource International Inc. - QC Department

(b) (4), (b) (6)

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(b) (4)

Report Supersedes (b) (4)

Client Code: (b) (4)
PO#: 5358

Received On: 10Aug2020
Reported On: 27Jul2021

(b) (4)

Client Sample Code: (b) (4)

Sample Description: Feed Enzyme-XYc

Sample Registration Date: 10Aug2020
Condition Upon Receipt: acceptable, 22.7°C

Sample Reference: Composite Lot#1

FSMRB - Mycotoxins

Reference
Anal.Bioanal.Chem. (2012) 402:2675-2686

Accreditation
ISO/IEC 17025:2017
A2LA 2918.01

Completed
26Aug2020

Sub
5

Parameter	Result
Aflatoxin B1	(b) (4)
Aflatoxin B2	
Aflatoxin G1	
Aflatoxin G2	
Aflatoxin M1	
Aflatoxin M2	
Deoxynivalenol	
Fumonisin B1	
Fumonisin B2	
HT-2 Toxin	
Ochratoxin A	
T-2 Toxin	
Zearalenone	

GU027 - PCR Qualitative-FMV 34S Promoter

Reference
Internal, real time PCR

Accreditation
ISO/IEC 17025:2017
A2LA 1940.01

Completed
28Aug2020

Sub
1

Parameter	Result
PCR Qualitative-FMV 34S Promoter	Negative

GU103 - PCR Qualitative-CaMV 35S Promoter

Reference
Internal, real time PCR

Accreditation
ISO/IEC 17025:2017
A2LA 1940.01

Completed
28Aug2020

Sub
1

Parameter	Result
PCR Qualitative-CaMV 35S Promoter	Negative

7/27/21 2:02 pm

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(b) (4), (b) (6)

ANALYTICAL REPORT

(b) (4)

Report Supersedes (b) (4)

Client Code: (b) (4)
PO#: 5358
Received On: 10Aug2020
Reported On: 27Jul2021

(b) (4)
Client Sample Code: (b) (4)
Sample Description: Feed Enzyme-XYc

Sample Registration Date: 10Aug2020
Condition Upon Receipt: acceptable, 22.7°C
Sample Reference: Composite Lot#1

GU105 - PCR Qualitative-NOS Terminator

Reference	Accreditation	Completed	Sub
Internal, real time PCR	ISO/IEC 17025:2017 A2LA 1940.01	28Aug2020	1

Parameter	Result
PCR Qualitative-NOS Terminator	Negative

QA101 - Aflatoxin B1 B2 G1 G2 (LC-MSMS)

Reference	Accreditation	Completed	Sub
AOAC 999.07 Modified	ISO/IEC 17025:2017 A2LA 2993.01	27Aug2020	2

Parameter	Result
Aflatoxin B1	(b) (4)
Adjusted LOQ for this matrix.	
Aflatoxin B2	
Aflatoxin G1	
Aflatoxin G2	
Aflatoxins total	
Adjusted LOQ for this matrix.	

QAA07 - Vomitoxin (Deoxynivalenol, DON) LC-MSMS

Reference	Accreditation	Completed	Sub
Food Addit Contam Part A, 2013:30(3),541-9.	A2LA ISO/IEC 17025:2005 2993-01	27Aug2020	2

Parameter	Result
Vomitoxin (Deoxynivalenol)	(b) (4)

QL005 - Dioxins and Furans: PCDD/F (17 Congeners)

Reference	Completed	Sub
EPA 1613B October 1994	19Jul2021	3

Parameter	Result
2,3,7,8-TetraCDD	(b) (4)
2,3,7,8-TetraCDF	
1,2,3,7,8-PentaCDD	
1,2,3,7,8-PentaCDF	
2,3,4,7,8-PentaCDF	
1,2,3,4,7,8-HexaCDD	
1,2,3,6,7,8-HexaCDD	
1,2,3,7,8,9-HexaCDD	
1,2,3,4,7,8-HexaCDF	
1,2,3,6,7,8-HexaCDF	
1,2,3,7,8,9-HexaCDF	
2,3,4,6,7,8-HexaCDF	
1,2,3,4,6,7,8-HeptaCDD	

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(b) (4), (b) (6)

ANALYTICAL REPORT

(b) (4)

Report Supersedes (b) (4)

Client Code: (b) (4)
PO#: 5358

Received On: 10Aug2020
Reported On: 27Jul2021

(b) (4)

Client Sample Code: (b) (4)

Sample Registration Date: 10Aug2020
Condition Upon Receipt: acceptable, 22.7°C

Sample Description: Feed Enzyme-XYc

Sample Reference: Composite Lot#1

QL005 - Dioxins and Furans: PCDD/F (17 Congeners)

Reference: EPA 1613B October 1994

Completed: 19Jul2021 Sub: 3

Parameter	Result
1,2,3,4,6,7,8-HeptaCDF	(b) (4)
1,2,3,4,7,8,9-HeptaCDF	
OctaCDD	
OctaCDF	
WHO(2005)-PCDD/F TEQ (lower-bound)	
WHO(2005)-PCDD/F TEQ (upper-bound)	

QL006 - Dioxin-like PCBs (12 WHO-PCBs)

Reference: EPA 1668 mod.

Completed: 19Jul2021 Sub: 3

Parameter	Result
PCB 77	(b) (4)
PCB 81	
PCB 105	
PCB 114	
PCB 118	
PCB 123	
PCB 126	
PCB 156	
PCB 157	
PCB 167	
PCB 169	
PCB 189	
WHO(2005)-PCB TEQ (lower-bound)	
WHO(2005)-PCB TEQ (upper-bound)	

QL007 - WHO-PCDD/F+PCB TEQ

Reference: EPA 1613B Modified

Completed: 19Jul2021 Sub: 3

Parameter	Result
WHO(2005)-PCDD/F+PCB TEQ (lower-bound)	(b) (4)
WHO(2005)-PCDD/F+PCB TEQ (upper-bound)	

QL008 - PCB ~6 ICES

Reference: EPA 1668 mod.

Completed: 05Sep2020 Sub: 3

Parameter	Result
PCB 28	(b) (4)
PCB 52	

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(b) (4), (b) (6)

ANALYTICAL REPORT

(b) (4)

Report Supersedes (b) (4)

Client Code: (b) (4)
PO#: 5358

Received On: 10Aug2020
Reported On: 27Jul2021

(b) (4)

Client Sample Code: (b) (4)

Sample Description: Feed Enzyme-XYc

Sample Registration Date: 10Aug2020
Condition Upon Receipt: acceptable, 22.7°C

Sample Reference: Composite Lot#1

QL008 - PCB ~6 ICES

Reference: EPA 1668 mod.

Completed: 05Sep2020
Sub: 3

Parameter	Result
PCB 101	(b) (4)
PCB 138	
PCB 153	
PCB 180	

TK015 - Arsenic (As) in Foods by ICP-MS

Reference: AOAC 2013.06

Accreditation: ISO/IEC 17025:2017 A2LA 4204.01

Completed: 21Aug2020
Sub: 4

Parameter	Result
Arsenic (As)	(b) (4)

TK024 - Cadmium (Cd) in Foods by ICP-MS

Reference: AOAC 2013.06

Accreditation: ISO/IEC 17025:2017 A2LA 4204.01

Completed: 21Aug2020
Sub: 4

Parameter	Result
Cadmium (Cd)	(b) (4)

TK048 - Mercury (Hg) in Foods by ICP-MS

Reference: AOAC 2013.06

Accreditation: ISO/IEC 17025:2017 A2LA 4204.01

Completed: 21Aug2020
Sub: 4

Parameter	Result
Mercury	(b) (4)

TK082 - Lead (Pb) in Foods by ICP-MS

Reference: AOAC 2013.06

Accreditation: ISO/IEC 17025:2017 A2LA 4204.01

Completed: 21Aug2020
Sub: 4

Parameter	Result
Lead (Pb)	(b) (4)

UM4BV - Yeast - FDA BAM Chapter 18 mod.

Reference: FDA BAM Chapter 18 mod.

Accreditation: A2LA ISO/IEC 17025:2005 3329.04

Completed: 15Aug2020

Parameter	Result
Yeast	(b) (4)
Moulds	(b) (4)

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(b) (4), (b) (6)

ANALYTICAL REPORT

(b) (4)

Report Supersedes (b) (4)

Client Code: (b) (4)
PO#: 5358

Received On: 10Aug2020
Reported On: 27Jul2021

(b) (4)

Client Sample Code: (b) (4)

Sample Description: Feed Enzyme-XYc

Sample Registration Date: 10Aug2020
Condition Upon Receipt: acceptable, 22.7°C

Sample Reference: Composite Lot#1

UMDTC - Salmonella spp. - AOAC-RI 121501

Reference
AOAC-RI 121501

Accreditation
ISO/IEC 17025:2017
A2LA 3329.04

Completed
11Aug2020

Parameter
Salmonella

Result
Not Detected per 25 g

UMJC3 - Total Coliforms - BAM Chapter 4

Reference
FDA BAM Chapter 4

Completed
11Aug2020

Parameter
Coliforms

Result
(b) (4)

Parameter
Escherichia coli

Result
(b) (4)

UMPD7 - Aerobic Plate Count - BAM Chapter 3

Reference
FDA BAM Chapter 3

Completed
12Aug2020

Parameter
Aerobic Plate Count

Result
(b) (4)

Comments:
Third version created to update WHO values (b) (4)

(b) (4)

Respectfully Submitted,

(b) (6)

Quality Specialist

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(b) (4), (b) (6)

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(b) (4)

Report Supersedes (b) (4)

Client Code: (b) (4)
PO#: 5358

Received On: 10Aug2020
Reported On: 27Jul2021

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Client Code: (b) (4)
PO#: 5358

Received On: 10Aug2020
Reported On: 27Jul2021

(b) (4)

Client Sample Code: (b) (4)

Sample Description: Feed Enzyme-XYc

Sample Registration Date: 10Aug2020
Condition Upon Receipt: acceptable, 22.7°C

Sample Reference: Composite Lot#2

FSMRB - Mycotoxins

Reference
Anal.Bioanal.Chem. (2012) 402:2675-2686

Accreditation
ISO/IEC 17025:2017
A2LA 2918.01

Completed
26Aug2020

Sub
5

Parameter	Result
Aflatoxin B1	(b) (4)
Aflatoxin B2	
Aflatoxin G1	
Aflatoxin G2	
Aflatoxin M1	
Aflatoxin M2	
Deoxynivalenol	
Fumonisin B1	
Fumonisin B2	
HT-2 Toxin	
Ochratoxin A	
T-2 Toxin	
Zearalenone	

GU027 - PCR Qualitative-FMV 34S Promoter

Reference
Internal, real time PCR

Accreditation
ISO/IEC 17025:2017
A2LA 1940.01

Completed
28Aug2020

Sub
1

Parameter	Result
PCR Qualitative-FMV 34S Promoter	Negative

GU103 - PCR Qualitative-CaMV 35S Promoter

Reference
Internal, real time PCR

Accreditation
ISO/IEC 17025:2017
A2LA 1940.01

Completed
28Aug2020

Sub
1

Parameter	Result
PCR Qualitative-CaMV 35S Promoter	Negative

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ANALYTICAL REPORT

(b) (4)

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Client Code: (b) (4)
PO#: 5358

Received On: 10Aug2020
Reported On: 27Jul2021

(b) (4)

Client Sample Code: (b) (4)

Sample Description: Feed Enzyme-XYc

Sample Registration Date: 10Aug2020
Condition Upon Receipt: acceptable, 22.7°C

Sample Reference: Composite Lot#2

GU105 - PCR Qualitative-NOS Terminator

Reference	Accreditation	Completed	Sub
Internal, real time PCR	ISO/IEC 17025:2017 A2LA 1940.01	28Aug2020	1

Parameter	Result
PCR Qualitative-NOS Terminator	Negative

QA101 - Aflatoxin B1 B2 G1 G2 (LC-MSMS)

Reference	Accreditation	Completed	Sub
AOAC 999.07 Modified	ISO/IEC 17025:2017 A2LA 2993.01	27Aug2020	2

Parameter	Result
Aflatoxin B1	(b) (4)
Adjusted LOQ for this matrix.	
Aflatoxin B2	
Aflatoxin G1	
Aflatoxin G2	
Aflatoxins total	
Adjusted LOQ for this matrix.	

QAA07 - Vomitoxin (Deoxynivalenol, DON) LC-MSMS

Reference	Accreditation	Completed	Sub
Food Addit Contam Part A, 2013:30(3),541-9.	A2LA ISO/IEC 17025:2005 2993-01	27Aug2020	2

Parameter	Result
Vomitoxin (Deoxynivalenol)	(b) (4)

QL005 - Dioxins and Furans: PCDD/F (17 Congeners)

Reference	Completed	Sub
EPA 1613B October 1994	19Jul2021	3

Parameter	Result
2,3,7,8-TetraCDD	(b) (4)
2,3,7,8-TetraCDF	
1,2,3,7,8-PentaCDD	
1,2,3,7,8-PentaCDF	
2,3,4,7,8-PentaCDF	
1,2,3,4,7,8-HexaCDD	
1,2,3,6,7,8-HexaCDD	
1,2,3,7,8,9-HexaCDD	
1,2,3,4,7,8-HexaCDF	
1,2,3,6,7,8-HexaCDF	
1,2,3,7,8,9-HexaCDF	
2,3,4,6,7,8-HexaCDF	
1,2,3,4,6,7,8-HeptaCDD	

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ANALYTICAL REPORT

(b) (4)

Report Supersedes (b) (4)

Client Code: (b) (4)4
PO#: 5358

Received On: 10Aug2020
Reported On: 27Jul2021

(b) (4)

Client Sample Code: (b) (4)

Sample Description: Feed Enzyme-XYc

Sample Registration Date: 10Aug2020
Condition Upon Receipt: acceptable, 22.7°C

Sample Reference: Composite Lot#2

QL005 - Dioxins and Furans: PCDD/F (17 Congeners)

Reference: EPA 1613B October 1994

Completed: 19Jul2021 | Sub: 3

Parameter	Result
1,2,3,4,6,7,8-HeptaCDF	(b) (4)
1,2,3,4,7,8,9-HeptaCDF	
OctaCDD	
OctaCDF	
WHO(2005)-PCDD/F TEQ (lower-bound)	
WHO(2005)-PCDD/F TEQ (upper-bound)	

QL006 - Dioxin-like PCBs (12 WHO-PCBs)

Reference: EPA 1668 mod.

Completed: 19Jul2021 | Sub: 3

Parameter	Result
PCB 77	(b) (4)
PCB 81	
PCB 105	
PCB 114	
PCB 118	
PCB 123	
PCB 126	
PCB 156	
PCB 157	
PCB 167	
PCB 169	
PCB 189	
WHO(2005)-PCB TEQ (lower-bound)	
WHO(2005)-PCB TEQ (upper-bound)	

QL007 - WHO-PCDD/F+PCB TEQ

Reference: EPA 1613B Modified

Completed: 19Jul2021 | Sub: 3

Parameter	Result
WHO(2005)-PCDD/F+PCB TEQ (lower-bound)	(b) (4)
WHO(2005)-PCDD/F+PCB TEQ (upper-bound)	

QL008 - PCB ~6 ICES

Reference: EPA 1668 mod.

Completed: 05Sep2020 | Sub: 3

Parameter	Result
PCB 28	(b) (4)
PCB 52	

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(b) (4), (b) (6)

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(b) (4)

Report Supersedes (b) (4)

Client Code: (b) (4)
PO#: 5358

Received On: 10Aug2020
Reported On: 27Jul2021

(b) (4)

Client Sample Code: (b) (4)

Sample Description: Feed Enzyme-XYc

Sample Registration Date: 10Aug2020
Condition Upon Receipt: acceptable, 22.7°C

Sample Reference: Composite Lot#2

QL008 - PCB ~6 ICES

Reference EPA 1668 mod.

Completed 05Sep2020 **Sub** 3

Parameter	Result
PCB 101	(b) (4)
PCB 138	
PCB 153	
PCB 180	

TK015 - Arsenic (As) in Foods by ICP-MS

Reference AOAC 2013.06

Accreditation ISO/IEC 17025:2017 A2LA 4204.01

Completed 21Aug2020 **Sub** 4

Parameter	Result
Arsenic (As)	(b) (4)

TK024 - Cadmium (Cd) in Foods by ICP-MS

Reference AOAC 2013.06

Accreditation ISO/IEC 17025:2017 A2LA 4204.01

Completed 21Aug2020 **Sub** 4

Parameter	Result
Cadmium (Cd)	(b) (4)

TK048 - Mercury (Hg) in Foods by ICP-MS

Reference AOAC 2013.06

Accreditation ISO/IEC 17025:2017 A2LA 4204.01

Completed 21Aug2020 **Sub** 4

Parameter	Result
Mercury	(b) (4)

TK082 - Lead (Pb) in Foods by ICP-MS

Reference AOAC 2013.06

Accreditation ISO/IEC 17025:2017 A2LA 4204.01

Completed 21Aug2020 **Sub** 4

Parameter	Result
Lead (Pb)	(b) (4)

UM4BV - Yeast - FDA BAM Chapter 18 mod.

Reference FDA BAM Chapter 18 mod.

Accreditation A2LA ISO/IEC 17025:2005 3329.04

Completed 15Aug2020

Parameter	Result
Yeast	(b) (4)
Moulds	(b) (4)

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(b) (4), (b) (6)

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(b) (4)
Report Supersedes (b) (4)

Client Code: (b) (4)

Received On: 10Aug2020
Reported On: 27Jul2021

(b) (4)
Client Sample Code: (b) (4)

Sample Description: Feed Enzyme-XYc

Sample Registration Date: 10Aug2020
Condition Upon Receipt: acceptable, 22.7°C

Sample Reference: Composite Lot#2

UMDTC - Salmonella spp. - AOAC-RI 121501

Reference
AOAC-RI 121501

Accreditation
ISO/IEC 17025:2017
A2LA 3329.04

Completed
11Aug2020

Parameter	Result
Salmonella	Not Detected per 25 g

UMJC3 - Total Coliforms - BAM Chapter 4

Reference
FDA BAM Chapter 4

Completed
11Aug2020

Parameter	Result
Coliforms	(b) (4)
Escherichia coli	(b) (4)

UMPD7 - Aerobic Plate Count - BAM Chapter 3

Reference
FDA BAM Chapter 3

Completed
12Aug2020

Parameter	Result
Aerobic Plate Count	(b) (4)

Comments:
Third version created to update WHO values. (b) (4)

(b) (4)

Respectfully Submitted,

(b) (6)

Quality Specialist

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PO#: 5358

Received On: 10Aug2020
Reported On: 27Jul2021

(b) (4)
Client Sample Code: (b) (4)
Sample Description: Feed Enzyme-XYc

Sample Registration Date: 10Aug2020
Condition Upon Receipt: acceptable, 22.7°C
Sample Reference: Composite Lot#3

FSMRB - Mycotoxins

Reference
Anal.Bioanal.Chem. (2012) 402:2675-2686

Accreditation
ISO/IEC 17025:2017 A2LA 2918.01

Completed 26Aug2020

Sub 5

Parameter	Result
Aflatoxin B1	(b) (4)
Aflatoxin B2	
Aflatoxin G1	
Aflatoxin G2	
Aflatoxin M1	
Aflatoxin M2	
Deoxynivalenol	
Fumonisin B1	
Fumonisin B2	
HT-2 Toxin	
Ochratoxin A	
T-2 Toxin	
Zearalenone	

GU027 - PCR Qualitative-FMV 34S Promoter

Reference
Internal, real time PCR

Accreditation
ISO/IEC 17025:2017 A2LA 1940.01

Completed 28Aug2020

Sub 1

Parameter	Result
PCR Qualitative-FMV 34S Promoter	Negative

GU103 - PCR Qualitative-CaMV 35S Promoter

Reference
Internal, real time PCR

Accreditation
ISO/IEC 17025:2017 A2LA 1940.01

Completed 28Aug2020

Sub 1

Parameter	Result
PCR Qualitative-CaMV 35S Promoter	Negative

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(b) (4), (b) (6)

ANALYTICAL REPORT

(b) (4)

Report Supersedes (b) (4)

Client Code: (b) (4)
PO#: 5358

Received On: 10Aug2020
Reported On: 27Jul2021

(b) (4)
Client Sample Code: (b) (4)

Sample Registration Date: 10Aug2020
Condition Upon Receipt: acceptable, 22.7°C

Sample Description: Feed Enzyme-XYc

Sample Reference: Composite Lot#3

GU105 - PCR Qualitative-NOS Terminator	**Reference** Internal, real time PCR	**Accreditation** ISO/IEC 17025:2017 A2LA 1940.01	**Completed** 28Aug2020	**Sub** 1

Parameter	**Result**
PCR Qualitative-NOS Terminator	Negative

QA101 - Aflatoxin B1 B2 G1 G2 (LC-MSMS)	**Reference** AOAC 999.07 Modified	**Accreditation** ISO/IEC 17025:2017 A2LA 2993.01	**Completed** 27Aug2020	**Sub** 2

Parameter	**Result**
Aflatoxin B1	(b) (4)
Aflatoxin B2	
Aflatoxin G1	
Aflatoxin G2	
Aflatoxins total	

QAA07 - Vomitoxin (Deoxynivalenol, DON) LC-MSMS	**Reference** Food Addit Contam Part A, 2013:30(3),541-9.	**Accreditation** A2LA ISO/IEC 17025:2005 2993-01	**Completed** 27Aug2020	**Sub** 2

Parameter	**Result**
Vomitoxin (Deoxynivalenol)	(b) (4)

QL005 - Dioxins and Furans: PCDD/F (17 Congeners)	**Reference** EPA 1613B October 1994		**Completed** 19Jul2021	**Sub** 3

Parameter	**Result**
2,3,7,8-TetraCDD	(b) (4)
2,3,7,8-TetraCDF	
1,2,3,7,8-PentaCDD	
1,2,3,7,8-PentaCDF	
2,3,4,7,8-PentaCDF	
1,2,3,4,7,8-HexaCDD	
1,2,3,6,7,8-HexaCDD	
1,2,3,7,8,9-HexaCDD	
1,2,3,4,7,8-HexaCDF	
1,2,3,6,7,8-HexaCDF	
1,2,3,7,8,9-HexaCDF	
2,3,4,6,7,8-HexaCDF	
1,2,3,4,6,7,8-HeptaCDD	
1,2,3,4,6,7,8-HeptaCDF	
1,2,3,4,7,8,9-HeptaCDF	

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(b) (4)

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Client Code: (b) (4)
PO#: 5358

Received On: 10Aug2020
Reported On: 27Jul2021

(b) (4)
Client Sample Code: (b) (4)
Sample Description: Feed Enzyme-XYc

Sample Registration Date: 10Aug2020
Condition Upon Receipt: acceptable, 22.7°C
Sample Reference: Composite Lot#3

QL005 - Dioxins and Furans: PCDD/F (17 Congeners)

Reference
EPA 1613B October 1994

Completed 19Jul2021
Sub 3

Parameter	Result
OctaCDD	(b) (4)
OctaCDF	
WHO(2005)-PCDD/F TEQ (lower-bound)	
WHO(2005)-PCDD/F TEQ (upper-bound)	

QL006 - Dioxin-like PCBs (12 WHO-PCBs)

Reference
EPA 1668 mod.

Completed 19Jul2021
Sub 3

Parameter	Result
PCB 77	(b) (4)
PCB 81	
PCB 105	
PCB 114	
PCB 118	
PCB 123	
PCB 126	
PCB 156	
PCB 157	
PCB 167	
PCB 169	
PCB 189	
WHO(2005)-PCB TEQ (lower-bound)	
WHO(2005)-PCB TEQ (upper-bound)	

QL007 - WHO-PCDD/F+PCB TEQ

Reference
EPA 1613B Modified

Completed 19Jul2021
Sub 3

Parameter	Result
WHO(2005)-PCDD/F+PCB TEQ (lower-bound)	(b) (4)
WHO(2005)-PCDD/F+PCB TEQ (upper-bound)	

QL008 - PCB ~6 ICES

Reference
EPA 1668 mod.

Completed 05Sep2020
Sub 3

Parameter	Result
PCB 28	(b) (4)
PCB 52	
PCB 101	
PCB 138	

BioResource International Inc. - QC Department

(b) (4), (b) (6)

ANALYTICAL REPORT

(b) (4)

Report Supersedes (b) (4)

Client Code: (b) (4)
PO#: 5358

Received On: 10Aug2020
Reported On: 27Jul2021

(b) (4)
Client Sample Code: (b) (4)
Sample Description: Feed Enzyme-XYc

Sample Registration Date: 10Aug2020
Condition Upon Receipt: acceptable, 22.7°C
Sample Reference: Composite Lot#3

QL008 - PCB ~6 ICES	**Reference** EPA 1668 mod.		**Completed** 05Sep2020	**Sub** 3
Parameter PCB 153 PCB 180	**Result** (b) (4)			
TK015 - Arsenic (As) in Foods by ICP-MS	**Reference** AOAC 2013.06	**Accreditation** ISO/IEC 17025:2017 A2LA 4204.01	**Completed** 21Aug2020	**Sub** 4
Parameter Arsenic (As)	**Result** (b) (4)			
TK024 - Cadmium (Cd) in Foods by ICP-MS	**Reference** AOAC 2013.06	**Accreditation** ISO/IEC 17025:2017 A2LA 4204.01	**Completed** 21Aug2020	**Sub** 4
Parameter Cadmium (Cd)	**Result** (b) (4)			
TK048 - Mercury (Hg) in Foods by ICP-MS	**Reference** AOAC 2013.06	**Accreditation** ISO/IEC 17025:2017 A2LA 4204.01	**Completed** 21Aug2020	**Sub** 4
Parameter Mercury	**Result** (b) (4)			
TK082 - Lead (Pb) in Foods by ICP-MS	**Reference** AOAC 2013.06	**Accreditation** ISO/IEC 17025:2017 A2LA 4204.01	**Completed** 21Aug2020	**Sub** 4
Parameter Lead (Pb)	**Result** (b) (4)			
UM4BV - Yeast - FDA BAM Chapter 18 mod.	**Reference** FDA BAM Chapter 18 mod.	**Accreditation** A2LA ISO/IEC 17025:2005 3329.04	**Completed** 15Aug2020	
Parameter Yeast	**Result** (b) (4)			
Parameter Moulds	**Result** (b) (4)			
UMDTC - Salmonella spp. - AOAC-RI 121501	**Reference** AOAC-RI 121501	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.04	**Completed** 11Aug2020	

BioResource International Inc. - QC Department

(b) (4), (b) (6)

ANALYTICAL REPORT

(b) (4)

Report Supersedes (b) (4)

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PO#: 5358

Received On: 10Aug2020
Reported On: 27Jul2021

(b) (4)

Client Sample Code: (b) (4)

Sample Description: Feed Enzyme-XYc

Sample Registration Date: 10Aug2020
Condition Upon Receipt: acceptable, 22.7°C

Sample Reference: Composite Lot#3

UMDTC - Salmonella spp. - AOAC-RI 121501	**Reference** AOAC-RI 121501	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.04	**Completed** 11Aug2020
Parameter Salmonella	**Result** Not Detected per 25 g		
UMJC3 - Total Coliforms - BAM Chapter 4	**Reference** FDA BAM Chapter 4		**Completed** 11Aug2020
Parameter Coliforms	**Result** (b) (4)		
Parameter Escherichia coli	**Result** (b) (4)		
UMPD7 - Aerobic Plate Count - BAM Chapter 3	**Reference** FDA BAM Chapter 3		**Completed** 12Aug2020
Parameter Aerobic Plate Count	**Result** (b) (4)		

Comments:
Third version created to update WHO values. (b) (4)

(b) (4)

Respectfully Submitted,

(b) (4)

Quality Specialist

7/27/21 2:01 pm

BioResource International Inc. - QC Department

(b) (4), (b) (6)

ANALYTICAL REPORT

(b) (4)

Report Supersedes (b) (4)

Client Code: (b) (4)
PO#: 5358

Received On: 10Aug2020
Reported On: 27Jul2021

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7/27/21 2:01 pm

(b) (4)

(b) (4)

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(b) (4), (b) (6)

ANALYTICAL REPORT

(b) (4)

Report Supersedes (b) (4)

Client Code: (b) (4)

Received On: 04Aug2021
Reported On: 03Sep2021

(b) (4)

Client Sample Code: (b) (4)

Sample Description: Feed Additive

Sample Registration Date: 04Aug2021
Condition Upon Receipt: acceptable, 21.4°C

Sample Reference:

FS087 - Residual Ethanol and Methanol

Reference
GC-FID*

Completed
11Aug2021

Sub
1

Parameter	Result
Ethanol	(b) (4)
Isopropanol	
Methanol	

UMJNL - Enterobacteriaceae - CMMEF Chapter 9.62

Reference
CMMEF Chapter 9.62

Completed
05Aug2021

Parameter	Result
Enterobacteriaceae	(b) (4)

Comments:
*Liquid samples can be analyzed directly or diluted with water prior to analysis. Solid samples should be weighed and diluted to a volume similar to that of the standards. A mass of 0.1g to 1.0g may be necessary. With increased sample mass, a matrix efffect is more likely to interfere with spike recoveries. The amount of each residual alcohol is determined by comparing the signal of the unknown sample, measured by the gas chromatograph and FID, with the signal of reference standard solutions.

This amended report was created to update the reference method from "Internal method" to "GC-FID," as well as to add a brief description of the method per the client's request.

Subcontracting partners:
(b) (4)

9/3/21 1:02 pm

BioResource International, Inc.
(b) (4), (b) (6)

ANALYTICAL REPORT

(b) (4)
Report Supersedes (b) (4)

Client Code: (b) (4)

Received On: 04Aug2021
Reported On: 03Sep2021

Respectfully Submitted,

(b) (6)

(b) (6)
Technican III Group Leader

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9/3/21 1:02 pm

(b) (4)

(b) (4)

BioResource International, Inc.

(b) (4), (b) (6)

ANALYTICAL REPORT

(b) (4)

Report Supersedes (b) (4)

Client Code: (b) (4)

Received On: 04Aug2021
Reported On: 03Sep2021

(b) (4)

Client Sample Code: (b) (4)

Sample Registration Date: 04Aug2021
Condition Upon Receipt: acceptable, 21.4°C

Sample Description: Feed Additive

Sample Reference:

FS087 - Residual Ethanol and Methanol

Reference GC-FID*

Completed 11Aug2021

Sub 1

Parameter	Result
Ethanol	(b) (4)
Isopropanol	
Methanol	

UMJNL - Enterobacteriaceae - CMMEF Chapter 9.62

Reference CMMEF Chapter 9.62

Completed 05Aug2021

Parameter	Result
Enterobacteriaceae	(b) (4)

Comments:
*Liquid samples can be analyzed directly or diluted with water prior to analysis. Solid samples should be weighed and diluted to a volume similar to that of the standards. A mass of 0.1g to 1.0g may be necessary. With increased sample mass, a matrix efffect is more likely to interfere with spike recoveries. The amount of each residual alcohol is determined by comparing the signal of the unknown sample, measured by the gas chromatograph and FID, with the signal of reference standard solutions.

This amended report was created to update the reference method from "Internal method" to "GC-FID," as well as to add a brief description of the method per the client's request.

(b) (4)

9/3/21 1:02 pm

BioResource International, Inc.

(b) (4), (b) (6)

ANALYTICAL REPORT

Report Supersedes (b) (4) (b) (4)

Client Code: (b) (4)

Received On: 04Aug2021
Reported On: 03Sep2021

Respectfully Submitted,

(b) (4)

(b) (4)
Technican III Group Leader

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9/3/21 1:02 pm

(b) (4)

(b) (4)

BioResource International, Inc.

(b) (4), (b) (6)

Client Code: (b) (4)

ANALYTICAL REPORT

(b) (4)

Report Supersedes (b) (4)

Received On: 04Aug2021
Reported On: 03Sep2021

(b) (4)

Client Sample Code: (b) (4)

Sample Registration Date: 04Aug2021
Condition Upon Receipt: acceptable, 21.4°C

Sample Description: Feed Additive

Sample Reference:

FS087 - Residual Ethanol and Methanol

Reference: GC-FID*

Completed: 11Aug2021
Sub: 1

Parameter	Result
Ethanol	(b) (4)
Isopropanol	
Methanol	

UMJNL - Enterobacteriaceae - CMMEF Chapter 9.62

Reference: CMMEF Chapter 9.62

Completed: 05Aug2021

Parameter	Result
Enterobacteriaceae	(b) (4)

Comments:

*Liquid samples can be analyzed directly or diluted with water prior to analysis. Solid samples should be weighed and diluted to a volume similar to that of the standards. A mass of 0.1g to 1.0g may be necessary. With increased sample mass, a matrix efffect is more likely to interfere with spike recoveries. The amount of each residual alcohol is determined by comparing the signal of the unknown sample, measured by the gas chromatograph and FID, with the signal of reference standard solutions.

This amended report was created to update the reference method from "Internal method" to "GC-FID," as well as to add a brief description of the method per the client's request.

(b) (4)

9/3/21 1:02 pm

BioResource International, Inc.

(b) (4), (b) (6)

ANALYTICAL REPORT

(b) (4)

Report Supersedes (b) (4)

Client Code: (b) (4)

Received On: 04Aug2021
Reported On: 03Sep2021

Respectfully Submitted,

(b) (4)

(b) (4)
Technican III Group Leader

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9/3/21 1:02 pm

(b) (4)

(b) (4)

BioResource International, Inc.

(b) (4), (b) (6)

Client Code: (b) (4)

ANALYTICAL REPORT

(b) (4)

Received On: 18Feb2021
Reported On: 01Mar2021

(b) (4)

Client Sample Code: (b)
Sample Description: Feed Additive

Sample Registration Date: 18Feb2021
Condition Upon Receipt: acceptable, 20.1°C
Sample Reference: XY18138

QA05L - Glycols (GC-MS)

Reference
ECAL Internal

Completed 01Mar2021
Sub 1

Parameter	Result
Diethylene glycol	(b) (4)
Ethylene glycol	
Propylene glycol	

(b) (4)

Respectfully Submitted,

(b) (6)

(b) (6)
Quality Specialist

3/1/21 7:54 pm

-

Safety Data Sheet

1. Identification

Product identifier	**Xylamax ®**
Other means of identification	None
Recommended use	Enzyme Feed Additive
Recommended restrictions	None known

Manufacturer/Importer/Supplier/Distributor Information

Company name	BioResource International, Inc.
Address	4222 Emperor Blvd, Suite 460 Durham, NC 27703 United States
Telephone	+1 (919)993-3389
Email	info@briworldwide.com
Contact person	Not available.
Emergency phone number	+1 (919)993-3389

2. Hazard(s) identification

Physical hazards	Not classified		
Health hazards	Sensitization, respiratory	Category 1	H334 – May cause allergy or asthma symptoms or breathing difficulties if inhaled

OSHA defined hazards Not classified

Label elements

Signal word Danger

Hazard statement **H334** – May cause allergy or asthma symptoms or breathing difficulties if inhaled.

Precautionary statements

Prevention	**P261** – Avoid breathing dust
Response	**P304 + P340** – If inhaled: If breathing is difficult, remove person to fresh air and keep comfortable for breathing
Storage	**P402** – Store in a dry place
Disposal	**P501** – Dispose of contents/ Container in accordance with local/regional/notional/international regulations

Hazard(s) not otherwise classified (HNOC) None known.

Supplemental information None.

3. Composition/information on ingredients

Substances

Chemical name	Common name and synonyms	CAS number	%
Pulverized limestone (CaCO3)		1317-65-3	70-90
Xylanase, endo-1, 4-		9025-57-4	10-30
Starch		9005-25-8	5-10

Composition comments

4.First-aid Measures

Inhalation

Move to fresh air. Get medical attention if irritation, allergic symptoms, or other symptoms develop and persist

Skin Contact

No first aid should be required. Wash with soap and water after handling. Seek medical attention if irritation or rash develops and persists.

Eye contact

In case of eye contact, flush eyes immediately with plenty of water. Remove contact lenses, if present and easy to do after the first 5 minutes. Continue rinsing. Seek medical attention if irritation persists

Ingestion

No first aid should be needed. Not intended for human consumption but no adverse effects are expected from ingestion.

Most important symptoms/effects, acute and delayed

Mild eye and skin irritation. Inhalation of dust from dried product may cause allergic respiratory reaction in sensitized individuals with symptoms of wheezing and difficulty breathing.

Indication of immediate medical attention and special treatment needed

Immediate medical attention is required for inhalation allergic reactions

General Information

Ensure that medical personnel are aware of the material(s) involved, and use precautions to protect themselves

5. Fire-fighting measures

Suitable extinguishing media

Use water spray, water fog, carbon dioxide, foam, or dry chemical. Water is most effective.

Specific hazards arising from the chemical

Dust generated in handling this material may present an inhalation hazard if suspended in air at high concentrations. Minimize the generation and accumulation of dust.

Special Protective equipment and precautions for firefighters

Firefighters should wear positive pressure self-contained breathing apparatus and protective gear.

Fire fighting equipment/instructions

In case of fire and/or explosion do not breath fumes. Move containers from fire area if you can do so without risk clothing to avoid contact

Specific methods

Use standards firefighting procedures and consider the hazards of other involved materials

General fire hazards

Avoid generating dust; fine dust dispersed in air in sufficient concentration, and in the presence of an ignition source is a potential dust explosion hazard

6. Accidental release measures

Personal precautions, protective equipment and emergency procedures	Keep unnecessary personnel away. Keep people away from and upwind of spill/leak. Use only non-sparking tools. Dust deposits should not be allowed to accumulate on surfaces, as these may form an explosive mixture if they are released into the atmosphere in sufficient concentration. Wear appropriate protective equipment and clothing during clean-up. Avoid inhalation of dust. Use a NIOSH/MSHA approved respirator if there is a risk of exposure to dust/fume at levels exceeding the exposure limits. Do not touch damaged containers or spill material unless wearing appropriate protective clothing. Ensure adequate ventilation. Local authorities should be advised if significant spillages cannot be contained. For personal protection, see section 8 of the SDS
Methods and materials for containment and cleaning up	Eliminate all ignition sources (no smoking, flares, sparks, or flames in immediate area). Take precautionary measures against static discharge. Use only non-sparking tools. Avoid dispersal of dust in the air (i.e. cleaning dust surfaces with compressed air). Minimize dust generation and accumulation. Collect dust using a vacuum cleaner equipped with HEPA filter. Stop the flow of material if this is without risk. Large spills: Wet down with water and dike for later disposal. Shovel the material into waste container. Absorb in vermiculite, dry sand or earth and place into containers. Following product recovery flush area with water. Small spills: Sweep up or vacuum up spillage and collect in suitable container for disposal. Wipe up with absorbent material (e.g. cloth, fleece). Clean surface thoroughly to remove residual contamination.
Environmental precaution	Never return spills to original containers for re-use. For waste disposal, see section 13 of the SDS. Avoid discharge into drains, water courses or onto the ground.

8. Exposure controls/Personal protection

Occupational exposure limits

US. OSHA Table Z-1 Limits for Air Contaminants (29 DFR 1910.1000)

Components	Type	Value	Form
Limestone (CAS 1317-65-3)	PEL/TWA as PNOC	5 mg/m3 to 15 mg/m3	Respirable fraction to Total dust
Xylanase, endo-1, 4- (CAS 9025-57-4)	PEL/TWA as PNOC	5 mg/m3 to 15 mg/m3	Respirable fraction to Total dust
Starch	PEL/TWA as PNOC	5 mg/m3 to 15 mg/m3	Respirable fraction to Total dust

Biological limit values	No biological exposure limits noted for the ingredient(s).
Exposure guidelines	If exposure limits have not been established, maintain airborne levels to an acceptable level.
Appropriate engineering controls	Explosion-proof general and local exhaust ventilation. Good general ventilation should be used. Ventilation rates should be matched to conditions. If applicable, use process enclosure, local exhaust ventilation, or other engineering controls to maintain airborne levels below recommended exposure limits. Eye wash facilities and emergency shower must be available when handling this product.

Individual protection measures, such as personal protective equipment	
Eye/face protection	Follow facility requirements. Safety glasses or dust goggles recommended to avoid eye contact.
Skin Protection **Hand protection**	Wear appropriate chemical resistant gloves. Suitable gloves can be recommended by the glove supplier.
Skin Protection **Other**	Wear appropriate chemical resistant clothing
Respiratory protection	Chemical respirator with organic vapor cartridge, full facepiece, dust and mist filter
Thermal hazards	Wear appropriate thermal protective clothing, when necessary
General hygiene considerations	When using, do not eat, drink, or smoke. Always observe good personal hygiene measures, such as washing after handling the material and before eating, drinking, and/or smoking. Routinely wash work clothing and protective equipment to remove contaminants.

9. Physical and chemical properties

Appearance	
Physical state	Solid.
Form	Powder.
Color	Grey
Odor	Neutral.
Odor Threshold	Not Available.
pH	Not Available.
Melting point/freezing point	Not Available.
Initial boiling point and boiling range	
Flash point	Not Available.
Evaporation rate	Not Available.
Flammability (solid, gas)	Not Available.
Upper/lower flammability or explosive limits	
Flammability Limit – lower (%)	Not Available.
Flammability Limit – upper (%)	Not Available.
Explosive limit – lower (%)	~100 g/m3 (fine dust
Explosive limit – upper (%)	Not Available.
Vapor pressure	Not Available.
Vapor density	Not Available.
Relative density	Not Available.
Solubility(ies)	
Solubility (water)	Not Available.
Partition coefficient (n-octanol/water)	Not Available.

Auto-ignition temperature	Not Available.
Decomposition temperature	Not Available.
Viscosity	Not Available.
Other information	
Explosive properties	No-data- Available.
Oxidizing properties	No-data- Available.

10. Stability and reactivity

Reactivity	The product is stable and non-reactive under normal condition of use, storage, anc transport.
Chemical stability	Stable under normal storage and handling conditions.
Possibility of hazardous reactions	No dangerous reaction known under conditions of normal use.
Conditions to avoid	Keep away from heat, sparks, and open flame. Minimize dust generation and accumulation. Contact with incompatible materials.
Incompatible materials	Strong oxidizing agents. Sensitive to moisture.
Hazardous decomposition products	Thermal decomposition will release oxides of carbon and nitrogen.

11. Toxicological information

Information on likely routes of exposure

Inhalation	May cause allergy or asthma symptoms or breathing difficulties in inhaled.
Skin contact	No adverse effects due to skin contact are expected.
Eye Contact	May cause mild irritation.
Ingestion	Not intended for human consumption but no adverse effects are expected from ingestion.
Symptoms related to the physical, chemical and toxicological characteristics	Coughing. Difficulty in breathing.

Information on toxicological effects

Acute toxicity	Not available.
Skin corrosion/irritation	Not classified.
Serious eye damage/eye irritation	Mild irritation possible.

Respiratory or skin sensitization

Respiratory sensitization	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
Skin sensitization	Not classified. Not classified.
Germ cell mutagenicity	May cause allergy or asthma symptoms or breathing difficulties if inhaled.

Carcinogenicity

IARC Monographs. Overall Evaluation of Carcinogenicity

Not listed.

NTP Report of Carcinogens

Not listed.

OSHA Specifically Regulated Substances (29 CFR 1910.1001-1050)

Not listed.

Reproductive toxicity	Not classified.
Specific target organ toxicity – single exposure	Not classified.
Specific target organ toxicity – repeat exposure	Not classified.

Aspiration hazard	Not classified.

12. Ecological information

Ecotoxicity	The product is not classified as environmentally hazardous. However, this does not exclude the possibility that large or frequent spills can have a harmful or damaging effect on the environment.
Persistence and degradability	No data is available on the degradability of this product.
Bioaccumulative Potential	No data available.
Mobility in soil	No data available.
Other adverse effects	None known.

13. Disposal considerations

Disposal Instructions	This product, if disposed as purchased would not meet the criteria of a RCRA hazardous waste.
Local disposal regulations	Dispose in accordance with all applicable regulations.
Hazardous waste code	The waste code should be assigned in discussion between the user, the producer, and the waste disposal company.
Waste from residues/unused products	Dispose of in accordance with local regulations. Empty containers or liners may retain some product residues. This material and its container must be disposed of in a safe manner (see: Disposal instructions).
Contaminated packaging	Since emptied containers may retain product residue, follow label warnings even after container is emptied. Empty containers should be taken to an approved waste handling site for recycling or disposal.

14. Transport information

DOT	Not regulated as dangerous goods.
IATA	Not regulated as dangerous goods.
IMDG	Not regulated as dangerous goods.
Transport in bulk according to Annex II of MARPOL 73/78 and the IBC code	Not applicable.

15. Regulatory information

US federal regulations The product is not hazardous under the criteria of the Federal OSHA Hazard Communication Standard (29 CFR 1910.1200).

TSCA Section 12(b) Export Notification (40 CFR 707, Subpt.D)
Not regulated

OSHA Specifically Regulated Substances (29 CFR 1910.1001-1050)
Not regulated

CERCLA Hazardous Substance List (40 CFR 302.4)
Not listed

Superfund Amendments and Reauthorization Act of 1986 (SARA)

Hazard Categories
Immediate Hazard – No
Delayed Hazard – No
Fire Hazard – No
Pressure Hazard – No
Reactivity – No

SARA 302 Extremely hazardous substance
Not listed.

SARA 313 (TRI reporting)
Not regulated
Other federal regulations
Clean Air Act (CAA) Section 112 Hazardous Air Pollutants (HAPs) List
Not Regulated.
Clean Air Act (CAA) Section 112(r) Accidental Release Prevention (40 CFR 68.130)
Not regulated.
Safe Drinking Water Act (SDWA) Not regulated.
US State regulations
US. Massachusetts RTK – substance List
Limestone (CAS 1317-65-3)
US. New Jersey Worker and Community Right-to-Know act
Limestone (CAS 1317-65-3)
US. Pennsylvania Worker and Community Right-to-Know Law
Limestone (CAS 1317-65-3)
US. Rhode Island RTK
Not regulated.
US. California Proposition 65
California Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65): This material is not known to contain any chemical currently listed as carcinogens or reproductive toxins.

16. Other information, including date of preparation or last revision

Issue date 12-March-2015
Revision date 18-January-2021
Version # 06
NFPA ratings

Disclaimer BioResource International, Inc. cannot anticipate all conditions under which this information and its product, or the products of other manufacturers in combination with its product, may be used. It is the user's responsibility to ensure safe conditions for handling, storage and disposal of the product, and to assume liability for loss, injury, damage or expense due to improper use. The information in the sheet was written based on the best knowledge and experience currently available.
Xylamax ® is a trademark of BioResource International, Inc. and are registered in the United States and other countries.

***** END OF APPENDIX 23 *****

Attachment B: AGRN 44—October 6, 2021
Utility

FDA Memo :

CVM: The notifier should expand its narrative to include relevant information supporting that the expressed protein has xylanase activity. A discussion to support the identity of the notified substance as a xylanase could include several different types of data and information, such as: enzyme kinetics, substrate specificity, enzyme specific activity, and quantification of the breakdown products from substrate hydrolysis.

The notifier states that it has concluded that the functionality of the notified xylanase does not relate to safety, but presents information in its notice concerning the mode of action of xylanases. Instead, the notifier's narrative needs to clearly explain how safety is not affected if the xylanase does not accomplish the stated effect in poultry and swine diets. This issue is particularly relevant because the notifier identifies xylan (substrate of xylanase action) as an antinutritional factor. The notifier should address why, if the enzyme fails to hydrolyze the xylan in diets formulated to contain xylanase, there are no adverse effects on the animal or its nutrition. Solely addressing the presence of xylan in feed would not address safety concerns when the notified substance is not functional. The focus of the narrative should be on how the notifier reached its conclusion that if the notified substance does not have its intended effect, there is no safety concern resulting from the lack of functionality. The notifier should re-write Section 2.5 to clearly address how a lack of enzyme functionality in xylanase containing diets would not impact animal safety rather than describe and support the mode of action of xylanase enzymes. The narrative should include citations to publicly available information and the scientific literature. CVM indicated that a discussion in one of the submitted references may be useful to the notifier in addressing this point.[5]

5 Although not identified in the meeting, the reference is: Flores et al. 2017. Evaluation of a thermotolerant xylanase on broiler growth performance and dietary ileal digestible energy value. J. Appl. Poult. Res. 26:60.

If the notifier has concluded that functionality is related safety, the utility section of the notice should be re-written and include use of traditional end points, such as digesta viscosity, to support functionality relying on publicly available information for the notified substance.

RESPONSE:

Section 2.5 is specific to the intended use of Xylanase and the notifiers determination that the functionality of xylanase is not related to safety.

The confirmation of identity GRAS substance, Xylanase enzyme product, is provided in section 2.1 of the GRAS conclusion., which also discusses the specificity of the enzyme preparation

(section 2.1.1). The activity of the Xylanase enzyme product is discussed in section 2.1.3 of the GRAS conclusion and the quantification of activity. Also, conformation of that the enzyme product is nearly identical to representative endo-β-1,4-xylanases is provided in section 2.1.2 of the GRAS conclusion. The GRAS conclusion includes a study (Duarte et al., 2019) that demonstrated that the use of the Xylanase enzyme product decreased disgesta viscosity which is one of the most accepted markers of in vitro activity of effective hemicelluloses, including Xylanase (AAFCO, 2021). The information within the GRAS conclusion, provides data that unequivocally demonstrates that the BioResource International enzyme preparation is an active endo-1,4-β-xylanase.

The intended use is described section 1.4 "BioResource International's Komagataella phaffii (P. pastoris) enzyme preparation (endo-1,4-β-xylanase or xylanase) is used for the hydrolysis of xylans , a component of hemicellulose in poultry and swine feed." The description of use (as found in section 2.5) states that the product will be used in nutritionally adequate feeds. The use will increase nutritional value from these feeds. In addition, this section discussed the fact that some consider non-starch polysaccharides to reduce availability of nutrients, hence decreasing the level of available nutrients from the feed. Hence, decreasing NPS will overall increase the value of the feed. However, Flores, et al., 2017, notes that the anti—nutritional effect of xylan has consistently been demonstrated in wheat-based diets, but not in the corn-soy diets fed in the United States. As such the use of xylanase in US swine and poultry feed is specific to increasing the energy value of the feed. The published study with the Xylanase enzyme preparation (Flores et al, 2017) demonstrated this increase in ileal digestible energy, supporting the hypothesis of the increase in energy utilization with the supplementation of the Xylanase enzyme preparation, as such this is a value-added product, allowing the bird to get more energy from the nutritionally complete animal diet provided. The use of the Xylanase preparation would be the same (increase in the energy value) of completed feed.

The FDA reviewer requested that notifier address why, if the enzyme fails to hydrolyze the xylan in diets formulated to contain xylanase, there are no adverse effects on the animal or its nutrition. We note in the Flores et al, 2017 studies the control birds (that did not receive xylanase supplementation—equivalent to a diet receiving xylanase (a protein) that failed to hydrolyze xylan) had no impact on body weight at slaughter. A recent article (Bedford, 2018) indicated that the function of non-starch polysaccharidases, in general, and xylanase specifically, is dependent on diet formulation, age of animal, microbiome composition, the small intestine endosperm cell wall content, as well as the heat treatment of the feed (or pelleting). Hence with any non-starch polysaccharide, the level of function of the enzyme may vary widely, but this variation will not impact the safety of the animal, as the intent is to allow digestion of xylans, that may escape digestion without adequate levels of xylanase in feeds.

We note that in the Final Rule (Federal Register: Vol. 81 54960) of the implementation of the GRAS regulations agency response to comment 144, discusses when utility studies are required to support GRAS conclusions. The FDA response is divided into nutrients and processing aids (specifically suggesting enzymes in this case). FDA states in this discussion" However, when

the function of an enzyme in animal food is well known, it is also common to use generally available and accepted data and information about the function of the enzyme in combination with animal feeding studies and stability studies to support the function of the enzyme (see section IV in CVM's experience document (Ref. 20))." The function and use of xylanase is well established, published, and accepted in the industry and by regulators (eg note the 8 sources of xylanase authorized for use in animal feed (AAFCO, Chapter 6, section 30.1). Five published studies with BRI Xylanase are cited in this conclusion, as well as many other xylanase products.

Reference:

Bedford, M.R. 2018. The evolution and application of enzymes in the animal feed industry: the role of data interpretation. British Poultry Science volume 59:486-493.

ATTACHMENT C- AGRN 44—October 6, 2021

Target Animal Safety (TAS)

CVM: The notifier's narrative does not do a sufficient job of explaining how the pieces of information are interpreted to allow conclusion of GRAS. CVM noted the TAS portion of the narrative should be expanded to include a summary description of relevant information from the published studies. The narrative should demonstrate how the notifier reached their conclusion of safety for the intended use of the substance. CVM stated the notifier needs to include, in its expanded narrative, description of the substance used in the published studies. Did the published studies use a xylanase produced using the same / current production process or a pilot scale (i.e., "tox lot" vs scaled up "market formulation")? If the substance in the studies is not produced under current manufacturing and formulation conditions, the notifier should explain how the published data are applicable to the notified substance.

CVM explained that the notifier needs to address the differences in proposed use rate (10,000 to 50,000 XU/kg feed) and the maximum doses in the published research articles (40,000 XU/kg feed for broilers and 45,000 XU/kg feed for weanling pigs). The notifier needs to explain why the demonstrated use rates can be extrapolated to address the proposed 50,000 XU/kg feed use rate.
CVM also noted that exposure calculations provided on page 32 of the notice are inconsistent and seem to have errors.

Response and sections to amend:

BRI has modified the maximum level of use to 40,000 XU / Kg of feed for poultry and swine.

For section 2.5.2. Use Levels: please update the text to ….maximum of 40,000 XU/kg feed.

For Part 3: Target Animal and Human Exposures, section: 3.1. Target Animal: in the first paragraph, please update the text to a maximum of 40,000 XU/Kg feed (40 XU/g feed)

For the exposure calculation under Part 3, section 3.1., please change the 20 XU to 40 XU in the locations illustrated below:

For broilers,….. Assuming 1 g of feed contains the maximum of 40 XU then the Estimated Daily intake of xylanase/kg bw /day…
93 grams feed /Kg body weight x 40 XU/g feed = 3720 XU/kg BW /day. This approximates 0.025 gram of Xylanase preparation/ kg BW each day.

For swine,….. Assuming 1 g of feed contains the maximum of 40 XU then the Estimated Daily Intake of xylanase/kg bw/day…
40 grams feed/Kg BW x 40 XU/g feed = 1600 XU/kg BW g/day. This approximates 0.01 gram of Xylanase preparation/ kg BW each day.

For the elaboration on part 3 narrative: Please add the following to Part 3, section 3.1. Target animal at the end of the section:

"The xylanase (endo-1,4-β-xylanase) contained in Xylamax is produced by the genetically modified production strain *K. phaffii* (b) (4) *K. phaffii* is listed as QPS when used for production. The *K. phaffii* (b) (4) has been thoroughly characterized, furthermore, absence of production strain cells and DNA has been proved. Therefore, xylanase production strain is presumed safe for the target animal species. The BRI xylanase preparation, has been used in the market for 5 years and no adverse effects have been reported. The BRI xylanase preparation product is currently marketed in Bangladesh, Egypt, Jordan, India, Indonesia, Mexico, Brazil, Chile, Nicaragua, Costa Rica, Bolivia, Columbia, Ecuador, El Salvador, Guatemala, Honduras, Panama, and Thailand. The long history of safe use of *K. phaffii* in feed enzyme manufacture and the absence of known pathogenic or toxigenic effects in *K. phaffii* demonstrates that it is a safe production organism for animal feed enzymes. *K. phaffii* (b) (4) genetic modification does not compromise the lack of toxigenic potential of the strain. Moreover, absence of production strain cells and DNA has been proved. Therefore, the xylanase contained in Xylamax should also be consider safe for the target animals.

Also, the xylanase will be degraded or inactivated during the passage through the digestive tract. Xylanase is a processing aid and is not expected to affect safety. As noted in the specifications (and the three batch analysis) there are extremely low levels of contaminants and the animal exposure is estimated at 0.01-0.025 grams/Kg bodyweight, demonstrating a safe product. Therefore, the use of Xylamax as a feed additive will not contribute to undesirable residues in animal products.

To support this conclusion, extensive literature search has been conducted, a summary table and review are provided herein, in this notice, 40,000 XU/Kg feed was set as the max dose due cost-benefit and marketing considerations rather than safety reasons, and to align with max inclusion rate used in published research conducted using Xylamax final product.

Table B. Published studies with the BRI Xylanase preparation

Title	Journal	Author	Animal category	Active substance	Dose enzyme
Evaluation of a thermotolerant xylanase on broiler growth performance and dietary ileal digestible energy value	Poultry Science Association Inc.	Flores C.A. et al, 2017	Broilers	Xylanase	20,000 XU/kg 40,000 XU/kg
Combination of Xylanase and Bacillus Direct-fed Microbials, as an Alternative to Antibiotic Growth Promoters, Improves Live Performance and Gut Health in Subclinical Challenged Broilers	International Journal of Poultry Science	Nusairat B. et al 2018	Broilers	Xylanase (alone and in combination with *Bacillus* spp)	15,000 XU/kg
Dietary supplementation of xylanase and protease on growth performance, digesta viscosity, nutrient digestibility, immune and oxidative stress status, and gut health of newly weaned pigs	Animal Nutrition	Duarte et al 2019	Pigs	Xylanase (alone and in combination with a protease)	45,000 XU/kg
Synbiotic Effects of Enzyme and Probiotics on Intestinal Health and Growth of Newly Weaned Pigs Challenged With Enterotoxigenic F18+ Escherichia coli	Frontiers in Veterinary Science	Duarte et al 2020	Pigs	Xylanase (alone and in combination with *Bacillus* spp)	10,000 XU/kg
Xylanase and Direct-Fed Microbials (DFM) Potential for Improvement of Live Performance, Energy Digestibility, and Reduction of Environmental Microbial Load of Broilers"	Frontiers in Veterinary Science	Nusairat and Wang 2020	Broilers	Xylanase (alone and in combination with *Bacillus* spp)	10,000 XU/kg

BRI has published five articles focused on testing the xylanase enzyme produced by the genetically modified strain *K. phaffii* (b) (4) using same production process (market formulation), in collaboration with multiple external research entities. These articles were published in several journals within the last five years. These published articles investigated xylanase activity ranging between 10,000 to 45,000 XU/Kg feed in both broiler chickens and pigs. In the article published by Flores et al., (2016), broilers raised for fattening were investigated in two trials; a total of 1260 broiler chickens were used in trial 1 and 1760 were used in trial 2, the maximum enzyme activity tested was 40,000 XU/kg feed, In both trials, birds consuming xylanase performed similar or superior to birds on control feed indicating that there were no adverse effects due to enzyme supplementation. Thus, it can be concluded that xylanase enzyme can be safely included in feed based on results of this published article.

In another article published on broiler chickens by Nusairat et al. (2018), researchers investigated the influence of xylanase supplemented at 15,000 XU/Kg feed when supplemented alone or in combination with *Bacillus spp.* probiotics. In this trial, 2,496 were used. Birds were subjected to a mild, subclinical challenge with two *Eimeria* species and *Clostridium perfringens* then necropsied for lesion score and general gut health evaluation. Mortality in birds receiving only 15,000 XU/Kg feed was significantly ($P<0.05$) lower than birds in negative control group indicating that adding xylanase to broiler feed even under disease challenge did not have any adverse effect on bird health. Lesion scores, *Salmonella* incidence, and counts for *E. coli*, aerobic plate count (APC), and *Clostridium perfringens* were estimated, birds consuming feed with xylanase enzyme had significantly ($P<0.05$) lower values compared to the negative control, and there were not any anomalies noted while performing the necropsy indicating that xylanase enzyme does not have any safety concerns when used in feed. Another study was published in broilers investigating xylanase enzyme supplementation at 10,000 XU/Kg feed either alone or in combination with *Bacillus spp* probiotic in broilers under a mild environmental challenge with *Clostridium perfringens* (Nusairat et al., 2020). A total of 2,496 broilers were used in this study. Results of this study did not show any negative effects of xylanase on bird performance and health, mortality was comparable among treatments which indirectly indicates that there were not any safety concerns due to xylanase inclusion in feed.

Xylanase was also investigated in pigs; Duarte et al. (2019) investigated the effect of supplementing xylanase at 45,000 XU/Kg feed. A total of 48 pigs were used, results showed that supplementing xylanase at this level did not result in any health concerns while performance and intestinal measurements were comparable to control. Furthermore, in another study on pigs published by Duarte et al. (2020), the effect of disease challenge was investigated on intestinal health and growth of newly weaned pigs. A total of 64 pigs were used in this study, xylanase inclusion was at 10,000 XU/Kg feed, results showed that there was no adverse effects observed in pigs receiving diets with xylanase which indicates that xylanase does not raise any safety concerns when used in feed. Moreover, outcomes of these published articles can indirectly indicate safety of Xylamax for use in all poultry and swine including broiler, layers, breeders for poultry, and nursery, fattening, finishing, lactating for swine.

In addition to the published research articles which clearly demonstrates the safety of using Xylamax final market product, the purity of the Xylamax final product has been demonstrated by analyzing for multiple contaminants in several batches of Xylamax, results of the analyses

demonstrated that Xylamax final product did not contain any chemical, mycotoxins, heavy metals, Dioxins/Furans and PCBs, or biological contaminants, indicating that the raw material going into the product formulation was also free of contaminants therefore, no safety concerns are raised due to impurities.

One of the main safety components in assessing an enzyme safety is the safety of the enzyme production organism, which has been demonstrated throughout this notice that *K. phaffii* meets the criteria for determining the safety of enzymes used in animal feed by Pariza and Cook (2010). Furthermore, the xylanase produced by the genetically modified *K. phaffii* is considered safe because the genetic modifications are well characterized and specific, and the incorporated DNA does not encode and express any known harmful or toxic substances.

Therefore, it can be concluded that xylanase produced by *K. phaffii* is safe for the use in poultry and swine. This conclusion is supported by the safety of the organism (*K. phaffii*) used for xylanase production, the safety of the genetic modification performed on *K. phaffii*, the history of safe xylanase use in poultry and swine feed of both Bioresource and other marketed xylanases, the safe nature of xylanase as an enzyme (protein) that functions as a digestion aid and is degraded or inactivated in the digestive tract, as well as the safety of the other ingredients going into the Xylamax final market product formulation. Altogether, support the safe use of the xylanase in poultry and swine."

Copies of Additional References in Attachment F

Nusairat, B., and J.-J., Wang. Xylanase and Direct-Fed Microbials (DFM) Potential for Improvement of Live Performance, Energy Digestibility, and Reduction of Environmental Microbial Load of Broilers. Front. Vet. Sci., 07 December 2020. DOI: https://doi.org/10.3389/fvets.2020.606415

Duarte, M., J. Tyus, and S. W. Kim. Synbiotic Effects of Enzyme and Probiotics on Intestinal Health and Growth of Newly Weaned Pigs Challenged With Enterotoxigenic F18+ Escherichia coli. Front. Vet. Sci., 09 September 2020. DOI: https://doi.org/10.3389/fvets.2020.00573

Nusairat, B., J. McNaughton, J. Tyus, and J.-J. Wang. 2018. Combination of Xylanase and Bacillus Direct-fed Microbials, as an Alternative to Antibiotic Growth Promoters, Improves Live Performance and Gut Health in Subclinical Challenged Broilers. International Journal of Poultry Science, 17: 362-366. DOI: 10.3923/ijps.2018.362.366

ATTACHMENT D: AGRN 44—October 6, 2021

Molecular Biology

CVM: CVM stated it is unclear whether the shift in molecular weight was due to a decrease in glycosylation or the reduction in the size of the secreted protein since the notifier deleted the 5′ and 3′ ends of the protein and added the starch binding domain. CVM used the amino acids sequence that was provided on page 9 and calculated that the secreted protein would have a molecular mass of 36.7 kilodaltons (kDa), which is slightly less than the value reported by the notifier (37.4 kDa). It is possible that the notifier and CVM used different formulas to calculate the molecular mass. The notifier should describe any other data that it has which demonstrates a reduction in glycosylation of the expressed protein.

Response:

BRI uses the sequences on page 9, which starts with the N terminal domain of the starch binding domain to the end of xylanase, with the sequences of

(b) (4)

", to do molecular weight analysis.

BRI originally used the DNAStar Lassergene 17 software to perform the molecular weight calculation. The result is 37405.14 Daltons.

Besides DNAStar, BRI also uses two websites to get the molecular weight calculation results:

The first website BRI uses is (b) (4).

The molecular weight result is 37405.14 dalton.

The second website BRI used is https://www.bioinformatics.org/sms/prot_mw.html

The molecular weight result we got is 37.41 kilodaltons.

The differences of the results with CVM's calculation may be due to the different formula used to calculate the molecular weight.

The SDS page gel shown on page 16 are the electrophoresis results of our xylanase plus the starch binding domain, which contains the above-mentioned sequences (b) (4) The left panel is the electrophoresis result of the (b) (4) protein with (b) (4) and (b) (4) in the original sequences (wild type), while in the right panel the protein contains the mutations of (b) (4) and (b) (4). The gel electrophoresis results showing that molecular weight differences, support the conclusion that the cause of molecular weight reduction is the remove of glycosylation due to the change of the (b) (4) amino acids, because no other sequences change occurred.

CVM: CVM noted the information provided in Table 5 on page 69 of the notice suggests that the promoter sequence for the (b) (4) cassette in the first plasmid insert was not incorporated into the genome. However, the information provided in Figure 4 and the nucleotide sequence that was provided in the appendix suggest that (b) (4) complete copies of (b) (4) were inserted into the genome of the host organism. The notifier should address this discrepancy.

Response:

The contents CVM mentioned are inside the whole genome analysis report of (b) (4), the production strain of Xylamax used by BRI. BRI contacted (b) (4) the company prepared the whole genome analysis of (b) (4) and found that the table was not correctly prepared. According to the results of genome sequencing, as the genetic elements drawn on Figure 4, Table 5 should have a row in the top showing the (b) (4) promoter for the (b) (4) cassette. (b) (4) has corrected the error in Table 5 and a new version of the report is attached with this answer (Corrected Appendix 2). Therefore, the promoter sequence for the (b) (4) cassette in the first plasmid insert was incorporated.

CVM: The notifier provides a study report for the antibiotic resistance gene transferability assay. This study report appears to be incomplete. The notifier should provide a limit of detection for the antibiotic resistance gene transferability assay.

Response:

BRI relies on the fact of production strain DNA is not present in the Xylanase product (Appendix 6), for assurances that antibiotic resistance is not a hazard. Since no DNA of the production strain can be found in the product, no DNA will be transferred to other organisms. Therefore, the purpose of proving our xylanase is not a source of antibiotic resistance, can rely on the study report found in appendix 6.

The antibiotic resistance gene transferability assay also was intended to support this portion of the safety assessment by demonstrating that the antibiotic resistance genes encoded inside the genome of the production strain are stable and not be able to transfer into other organisms, such as E. coli. However, we agree the study report is incomplete and we do not have the ability to rectify the missing information.

It is BRI's conclusion that the fact that the of production strain DNA is not present in the Xylanase product, assures that Xylanase product will not be a source of antibiotic resistance.

CVM: The notifier states that plasmid (b) (4) was integrated (b) (4) (b) (4) The mode of insertion is unclear since the plasmid does not contain homology regions that are upstream and downstream of the (b) (4) expression cassettes (b) (4)). The notifier should address this issue.

Response:

(b) (4)

(b) (4)

CVM: In addition, CVM stated it is unclear what the notifier means on page 85 of the notice when it states "there are too many copies of tandem repeat integration" to determine if the (b) (4) promoter was intact or not. This statement suggests that more than (b) (4) copies of the (b) (4) plasmid were inserted (b) (4). The notifier should resolve the discrepancies between what was reported for its whole genome sequencing and this Southern blot analysis.

Response:

Appendix 5 (b) (4)

Appendix:

Corrected Appendix 2

(b) (4)

Microbial Safety

CVM: CVM explained the notifier identifies the host strain as *K. phaffii* BG10 and provides a whole genome sequencing (WGS) report in Appendix 1 to support identity. However, Appendix 1 is marked as confidential business information (CBI) and the narrative of the notice does not include any information on confirmation of the identity of the host strain except for the reference to Appendix 1. Identity of host strain is crucial for addressing microbial safety. The notifier needs to include a summary of Appendix 1 in the narrative of the notice including the methods and various approaches used in conclusively establishing the identity of the host strain. In this narrative, the notifier should also link the host strain's identity to the microbial safety information for the genus species to establish that host organism is safe for this use.

CVM noted that the notifier also includes a statement in Section 6 of the notice which reads as follows: "Data and information cited in this notification is not generally available and Part 6 contains information that is exempt from disclosure under the FOIA". The notifier does not identify any subsections in Section 6 as confidential. The notifier should provide clarification about this sentence as it calls into question the general availability of safety data which is the primary basis for a GRAS conclusion.

Response:

Add the following text to section 2.2.2. host strain after the Komagataella phaffii (Pichia pastoris) taxonomy list:

"Four different approaches were used for the taxonomic identification (Appendix 1). The production strain was unequivocally identified as Komagataella phaffii. 1) The (b) (4) was analysed using megablast searches against the NCBI RefSeq database of (b) (4) sequence data of microbial type strains. The best match was with K. phaffii NRRL Y-7556. 2) Completeness of the BG10 genome was assessed against the K. phaffii CBS 7435 by (b) (4) alignment using the MCM algorithm in Mauve. The production strain aligned very well with the chosen reference genome. 3) The alignment-free genome distance estimation analysis with (b) (4) supported K. phaffii GS115 (GCF_000027005.1) as the closest genome. 4) (b) (4) grouped BG10 with other K. phaffii strains. The strain is not genetically modified. As a species, K. phaffii qualifies as QPS (Qualified Presumption of Safety) when used for enzyme production. Furthermore, it is a common host for enzyme production (also referred to as Pichia pastoris) as found in the listing of authorized feed enzymes (AAFCO Chapter 6, section 30)."

The Chapter 6 narrative does not contain confidential business information, and BRI vacates that statement of confidentiality in this section of the GRAS notice.

Cerrito, Chelsea

From: Kristi Smedley <smedley@cfr-services.com>
Sent: Thursday, October 07, 2021 12:55 PM
To: Animalfood-premarket
Cc: 'Rasha Qudsieh'; Conway, Charlotte
Subject: RE: [EXTERNAL] RE: GRAS Notice No. AGRN 44 - Call Regarding Xylanase enzyme to be used in swine and poultry feeds--REFERENCES
Attachments: Attachemnt F-Additional References.docx; Bedford2018The evolution and application of enzymes in the animal feed industry the role of data interpretation.pdf; Duarte_et_al_2020.pdf; Li2007 _Article_ExpressionOfRecombinantProtein.pdf; Nusairat_et_al_2018.pdf; Nusairat_et_al_2020.pdf

I am attaching the list of new references, as well as copies of the references to the AGRN 44 amendment.

Kristi O. Smedley, Ph.D.

Center for Regulatory Services, Inc.
5200 Wolf Run Shoals Rd.
Woodbridge, VA 22192

Ph. 703-590-7337
Cell (b) (6)
Fax 703-580-8637

From: Kristi Smedley [mailto:smedley@cfr-services.com]
Sent: Thursday, October 07, 2021 12:54 PM
To: 'Animalfood-premarket'
Cc: 'Rasha Qudsieh'; 'Conway, Charlotte'
Subject: RE: [EXTERNAL] RE: GRAS Notice No. AGRN 44 - Call Regarding Xylanase enzyme to be used in swine and poultry feeds

Please see attachments to this email that provides the response to concerns raised by the Division specific to AGRN 44.

Should have any problems receiving these attachments or on the provided information, please contact me.

Kristi O. Smedley, Ph.D.

Center for Regulatory Services, Inc.
5200 Wolf Run Shoals Rd.
Woodbridge, VA 22192

Ph. 703-590-7337
Cell (b) (6)
Fax 703-580-8637

From: Animalfood-premarket [mailto:Animalfood-premarket@fda.hhs.gov]
Sent: Wednesday, September 22, 2021 12:38 PM
To: Kristi Smedley
Cc: Rasha Qudsieh; Animalfood-premarket; Conway, Charlotte
Subject: RE: [EXTERNAL] RE: GRAS Notice No. AGRN 44 - Call Regarding Xylanase enzyme to be used in swine and poultry feeds

Dear Dr. Smedley,

With regards to GRAS Notice No. AGRN 44, please find attached our meeting minutes from the September 15, 2021 teleconference and response to your request for the meeting minutes. Please let us know if you have any questions.

Kind regards,
Chelsea

From: Animalfood-premarket <Animalfood-premarket@fda.hhs.gov>
Sent: Wednesday, September 15, 2021 12:44 PM
To: Kristi Smedley <smedley@cfr-services.com>
Cc: Animalfood-premarket <Animalfood-premarket@fda.hhs.gov>; Rasha Qudsieh <rQudsieh@briworldwide.com>
Subject: RE: [EXTERNAL] RE: GRAS Notice No. AGRN 44 - Call Regarding Xylanase enzyme to be used in swine and poultry feeds

Dear Kristi,

Thank you for your request for minutes. A copy will be e-mailed to you and Dr. Qudsieh as soon they are available.

Kind regards,
Chelsea

From: Kristi Smedley <smedley@cfr-services.com>
Sent: Wednesday, September 15, 2021 12:10 PM
To: Animalfood-premarket <Animalfood-premarket@fda.hhs.gov>
Cc: Rasha Qudsieh <rQudsieh@briworldwide.com>
Subject: RE: [EXTERNAL] RE: GRAS Notice No. AGRN 44 - Call Regarding Xylanase enzyme to be used in swine and poultry feeds

Chelsea:

Thank you for organizing this meeting. It was very beneficial, and we will be working on our amendment.

We are requesting the notes of this meeting. I am requesting that they be sent by email to both Rasha (Rasha Qudsieh (rQudsieh@briworldwide.com)) and I.

Kristi O. Smedley, Ph.D.

Center for Regulatory Services, Inc.
5200 Wolf Run Shoals Rd.
Woodbridge, VA 22192

Ph. 703-590-7337
Cell (b) (6)
Fax 703-580-8637

From: Animalfood-premarket [mailto:Animalfood-premarket@fda.hhs.gov]
Sent: Wednesday, September 08, 2021 9:08 AM
To: Kristi Smedley
Cc: Animalfood-premarket
Subject: RE: [EXTERNAL] RE: GRAS Notice No. AGRN 44 - Call Regarding Xylanase enzyme to be used in swine and poultry feeds

Dear Dr. Smedley,

I have scheduled the call for Wednesday, September 15 from 10-11 am US Eastern Time.

Below are the Zoom details for the call. Once you click on the hyperlink "Join Zoom Meeting", you will be prompted to connect your audio either by using your computer audio or dialing in by phone (will require entering the meeting ID and passcode (b) (6)).

Please let me know if you have any questions.

Kind regards,
Chelsea

Join Zoom Meeting

One tap mobile: (b) (6)

Meeting URL:
Meeting ID:
Passcode:

Join by Telephone

For higher quality, dial a number based on your current location.

Dial: (b) (6)

Meeting ID:

Passcode:

International numbers

From: Kristi Smedley <smedley@cfr-services.com>
Sent: Tuesday, September 07, 2021 11:51 AM
To: Animalfood-premarket <Animalfood-premarket@fda.hhs.gov>
Subject: [EXTERNAL] RE: GRAS Notice No. AGRN 44 - Call Regarding Xylanase enzyme to be used in swine and poultry feeds

Thank you, we prefer Wednesday, September 15 from 10 – 11 am.

Kristi O. Smedley, Ph.D.

Center for Regulatory Services, Inc.
5200 Wolf Run Shoals Rd.
Woodbridge, VA 22192

Ph. 703-590-7337
Cel (b) (6)
Fax 703-580-8637

From: Animalfood-premarket [mailto:Animalfood-premarket@fda.hhs.gov]
Sent: Tuesday, September 07, 2021 9:01 AM
To: Kristi Smedley (smedley@cfr-services.com)
Cc: Animalfood-premarket
Subject: GRAS Notice No. AGRN 44 - Call Regarding Xylanase enzyme to be used in swine and poultry feeds

Dear Dr. Smedley,

I hope this e-mail finds you well. We would like to schedule a call with you, as well as any others from BioResource International, Inc., to discuss the GRAS notice. We are available during the following dates and times (US Eastern):

1. Wednesday, September 15 from 10 – 11 am
2. Thursday, September 16 from 12 – 1 pm

Please let me know if one of these options works or if I should look for more options. I will send Zoom information for the call once it has been scheduled.

Kind regards,
Chelsea

Chelsea Cerrito, MAS
Animal Scientist, Division of Animal Feeds (DAF)
Center for Veterinary Medicine

Office of Surveillance and Compliance
U.S. Food and Drug Administration
Tel: 240-402-6729
Personal e-mail address: Chelsea.Cerrito@fda.hhs.gov
To schedule a meeting with DAF, please e-mail: animalfood-premarket@fda.hhs.gov

ATTACHMENT F

Additional References

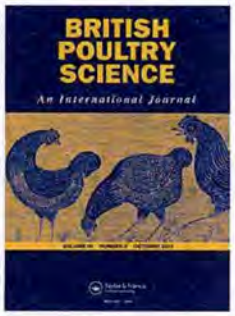
Bedford, M.R. 2018. The evolution and application of enzymes in the animal feed industry: the role of data interpretation. British Poultry Science volume 59:486-493.

Li P, Anumanthan A, Gao XG, Ilangovan K, Suzara VV, Düzgüneş N, Renugopalakrishnan V. Expression of recombinant proteins in Pichia pastoris. Appl Biochem Biotechnol. 2007 Aug;142(2):105-24. doi: 10.1007/s12010-007-0003-x. PMID: 18025573.

Nusairat, B., and J.-J., Wang. Xylanase and Direct-Fed Microbials (DFM) Potential for Improvement of Live Performance, Energy Digestibility, and Reduction of Environmental Microbial Load of Broilers. Front. Vet. Sci., 07 December 2020. DOI: https://doi.org/10.3389/fvets.2020.606415

Duarte, M., J. Tyus, and S. W. Kim. Synbiotic Effects of Enzyme and Probiotics on Intestinal Health and Growth of Newly Weaned Pigs Challenged With Enterotoxigenic F18+ Escherichia coli. Front. Vet. Sci., 09 September 2020. DOI: https://doi.org/10.3389/fvets.2020.00573

Nusairat, B., J. McNaughton, J. Tyus, and J.-J. Wang. 2018. Combination of Xylanase and Bacillus Direct-fed Microbials, as an Alternative to Antibiotic Growth Promoters, Improves Live Performance and Gut Health in Subclinical Challenged Broilers. International Journal of Poultry Science, 17: 362-366. DOI: 10.3923/ijps.2018.362.366



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The evolution and application of enzymes in the animal feed industry: the role of data interpretation

Michael R. Bedford

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GORDON MEMORIAL LECTURE

The evolution and application of enzymes in the animal feed industry: the role of data interpretation

Michael R. Bedford

R&D, AB Vista, Marlborough, UK

ABSTRACT

1. Enzymes have been used commercially for nearly 40 years and save significant costs through sparing of expensive nutrients but the mechanism by which this is achieved is still debated.
2. The research focused on non-starch polysaccharidase (NSPase) enzymes is used as an example of where greater progress could have been made if the details of the work had been described more fully and the analysis of the data generated had been broader in scope and more critical.
3. Lack of standardisation of the details presented in the materials and methods has been identified as a significant barrier to meaningful retrospective analysis and thus limits advances in the understanding of the mode of action of these enzymes.
4. The identity of the enzyme employed and its activity is often lacking, and more importantly the purity is rarely disclosed. Contaminant activities which are neither listed nor assayed could play a significant role in the responses observed.
5. The dose optimum of most enzymes is often considerably higher than that employed in most studies. Thus studies claiming synergy between two 'activities' should ensure that the response is not related to each enzyme simply augmenting the dose of just one activity in the finished feed. This is a common problem, and coupled with the lack of factorial experiments to justify the presence of each enzyme in a multi-enzyme product, it is not surprising that there is still debate as to whether single or multi-enzymes are best suited poultry rations.
6. The three proposed mechanisms for NSPases (viscosity, cell wall and prebiotic) are discussed, and along with their strengths and weaknesses it is suggested that a re-evaluation of each is needed. Viscosity may have to be re-evaluated as being a function not only of the cereal being fed, but of the age of the animal as well. The cell wall theory as described is poorly modelled *in vitro* and hence the validity of these data is questioned. The prebiotic theory may need significant modification as it appears that the quantities of oligomers produced are insufficient to generate the additional volatile fatty acids (VFA)'s reported. It is likely that all three mechanisms play a role in the responses observed, but the prebiotic mechanism probably plays by far the most important part in low viscosity diets.
7. Future research would be improved if it considered all potential mechanisms when designing a trial. Significant failings are apparent as a result of adherence to tenets in explanation of the results. Most importantly, it should be emphasised that a hypothesis is there to be tested, not defended.

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KEYWORDS
Cell wall theory; feed enzymes; mechanism of action; NSPase; prebiotic; viscosity

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Expression of Recombinant Proteins in *Pichia Pastoris*

Pingzuo Li · Anukanth Anumanthan · Xiu-Gong Gao · Kuppusamy Ilangovan · Vincent V. Suzara · Nejat Düzgüneş · V. Renugopalakrishnan

Received: 14 April 2006 / Revised: 16 May 2006 / Accepted: 23 May 2006 /
Published online: 25 April 2007

Abstract *Pichia pastoris* has been used extensively and successfully to express recombinant proteins. In this review, we summarize the elements required for expressing heterologous proteins, and discuss various factors in applying this system for protein expression. These elements include vectors, host strains, heterologous gene integration into the genome, secretion factors, and the glycosylation profile. In particular, we discuss and evaluate the recent progress

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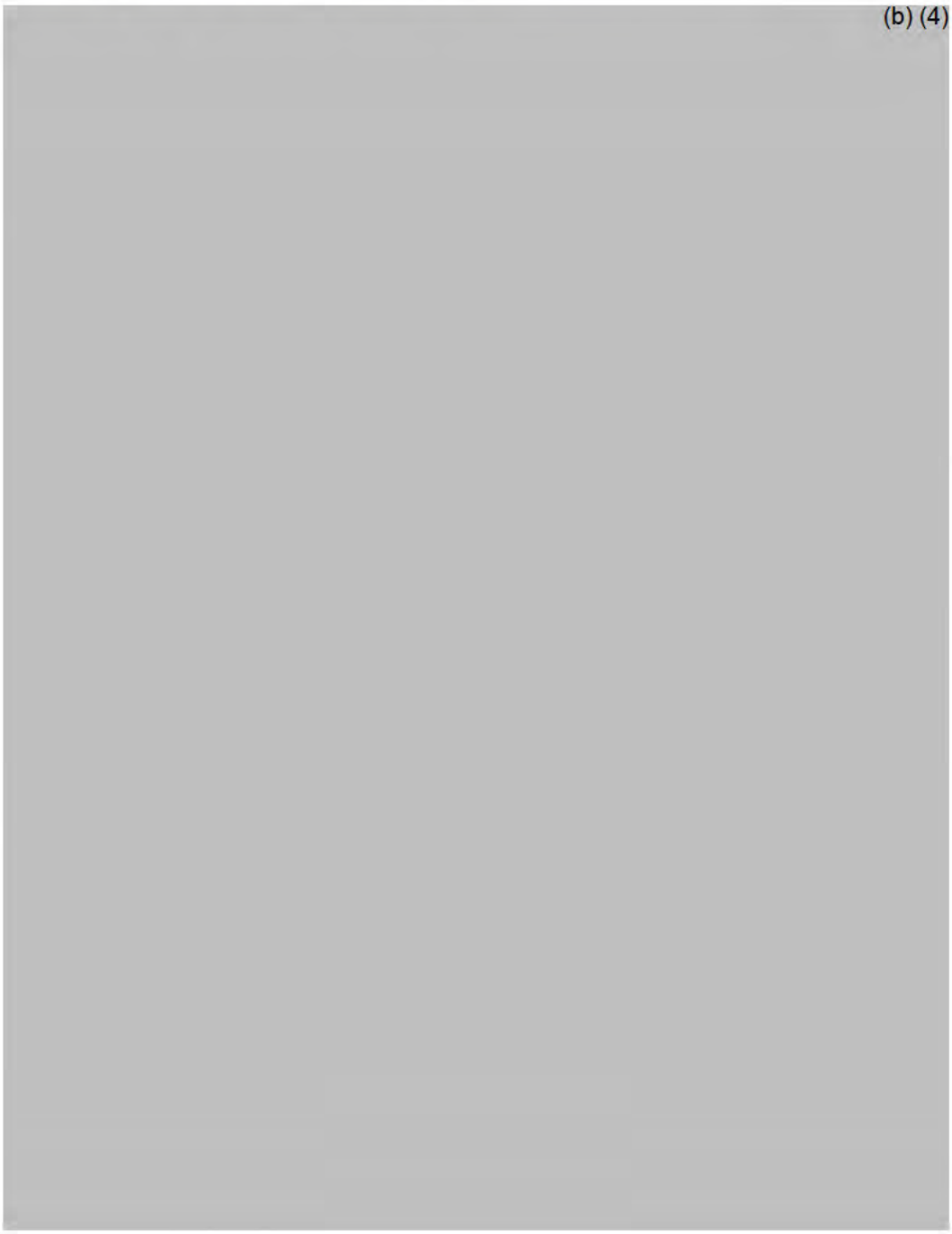
in optimizing the fermentation process to improve the yield and stability of expressed proteins. Optimization can be achieved by controlling the medium composition, pH, temperature, and dissolved oxygen, as well as by methanol induction and feed mode.

Keywords *Pichia pastoris* · Protein expression · Methanol induction · Dissolved oxygen · Gene integration · Alcohol oxidase promoter · AOX1



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Research Article

Combination of Xylanase and *Bacillus* Direct-fed Microbials, as an Alternative to Antibiotic Growth Promoters, Improves Live Performance and Gut Health in Subclinical Challenged Broilers

[1]Basheer Nusairat, [2]James McNaughton, [1]James Tyus and [1]Jeng-Jie Wang

[1]BioResource International, Inc. 4222 Emperor Blvd, Suite 460 Durham, NC 27703, USA
[2]AHPharma, Inc. 27013 E Lillian St, Hebron, MD 21830, USA

Abstract

Objective: This study evaluated the effect of xylanase, *Bacillus* direct-fed microbials (DFM) and their combination on performance under a mild, subclinical challenge with two *Eimeria* species and *Clostridium perfringens* in broilers raised to 42 days. **Materials and Methods:** A total of 6 dietary treatments were used throughout the trial. Diets were supplemented with one of the following; no xylanase or *Bacillus* (control), xylanase only, *Bacillus* L. only, *Bacillus* A. only, xylanase plus *Bacillus* L. or xylanase plus *Bacillus* A. Data were analyzed as randomized complete block design. **Results:** When compared to control at 42 days, the xylanase, *Bacillus* L. and *Bacillus* A. improved ($p \leq 0.05$) BW gain by 93, 94 and 53 g, respectively and FCR by 4, 4 and 6 points, respectively. When compared to control at 42 days, the combination of xylanase and *Bacillus* L. or *Bacillus* A. improved ($p \leq 0.05$) BW gain by 142 or 147 g, respectively and FCR by 9 or 11 points, respectively. The combination of xylanase and *Bacillus* L. or *Bacillus* A. reduced ($p \leq 0.05$) BW coefficient of variation from 15.09% (control) to 8.27 or 8.22%, respectively at 42 days. The combination of xylanase and *Bacillus* L. or *Bacillus* A. reduced ($p \leq 0.05$) gross lesion scores in small intestine and *C. perfringens* count at 42 days compared to control. **Conclusion:** Results suggest that xylanase and *Bacillus* alone may improve broiler performance and reduce the severity of intestinal lesions due to *Eimeria* and *C. perfringens* challenges and that the effect of xylanase and *Bacillus* DFM are additive.

Key words: Broiler, xylanase, direct-fed microbial, *Clostridium perfringens*, *Eimeria*

Received: May 09, 2018 Accepted: June 28, 2018 Published: July 15, 2018

Citation: Basheer Nusairat, James McNaughton, James Tyus and Jeng-Jie Wang, 2018. Combination of xylanase and *Bacillus* direct-fed microbials, as an alternative to antibiotic growth promoters, improves live performance and gut health in sub-clinical challenged broilers. Int. J. Poult. Sci., 17: 362-366.

Corresponding Author: Basheer Nusairat, BioResource International, Inc. 4222 Emperor Blvd, Suite 460 Durham, NC 27703, USA

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

frontiers in Veterinary Science

ORIGINAL RESEARCH
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doi: 10.3389/fvets.2020.00573

Synbiotic Effects of Enzyme and Probiotics on Intestinal Health and Growth of Newly Weaned Pigs Challenged With Enterotoxigenic F18$^+$ *Escherichia coli*

Marcos Elias Duarte[1], *James Tyus*[2] *and Sung Woo Kim*[1]*

[1] *Department of Animal Science, North Carolina State University, Raleigh, NC, United States,* [2] *BioResource International, Inc., Durham, NC, United States*

This study aimed to investigate the effect of dietary supplementation with xylanase and probiotics on growth performance and intestinal health of nursery pigs challenged with enterotoxigenic *Escherichia coli* (ETEC). Sixty-four newly weaned pigs (32 barrows and 32 gilts with 7.9 ± 0.4 kg BW) were allotted in a randomized complete block design (2 × 2 factorial). Two factors were ETEC challenge (oral inoculation of saline solution or *E. coli* F18$^+$ at 6 × 10^9 CFU) and synbiotics (none or a combination of xylanase 10,000 XU/kg and *Bacillus* sp. 2 × 10^8 CFU/kg). All pigs were fed experimental diets following NRC (2012) in two phases (P1 for 10 d and P2 for 11 d). The ETEC was orally inoculated on d 7 after weaning. Feed intake and BW were measured on d 7, 10, 15, and 20. On d 20, pigs were euthanized to collect samples to measure gut health parameters and microbiome. Synbiotics increased ($P < 0.05$) ADG in phase 1 and ETEC reduced ($P < 0.05$) ADG and G:F in the post-challenge period. ETEC increased ($P < 0.05$) the fecal score of pigs from d 7 to 13; however, synbiotics reduced ($P < 0.05$) it at d 9 and 11 in challenged pigs. ETEC increased ($P < 0.05$) mucosal MDA, IL-6, Ki-67$^+$, and crypt depth, whereas synbiotics tended to reduce TNFα ($P = 0.093$), protein carbonyl ($P = 0.065$), and IL-6 ($P = 0.064$); reduced ($P < 0.05$) crypt depth and Ki-67$^+$; and increased ($P < 0.05$) villus height. ETEC reduced ($P < 0.05$) the relative abundance of Bacteroidetes and Firmicutes and increased ($P < 0.05$) the relative abundance of Proteobacteria. In conclusion, ETEC challenge reduced growth performance by affecting microbiome, immune response, and oxidative stress in the jejunum. Synbiotics enhanced growth performance by reducing diarrhea, immune response, and oxidative stress in the jejunum.

Keywords: ***Escherichia coli*, growth performance, intestinal health, newly weaned pigs, probiotics, synbiotics, xylanase**

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INTRODUCTION

(b) (4)

frontiers in Veterinary Science

ORIGINAL RESEARCH
published: 07 December 2020
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Xylanase and Direct-Fed Microbials (DFM) Potential for Improvement of Live Performance, Energy Digestibility, and Reduction of Environmental Microbial Load of Broilers

Basheer Nusairat[1]* *and Jeng-Jie Wang*[2]

[1] Department of Animal Production, College of Agriculture, Jordan University of Science and Technology, Irbid, Jordan, [2] BioResource International, Inc., Durham, NC, United States

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The challenge of identifying alternatives to subtherapeutic levels of antibiotic growth promoters (AGP) in animal feed has led to increased interest in feed additives such as exogenous enzymes and direct-fed microbials (DFM). Six corn soy-based dietary treatments were designed to investigate the effect of high-efficiency xylanase alone, *Bacillus* spp. probiotics alone, and their combination vs. a commonly used antibiotic growth promoter (bacitracin methylene disalicylate; BMD) on live performance and environmental *Clostridium perfringens* load of broiler chickens with eight replicate pens per treatment. Diets were as follows: standard diet (positive control; PC); 130 kcal/kg reduced-energy diet (negative control; NC); NC with xylanase (NC + Xy); NC with probiotics (NC + Pro); NC with xylanase and probiotics mix (NC + XyPro); and NC with BMD (NC + BMD). Data were analyzed as one-way ANOVA. At 35 and 42 days, birds fed with NC + XyPro and NC + BMD were heavier ($P < 0.05$) than birds fed with NC. Improvement in feed conversion ratio (FCR) ($P = 0.0001$) was observed from 1 to 42 days by ~3 points in both NC + XyPro and NC + BMD compared to NC. The NC + XyPro reduced lesion scores by 66% compared to PC and NC. Litter *C. perfringens* cell count was reduced by ~16% with supplementation of XyPro or BMD. It can be concluded that a blend of xylanase (10 XU/g feed) and *Bacillus* spp. [1×10^5 colony forming units (CFU)/g feed] can be used as an alternative to AGP in low-energy broiler diets.

Keywords: xylanase, *Bacillus* spp., DFM, antibiotic-free, energy digestibility, broiler

INTRODUCTION

(b) (4)

Cerrito, Chelsea

From: Kristi Smedley <smedley@cfr-services.com>
Sent: Tuesday, November 02, 2021 9:37 AM
To: Animalfood-premarket
Cc: 'Rasha Qudsieh'
Subject: RE: [EXTERNAL] RE: GRAS Notice No. AGRN 44 - Call Regarding Xylanase enzyme to be used in swine and poultry feeds
Attachments: Attachment D Appendix 2 corrected -esigned.pdf

Chelsea:

The corrected appendix 2 is attached. I apologize for the oversight.

Kristi O. Smedley, Ph.D.

Center for Regulatory Services, Inc.
5200 Wolf Run Shoals Rd.
Woodbridge, VA 22192

Ph. 703-590-7337
Cell (b) (4)
Fax 703-580-8637

From: Animalfood-premarket [mailto:Animalfood-premarket@fda.hhs.gov]
Sent: Tuesday, November 02, 2021 9:29 AM
To: Kristi Smedley
Cc: 'Rasha Qudsieh'; Animalfood-premarket
Subject: RE: [EXTERNAL] RE: GRAS Notice No. AGRN 44 - Call Regarding Xylanase enzyme to be used in swine and poultry feeds

Good morning Kristi,

In the attachment titled "Attachment D. Molecular Biology- BRI Xylanase GRAS – Response to FDA", we note that it mentions a "Corrected Appendix 2" document. We are unable to locate this appendix in the attachments/e-mail below, as well as the second e-mail containing the new of new references, as well as copies of the references to the AGRN 44 amendment. We are requesting a copy of "Corrected Appendix 2" be provided.

Kind regards,
Chelsea

From: Kristi Smedley <smedley@cfr-services.com>
Sent: Thursday, October 07, 2021 12:54 PM
To: Animalfood-premarket <Animalfood-premarket@fda.hhs.gov>
Cc: 'Rasha Qudsieh' <rQudsieh@briworldwide.com>; Conway, Charlotte <Charlotte.Conway@fda.hhs.gov>

Subject: RE: [EXTERNAL] RE: GRAS Notice No. AGRN 44 - Call Regarding Xylanase enzyme to be used in swine and poultry feeds

Please see attachments to this email that provides the response to concerns raised by the Division specific to AGRN 44.

Should have any problems receiving these attachments or on the provided information, please contact me.

Kristi O. Smedley, Ph.D.

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STUDY REPORT

Whole genome sequencing -based taxonomic identification and description of genetic modifications in *Komagataella phaffii* (b) (4). Secondary metabolite pathway search.

Author(s)
(b) (6) PhD, Project Manager
hD, Bioinformatician

(b) (6) **proved by**
, Prof., Scientific Director
(b) (4)

Requestor
BioResource International Inc
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27703 Durham, NC
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This report supersedes the previous report (b) (4)

This report contains scientific interpretation of the received data by the named scientists from (b) (4) It does not necessarily represent the official views of the competent authorities.

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AMENDMENT TO PREVIOUS VERSION (b) (4)

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EXECUTIVE SUMMARY

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1 INTRODUCTION

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2.4 Annotation

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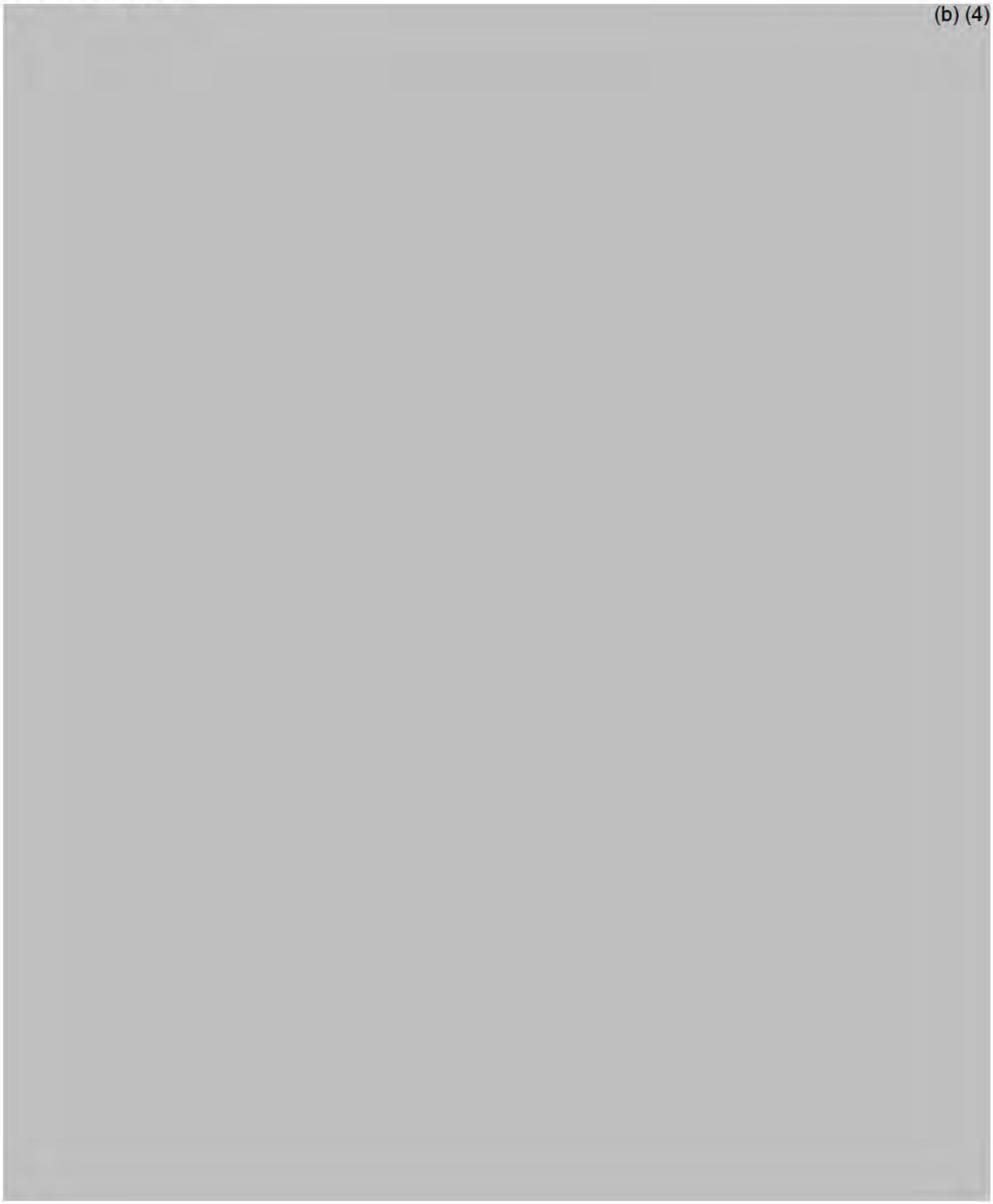

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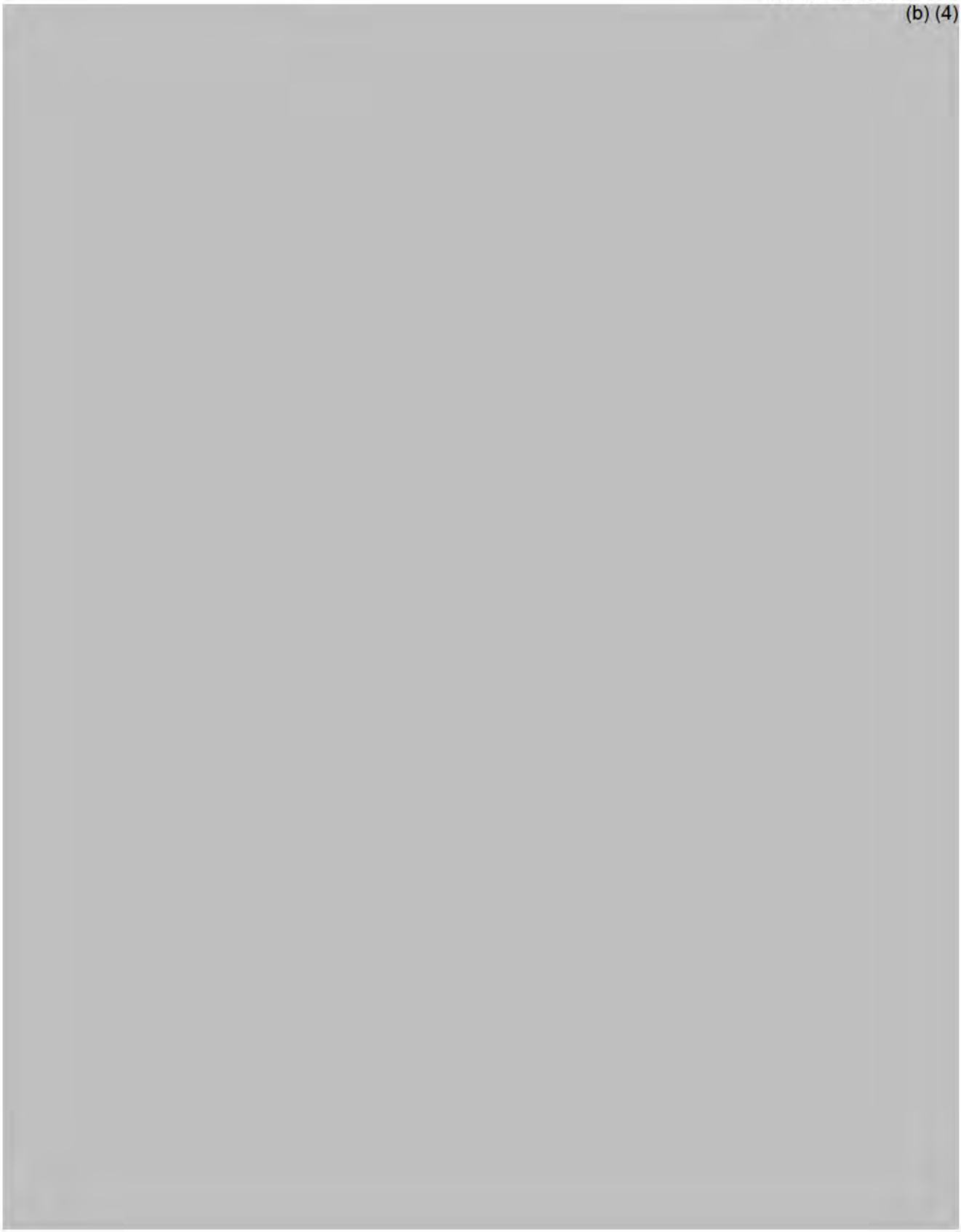

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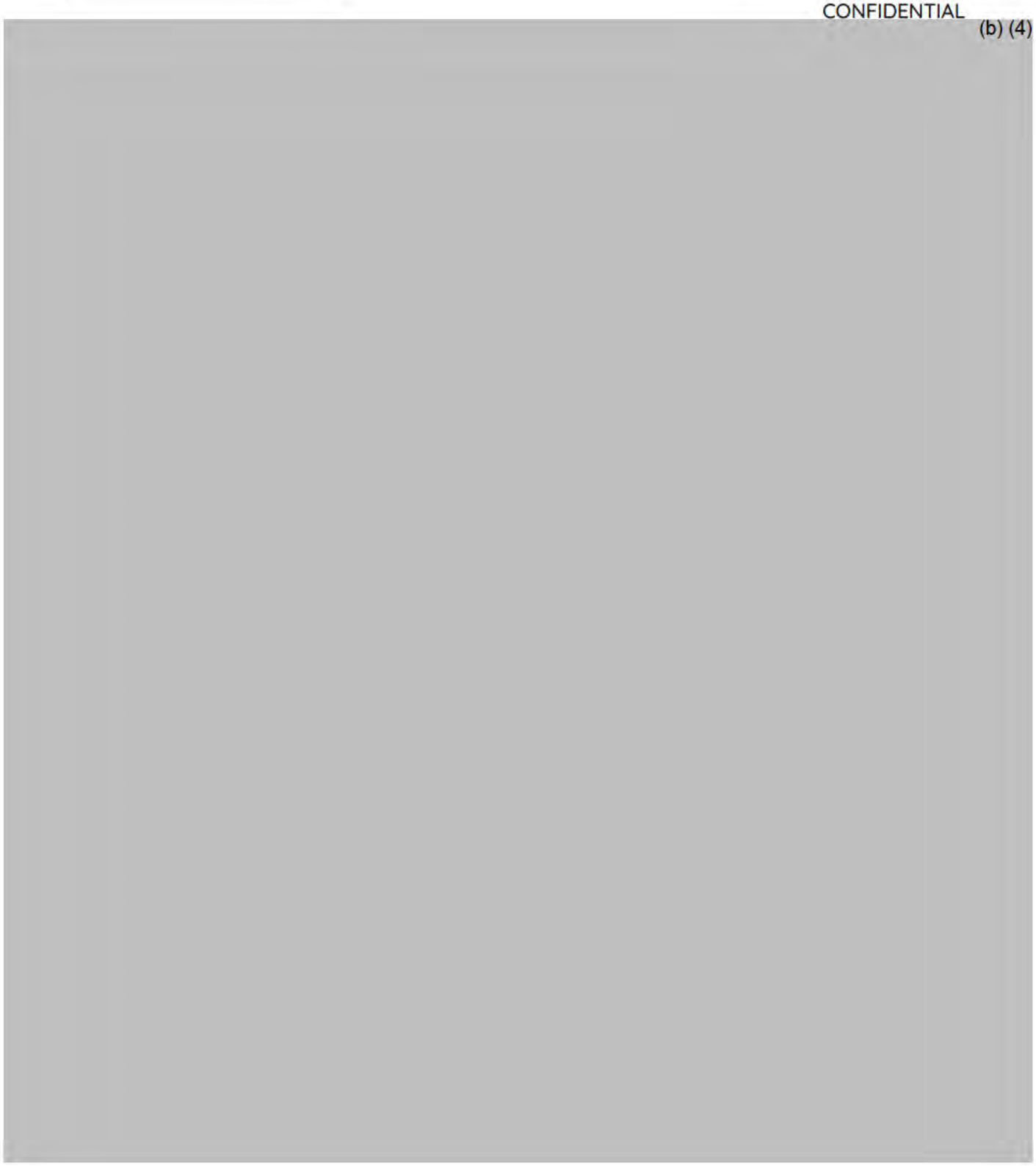

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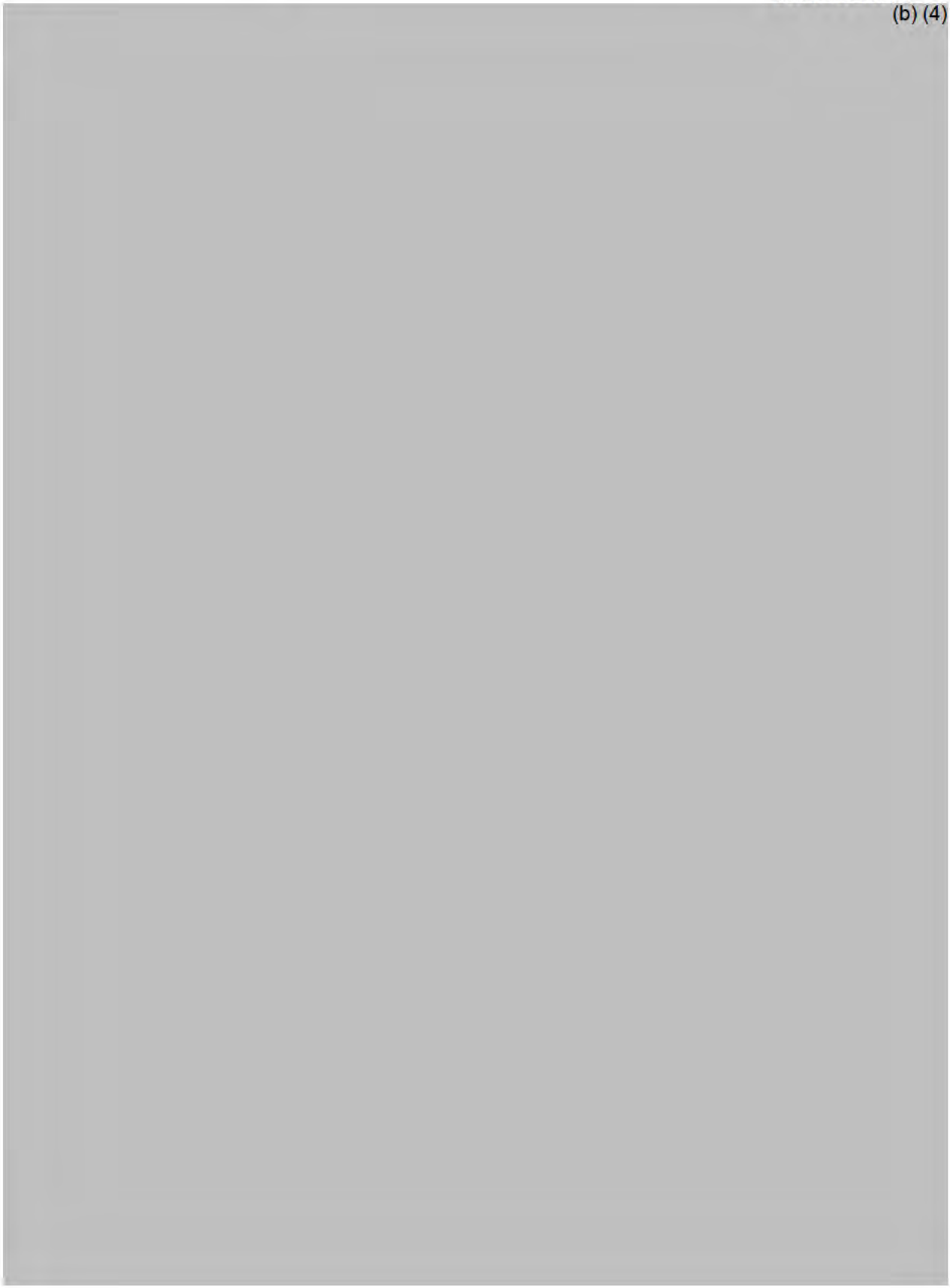

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T-0005

Cerrito, Chelsea

From: Kristi Smedley <smedley@cfr-services.com>
Sent: Thursday, October 07, 2021 12:54 PM
To: Animalfood-premarket
Cc: 'Rasha Qudsieh'; Conway, Charlotte
Subject: RE: [EXTERNAL] RE: GRAS Notice No. AGRN 44 - Call Regarding Xylanase enzyme to be used in swine and poultry feeds
Attachments: CFR Services Cover Letter BRI AGRN 44 amendment Oct 7 2021.pdf; Attachment A. Chemistry Manufacturing --BRI Xylanase GRAS - Response to FDA.pdf; Attachment A-Revised Appendix 7.pdf; Attachment A. Addendum to Appendix 21.pdf; Attachment A. Addendum to Appendix 22.pdf; Attachment A. New Appendix 24. GRAS XY purity (b) (4)df; Attachment A-Revised Appendix 23.pdf; Attachment B.Utility- BRI Xylanase GRAS - Response to FDA.pdf; Attachment C. TAS-BRI Xylanase GRAS - Response to FDA.pdf; Attachment D. Molecular Biology- BRI Xylanase GRAS - Response to FDA.pdf; Attachment D. Molecular Biology- BRI Xylanase GRAS - Response to FDA.pdf; Attachment E. Microbial Safety-BRI Xylanase GRAS - Response to FDA.pdf

Please see attachments to this email that provides the response to concerns raised by the Division specific to AGRN 44.

Should have any problems receiving these attachments or on the provided information, please contact me.

Kristi O. Smedley, Ph.D.

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From: Animalfood-premarket [mailto:Animalfood-premarket@fda.hhs.gov]
Sent: Wednesday, September 22, 2021 12:38 PM
To: Kristi Smedley
Cc: Rasha Qudsieh; Animalfood-premarket; Conway, Charlotte
Subject: RE: [EXTERNAL] RE: GRAS Notice No. AGRN 44 - Call Regarding Xylanase enzyme to be used in swine and poultry feeds

Dear Dr. Smedley,

With regards to GRAS Notice No. AGRN 44, please find attached our meeting minutes from the September 15, 2021 teleconference and response to your request for the meeting minutes. Please let us know if you have any questions.

Kind regards,
Chelsea

From: Animalfood-premarket <Animalfood-premarket@fda.hhs.gov>
Sent: Wednesday, September 15, 2021 12:44 PM
To: Kristi Smedley <smedley@cfr-services.com>
Cc: Animalfood-premarket <Animalfood-premarket@fda.hhs.gov>; Rasha Qudsieh <rQudsieh@briworldwide.com>
Subject: RE: [EXTERNAL] RE: GRAS Notice No. AGRN 44 - Call Regarding Xylanase enzyme to be used in swine and poultry feeds

Dear Kristi,

Thank you for your request for minutes. A copy will be e-mailed to you and Dr. Qudsieh as soon they are available.

Kind regards,
Chelsea

From: Kristi Smedley <smedley@cfr-services.com>
Sent: Wednesday, September 15, 2021 12:10 PM
To: Animalfood-premarket <Animalfood-premarket@fda.hhs.gov>
Cc: Rasha Qudsieh <rQudsieh@briworldwide.com>
Subject: RE: [EXTERNAL] RE: GRAS Notice No. AGRN 44 - Call Regarding Xylanase enzyme to be used in swine and poultry feeds

Chelsea:

Thank you for organizing this meeting. It was very beneficial, and we will be working on our amendment.

We are requesting the notes of this meeting. I am requesting that they be sent by email to both Rasha (Rasha Qudsieh (rQudsieh@briworldwide.com)) and I.

Kristi O. Smedley, Ph.D.

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From: Animalfood-premarket [mailto:Animalfood-premarket@fda.hhs.gov]
Sent: Wednesday, September 08, 2021 9:08 AM
To: Kristi Smedley
Cc: Animalfood-premarket
Subject: RE: [EXTERNAL] RE: GRAS Notice No. AGRN 44 - Call Regarding Xylanase enzyme to be used in swine and poultry feeds

Dear Dr. Smedley,

I have scheduled the call for Wednesday, September 15 from 10-11 am US Eastern Time.

Below are the Zoom details for the call. Once you click on the hyperlink "Join Zoom Meeting", you will be prompted to connect your audio either by using your computer audio or dialing in by phone (will require entering the meeting ID and passcode (b) (6).

Please let me know if you have any questions.

Kind regards,
Chelsea

Join Zoom Meeting

One tap mobile: (b) (6)

Meeting URL:
Meeting ID:
Passcode:

Join by Telephone

For higher quality, dial a number based on your current location.
Dial: (b) (6)

Meeting ID:

Passcode:

International numbers

From: Kristi Smedley <smedley@cfr-services.com>
Sent: Tuesday, September 07, 2021 11:51 AM
To: Animalfood-premarket <Animalfood-premarket@fda.hhs.gov>
Subject: [EXTERNAL] RE: GRAS Notice No. AGRN 44 - Call Regarding Xylanase enzyme to be used in swine and poultry feeds

Thank you, we prefer Wednesday, September 15 from 10 – 11 am.

Kristi O. Smedley, Ph.D.

Center for Regulatory Services, Inc.
5200 Wolf Run Shoals Rd.
Woodbridge, VA 22192

Ph. 703-590-7337
Cell (b) (6)
Fax 703-580-8637

From: Animalfood-premarket [mailto:Animalfood-premarket@fda.hhs.gov]
Sent: Tuesday, September 07, 2021 9:01 AM
To: Kristi Smedley (smedley@cfr-services.com)
Cc: Animalfood-premarket
Subject: GRAS Notice No. AGRN 44 - Call Regarding Xylanase enzyme to be used in swine and poultry feeds

Dear Dr. Smedley,

I hope this e-mail finds you well. We would like to schedule a call with you, as well as any others from BioResource International, Inc., to discuss the GRAS notice. We are available during the following dates and times (US Eastern):

1. Wednesday, September 15 from 10 – 11 am
2. Thursday, September 16 from 12 – 1 pm

Please let me know if one of these options works or if I should look for more options. I will send Zoom information for the call once it has been scheduled.

Kind regards,
Chelsea

Chelsea Cerrito, MAS
Animal Scientist, Division of Animal Feeds (DAF)

Center for Veterinary Medicine
Office of Surveillance and Compliance
U.S. Food and Drug Administration
Tel: 240-402-6729
Personal e-mail address: Chelsea.Cerrito@fda.hhs.gov
To schedule a meeting with DAF, please e-mail: animalfood-premarket@fda.hhs.gov

Center for Regulatory Services, Inc.
5200 Wolf Run Shoals Road
Woodbridge, VA 22192-575.5
703 590 7337 (Fax 703 580 8637)
CFR@c-fr-services.com

Since 1973
consultants to the regulated industry

October 7, 2021

David Edwards, Director
Division of Animal Feeds (HFV- 220)
Center for Veterinary Medicine
Food and Drug Administration
7519 Standish Pl.
Rockville, MD 20855

Subject: Response to Division of Animal Feeds Concerns—September 22 2021
Xylanase preparation for the use in swine and poultry feed
AGRN 44

Notifier: BioResource International, Inc.
4222 Emperor Blvd., Suite 460
Durham, NC USA 27703

Dear Dr. Edwards:

On behalf of BioResource International, Inc., I am providing responses to the clarification issues as raised by animal GRAS notice for the use of Xylanase prepared from *Komagataella phaffii* expressing the gene encoding xylanase from *Orpinomyces sp.* for use in poultry and swine diets. These issues were raised in a teleconference of September 15, 2021 and the letter issued on September 22, 2021.

We have provided 6 sections of attachments (some include revised or new appendices) to this letter each one covering one area of the provided concerns as covered in the September 22, 2021 memo:

A. Chemistry and Manufacturing Control Issues
B. Utility
C. Target Animal Safety
D. Molecular Biology
E. Microbial Safety
F. New references

Please note that we have revised the maximum suggested use level of the Xylanase preparation to 40,000 XU/Kg feed.

Should you have any questions on the filing, please contact me directly. We are providing by email.

Sincerely,

(b) (4)

Kristi O, Smedley
Consultant to BioResource International, Inc.

Cc: Rasha Qudsieh, BRI

ATTACHMENTS:

As described in the letter

ATTACHMENT A- AGRN 44—October 6, 2021

Chemistry, Manufacturing, and Controls (CMC)

Specifications

CVM: CVM requested the notifier to address and clarify the presence of other potential contaminants[3], as appropriate, in the amendment. CVM requested the notifier to clarify the specification limits for some of the parameters, including clarifying how those limits were established. For example, for lead, where the limits are higher than the values supported by the batch analyses or for mold where the specification is set at ≤1000 colony forming units per gram (CFU/g) but it is not clear how the data submitted for mold relates to any batches notified substance. The notifier should also explain why it is not necessary to analyze for the presence of formaldehyde, formic acid, or other potential contaminants. The notifier should provide calculation for total organic solids (TOS) referred to in the submission and clearly state if the TOS value is for the enzyme or its marketed form(s).

[3]: As a follow-up to the September 15, 2021 teleconference, CVM clarifies that it requests the method used and results of three representative batches of each market formulation be provided for these specifications.

Response:
Information listed in Table 2 and Appendix 12 have been revised and an updated Table 2 and Appendix 12 are provided in the amendment to explain the levels of potential contaminants in 3 batches of the final product Xylamax. Specification limits are provided in the revised Table 2. Based on the nature of the substances and raw material specifications used in the entire process of Xylamax manufacturing, it is not expected to have any contaminants in the final market product. To ensure that the assessment is correct, several additional contaminants (not part of the final product specification testing) were tested in multiple Xylamax final product batches as a confirmation, the results are listed as part of Appendix 12 table and in Table A. In addition, these additional contaminants will be re-evaluated on a yearly basis to make sure the assessment remains correct and final product is not contaminated. For the heavy metals tested, the detected levels are very low therefore are not considered a safety concern. Furthermore, the pre-established quality control standards are in place to ensure quality of material going into final product are safe and comply with standards. As indicated in ICCF Guidance # 4, the specifications should be based on analysis of multiple batches and should reflect the identity, safety, quality (including the purity) and intended effect of the feed ingredient. In addition this guidance suggests that the specification for fermentation products should be most specific on the microbial and mycotoxin contamination. As demonstrated in our multiple batch analysis, mycotoxin and microbial assessment was below the LOQ of the assay. As such, BRI has chosen specific mycotoxins, heavy metals, and microbial speciation's, to assure their product is safe and manufactured under controlled process. The limits set are based on the results of the three batch analysis as well as the safety of the contaminant (importantly this product will be incorporated in the feed at very low levels, a maximum of 0.025 grams xylanase prepation/Kg body weight. Hence there is no safety concern related to the established specifications.

For the analyses on presence of formaldehyde, formic acid, or other potential contaminant. The analysis of *Enterobacteriaceae*, ethanol, isopropanol, and methanol was performed on an additional 3 samples of Xylamax final products, and for Glycols (diethylene glycol, ethylene glycol, and propylene) on an additional final Xylamax product, results are summarized in Table A below. Results illustrate that for these analyses, the values were below the limit of quantification (LOQ) of each analytical procedure, therefore, none of these contaminants were detected in the final product which indicates that none were present in any of the raw material used in manufacturing, furthermore, the absence of methanol contamination indirectly illustrates that formaldehyde is not a contamination concern neither in raw material nor in final manufactured product. Certificates of Analysis from the analytical labs are provided as a supplement in Attachment A (Attachment A. New Appendix 24. GRAS XY purity (b) (4)) providing the method used for determining each analyte. We do not believe a specification for these contaminants is warranted.

Table A. Additional Xylamax purity analyses

	Batch No XY20281	Batch No XY20288	Batch No XY20295
	cfu/g		
Enterobacteriaceae	< 10	< 10	< 10
	ppm		
Ethanol	< 10	< 10	< 10
Isopropanol	< 10	< 10	< 10
Methanol	< 10	< 10	< 10
Glycols	Batch No XY18138		
Diethylene glycol	< 0.01%		
Ethylene glycol	< 0.01%		
Propylene glycol	< 0.01%		

The Total Organic Solids (TOS) parameter is calculated as a mean for standardizing the quantity of material derived from the enzyme source in order to assess its toxicological significance. It is defined as the sum of the organic compounds excluding diluents, and it was calculated for the final product formulation (Xylamax), the calculation was performed according to the following equation:
% TOS = 100 - (A + W + D), where
A = ash; W = Water; D = diluent and carrier
Ash was determined according to analytical method of AOAC Official Method 942.05; while moisture (water) was determined according to AOAC Official Method 934.01, 2006, vacuum oven. Since this was performed on a final product formulation, no further dilution via carrier was performed.

Sections to be replaced/updated in the dossier:
For section 2.4.2. specifications
Update text to reflect Xylamax product formulation instead of endo-1,4-β-xylanase:

"Three different batches of Xylamax product formulation were assessed…."

Please replace existing Table (Table 2. Enzyme production specification and frequency of testing) with Table 2 below (Table 2. Enzyme production specification), and replace existing table in Appendix 12 (Appendix 12. Specifications of Xylamax Product) with Appendix below (Appendix 12. Specifications and contaminant testing of Xylamax Product).

Revised Table 2. Xylamax specification

Property	Specification	Test method
Appearance	Light Grey powde	Visual inspection
Moisture, %	< 3 %	Loss on drying assay (BRI SAP)
Xylanase activity, XU/	≥ 150,000 XU/g	DNS reducing sugar assay (BRI SAP)
Mycotoxins, ppb		
Aflatoxin B1	<0.5	LC-MS/MS: detection in ppb (1 ppb = 1 μg/kg)
Aflatoxin B2	<0.5	
Aflatoxin G1	<0.5	
Aflatoxin G2	<0.5	
Heavy Metals, mg/kg		
Arsenic	<3	ICP-MS AOAC 2013.06
Lead	<3	
Microbial contaminants, CFU/g		
Coliforms	<10	E-Cultural (Non-chromogenic Media) / VRB+MUG FDA BAM Chapter
E. Coli	<10	
Salmonella	ABSENCE in 25 g	RT-PCR AOAC-RI 121501
Molds	<10	E-Cultural (Chromogenic Media) / FDA BAM Chapter 18

Revised Appendix 12. Three Batch analysis testing of Xylamax Product

Analysis	Batch No XY-C001	Batch No XY-C002	Batch No XY-C003
Production Date	April 2019	November 2019	January 2020
Best Before	April 2021	November 2021	January 2022
Appearance	Light grey powde	Light grey powde	Light grey powde
Moisture, %	1.5	1.4	1.5
Xylanase activity, XU/g	151,494	163,562	168,232
Mycotoxins	Ppb (LOQ)		
Ochratoxin	nq(<1)	nq(<1)	nq(<1)
Aflatoxin B1	nq(<0.5)	nq(<0.5)	nq(<0.5)
Aflatoxin B2	nq(<0.5)	nq(<0.5)	nq(<0.5)
Aflatoxin G1	nq(<0.5)	nq(<0.5)	nq(<0.5)
Aflatoxin G2	nq(<0.5)	nq(<0.5)	nq(<0.5)
Deoxynivalenol	nq(<100)	nq(<100)	nq(<100)
Fumonisin B1	nq(<25)	nq(<25)	nq(<25)
Fumonisin B2	nq(<25)	nq(<25)	nq(<25)
Zearalenone	nq(<30)	nq(<30)	nq(<30)
Heavy Metals	mg/kg (LOQ)		
Arsenic	0.306	0.292	0.373
Lead	0.215	0.218	0.295
Cadmium	0.388	0.360	0.381
Mercury	nq (<0.018)	nq (<0.018)	nq (<0.018)
Dioxins,/Furans and PCBs	ng/kg (LOQ)		
Dioxins and furans: WHO(2005)-PCDD/F TEQ (upper-bound)	0.0443	0.032	0.198
WHO(2005)- PCB TEQ (upper-bound)	0.0011	0.00137	0.00126
Microbial contaminants	CFU/g		
Coliforms	nq (<10)	nq (<10)	nq (<10)
E. Coli	nq (<10)	nq (<10)	nq (<10)
Salmonella	ABSENCE in 25 g	ABSENCE in 25 g	ABSENCE in 25 g
Molds	nq (<10)	nq (<10)	nq (<10)

nq: not quantifiable (< limit of quantification)

Manufacturing

CVM: CVM noted that some raw materials (for example,) are mentioned in the narrative of the notice (titled "20201229_Xylanase FDA GRAS Dossier final Dec 29 2020") but are not listed as raw materials in the table provided. The notifier should provide a table listing all the raw ingredients along with applicable regulatory status of each ingredient categorized by each manufacturing process stage, such as production tank, recovery, formulation, etc., and describe which ingredient is used for which part of the manufacturing process such that CVM can understand at which stage of the manufacturing process these ingredients are added (for example, in fermenter or market formulation). The notifier should clearly state that no other raw materials are used for the manufacturing process. The notifier provided a composition table for the enzyme and should clarify if this table is for the marketed formulation of the enzyme. The notifier should clarify if Xylamax is their market formulation and provide its composition, including all ingredients added to the formulation.

Response:
Appendices 7 to 10 contain detailed information about raw material used at each step of the production. Appendix 7 (Raw Material Used in Fermentation and Downstream Processing) provides an overview of each raw material used in the process of fermentation and manufacturing, in which media the raw material was used, the regulatory status, and a detailed description and specification of each raw material component. Appendix 7 has been updated to include the missing raw materials used during the fermentation/manufacturing process (b) (4) and which stage of manufacturing each raw material was used at. An updated Appendix 7 is provided below to be used in the amendment with changes highlighted. Appendix 9 (Growth Media and Reagents) provides information on the raw materials including percentages/quantities included in each growth media preparation in details. Appendix 10 (Fermentation and Downstream Processing) provides further details about the raw materials used in fermentation and downstream processing with quantities and steps further explained, in a step by step approach, how each media is mixed and prepared.

For composition Table (Table 1. Product composition), the submitted text, table (and title) were amended, and an updated/amended version is provided below (section 2.4.1). The text preceding the Table have been updated to make it clear that this composition represents the market formulation of Xylamax. To clarify, Xylamax is the only formulation product containing xylanase as the active agent, with a minimum enzyme activity of 150,000 XU/g, is a subject of this notice.

Sections to be replaced/updated in the dossier:

For Appendix 7. Raw Material Used in Fermentation and Downstream Processing (CONFIDENTIAL). Please use updated tables provided in Attachment A-Revised Appendix 7

For Appendix 23. Safety Data Sheet of Xylamax, please use amended SDS provided in Attachment A. Revised Appendix 23

For section 2.3.1. Raw Materials (CONFIDENTIAL)
Please replace the existing text under this section with the following: (b) (4)

For section 2.4.1. please replace existing text and Table 1 with the text and Table 1 below:

2.4.1. Quantitative Composition
The composition of the Xylamax market formulation is detailed in Table 1. Xylamax is the formulated product containing xylanase as the active agent, with a minimum enzyme activity of 150,000 XU/g as covered by this notice.

(b) (4)

Table 1. Composition of Xylamax market formulation

Component	CAS Number	Percent (w/w)
Active agent: Endo-1,4-β-xylanase	9025-57-4	10-30
Excipient: Limestone CaCO3	1317-65-3	70-90
Excipient: Starch	9005-25-8	5-10

Stability and Homogeneity

CVM: CVM stated that the notifier should clarify if the xylanase shelf-life stability study was performed on the market formulation of the enzyme. The notifier stated for stability of the substance that the storage temperature does not exceed 25 °C. The notifier provided a stability study at 30 °C and used a reference at 25 °C. The notifier should provide a justification for performing the study at 30 °C and clarify if the reference sample is for the 0-month/initial time point. The notifier has referred to the activity of the enzyme in XU/g and U/g; the notifier should clarify if units per gram (U/g) is same as xylanase units per gram (XU/g). The notifier provided a table for average xylanase storage and should clarify if the enzyme activity values shown are average of two or more replicates per time point. The notifier should provide an explanation for the variation in the percent activity of enzyme stability in feed.

CVM noted that stability of enzyme in premix was not provided in the notice. CVM stated that if a premix for the enzyme is used then information on the stability and homogeneity, if applicable, of enzyme in the premix may be required. The notifier stated that their enzyme market formulation will be added directly to finished feed and not through a premix.[4]

[4] As a follow-up to the September 15, 2021 teleconference, CVM notes that in the homogeneity study the notifier refers to a premix before adding the enzyme to the feed.

The notifier stated that the samples in the homogeneity study were analyzed in triplicates and they should clarify if the results are average of triplicates. The notifier stated that the enzyme is added at an inclusion rate of 70 grams per metric ton (g/MT) for the homogeneity study and they should clarify if this is a representative inclusion rate in the feed. Also, the notifier should address the effect of pelleting on the stability of the enzyme in the feed ingredient, as appropriate. Typically, CVM requires pelleted feed stability for three batches of the substance and data from before and after pelleting (pelleting conditions should be representative of United States conditions) to demonstrate pelleted feed stability.

Response:

The xylanase shelf-life stability was performed on Xylamax market formulation. It is guaranteed that the market product formulation will meet its minimum enzyme activity of 150,000 XU/g product if stored at the recommended conditions (25°C for 24 months). However, the shelf-life stability data is provided under the simulation of temperature abuse conditions (to show additional guarantee), reference sample indicated in Appendix 17 is for the 0-month/initial time point activity measurement. Expression of xylanase activity units per gram (U/g) is same as xylanase units per gram (XU/g) so both terms were used interchangeably. In both shelf-life stability trials, each sample bag (representing

one time point) was analyzed in triplicate. The values in summary tables each represent the average of 3 (triplicate) samples.

With regards to the variation in the percent activity of enzyme stability in feed, data in Appendix 18 study 1 presents in-feed stability data in mash feed prepared from 3 different lots of Xylamax final product, the feed was stored for 24 weeks (6 months) which is in practical industry sitting not expected, because in reality, complete feed would not be stored for more than 4 weeks (1 month) due to multiple reasons including stability of other more susceptible feed ingredients such as fat/oil which will cause rancidity if stored for prolonged period of time. Therefore, the important time period to emphasize for Xylamax stability in feed would be for the first 4 weeks as that is the industry representative timeframe of feed storage (at max). Data show that there was no large variation in enzyme recovery in the mash feed during the first 4 weeks, minimum enzyme recovery was at 100% and max recovery was an average of 115% which is acceptable, these variations could be due to having variation among different analyst perform the assay. Data beyond 4 weeks does not represent a real-life situation in relation to in-feed stability therefore will not be considered as part of making the conclusion on the stability of Xylamax in-feed. Variation after that time period is more likely an anlysis/analyst issue, as the data as a whole does not suggest xylanase degradation in activity.

Enzyme market product is not intended to be used in a premix, the product will be directly mixed into feed. The note about the product being added to a premix in the homogeneity study is referring to mixing all micro-ingredients (vitamins, minerals, synthetic amino acids, and enzyme) as a feed manufacturing practice rather than a product specification requirement. The term premix used in the homogeneity study was referring to the marketed product .

For the homogeneity study, each collected sample was analyzed in triplicate, the data provided in table is an average of 3 (triplicate) samples for both mash and pelleted feed homogeneity. The enzyme is added at an inclusion rate of 70 grams per metric ton (g/MT) which is a representative minimum inclusion rate in the feed.

For the effect of pelleting on the stability of Xylamax in feed, was not included in the original notice submission because data was not complete when dossier was first submitted, however, both mash and pellet samples were collected from study 2 in Appendix 18 (Appendix 18. Xylanase in-Feed Stability and homogeneity: study 1 and 2) and stored for 3 months (each timepoint had a designated pre-labelled bag), each sample was analyzed in triplicates and average values are recorded in summary table for both mash and pellet. Text added below to be included as an addition to Appendix 18.

Feed manufacturing performed in this study followed the United States manufacturing conditions by pelleting at 85°C and conditioning the feed for 30 sec retention time. The analyzed activity in mash feed was above the minimum activity and was maintained for 3 months of feed storage, while in the pelleted feed, the activity was slightly reduced but maintained the activity during the 3 month storage.

BioResource has concluded that the studies were robust and satisfy their marketing requirements to assure that the product is stable in the marketed container, stable once added to feed, stable in pelleted feed, and can be homogenously mixed. These data support the utility of the enzyme preparation.

Sections to be replaced/updated in the dossier:

***** The text/Tables below are to be added to the end of Appendix 18. Xylanase in-Feed Stability and homogeneity: study 1, 2, and 3 *****

In addition to determination of homogeneity and recovery of Xylamax, study 2 aimed to determine the effect of time on Xylamax stability in feedstuffs based on testing the stability of processing from mash to pellet at 85 °C, as well as monthly testing for Xylamax active ingredient (xylanase) in feed samples (mash and pellet) stored for 3 months at ambient conditions (25° C ± 2°C and 60% RH) packed in labelled paper bags to mimic storage in feed bags. Samples were tested at time zero (homogeneity data) and after 1, 2, and 3 months and the active substances determined (xylanase activity). Feed is not expected to be stored for more than one month in a practical industry situation therefore, 3 month in-feed stability data should be more than sufficient to demonstrate xylanase stability in feed over time. Each sample was analyzed in triplicate and average values were reported. The methods used for determination of

xylanase activity xylanase in-feed assay in mash and pelleted feed. Xylamax enzyme activity was reduced by 29.9% after pelleting at 85 °C. Stability results are summarized in tables below:

Stability of Xylamax in mash and pelleted feed (study 2)-Expressed as XU/Kg

Sample	Expected (XU/kg)	0 months	1 months	2 months	3 months
Mash	10,000	11,633	10,802	10,830	11,052
Pellet	10,000	8,152	8,072	7,825	7,782
Enzyme loss % compared to time 0 (mash)			5.0%		
Enzyme loss % compared to time 0 (pellets)			4.5%		

BRI has concluded that Xylanase activity is not impacted by storage at ambient conditions, in mash or pelleted feeds.

Study 3:

General objective:
To determine xylanase enzyme recovery after pelleting at 85 °C when xylamax is added at 2 different rates to feed (70 g/MT and 140 g/MT).

Ingredient Composition of the feed:

2 different lots of Xylamax (XY20355 and XY20356) final product were tested in a 2000-lb batch, Table below illustrates the corn-SBM-based feed formula, feed was conditioned to 85°C with 30 sec retention time, then pelleted using (b) (4) pellet mill.

Composition of basal diet.

Ingredient	%	Ingredient	%
Corn	64.28	Choline chloride, 60%	0.20
Soybean meal	30.40	Mineral premix	0.20
Poultry fat	2.00	Vitamin premix	0.05
Defluorinated Phosphate	1.91	L-lysine	0.11
Calcium carbonate	0.25	Selenium premix	0.05
Sodium chloride	0.28	L-Threonine	0.09
DL-Methionine	0.18		

Pelleting Parameters
Treatments were pelleted in a (b) (4) pellet mill (model: (b) (4). The target conditioning temperature is 85 °C. The target conditioning time was 30 seconds, with a production rate of (b) (4) per hour and a die size of (b) (4) with a (b) (4) effective thickness

Sampling Procedure
1. Mash Feed

3 samples (~ 500 g each) per feed batch of the mash feed were collected at 20 sec intervals as mash feed is dispatched from the mixer to the conditioner. Samples were collected in pre-labelled zip top bags.

2. Hot Pellet Samples

3 (~1000 g each) hot pellet samples were collected at 30 sec intervals in mesh-lined trays at the exit chute of the pellet mill before they reach the cooler. The samples with trays were placed immediately into a portable air cooler, collected samples were numbered according to their collection sequence and labelled with the hot pellet temperature measured at time of collection. The cooler draws air from underneath the box to the top of the container. The mesh-lined trays

allow the air to be drawn around and through the pellets to facilitate cooling of the samples. Samples were cooled for approximately 10 minutes to reach a temperature within +/- 5 °C of ambient. After the samples were cooled, a 500 g sample of each was collected in pre-labelled zip top bags and stored in cooler until delivering samples to R&D lab.

All 3 samples from mash and pellets were analyzed in triplicates (total 9 replicates per feed batch) using the xylanase in-feed assay listed in Appendix 20. Average recovery of xylanase enzyme from the feed batches are summarized in Table below.
Based on the analyzed recoveries of xylanase enzyme in pelleted feed (from studies 2 and 3), it can be concluded that xylanase enzyme loss on pelleting had an average of 27.8% loss, therefore, it can be recovered with an approximate average of 72% when subjected to conditioning temperature of 85 °C during pelleted feed manufacturing process.

Stability of Xylamax under processing at 85° C (Compilation of both study 2 and study 3)

Xylamax lot #	XY20281	XY20355	XY20356
	XU/Kg feed		
Activity in mash prior pelleting	11,633	10,239	21,261
Activity in pellets	8,151	6,907	16,789
Activity loss	-29.9%	-32.5%	-21.0%

BRI has concluded that the pelleting process decreased the Xylanase activity approximately 30%, and the label will reflect that information.

***** END OF ADDED SECTION TO APPENDIX 18 *****

Analytical Method

CVM:CVM noted that "beechwood xylan" is identified in the definition of the enzyme activity. "Xylan" is also mentioned in the analytical methods. The notifier should revise the analytical procedures and validations to specify beechwood xylan or provide a statement clarifying that the xylan referred to in the analytical methods and validations is beechwood xylan. CVM requested the notifier to explain the analytical methods and the purpose of the materials (for example, α-amylase, used in the analytical methods). The notifier should provide explanation and information for the analytical method validations.

The notifier should clarify if pages 145 and 171 of 171 in the notice were intentionally left blank.

Response:
The active substance in Xylamax is endo-1,4-β-xylanase. There is currently no available standard method for quantifying xylanase activity. Thus BRI has validated and further verified (at a third party lab) the internal methods for xylanase determination in product and feed.

There are 2 standard analytical procedures (SAPs) to quantify xylanase activity: one in product (Appendix 19. Standard Analytical Procedure for xylanase in product activity) and one in feed (Appendix 20. Standard Analytical Procedure for xylanase in-feed activity), both were validated, then verified in a second laboratory. Based on the performance characteristics, these methods are considered valid for the proposed use. Thus, BRI suggests the use of DNS Reducing Sugar Method for official control of xylanase in product and the XylX6 method for official control of xylanase activity in feed.

For both SAPs, one unit of Endo-1,4- β- Xylanase activity (XU) is defined as the amount of enzyme needed for the release of 1 nanomole of reducing sugars (xylose equivalents) per second from 0.5% xylan at 50°C in 50 mM trisodium citrate buffer pH 6.0. The xylan referred to in the definition, analytical methods and validations is beechwood xylan.

The purpose of using α-amylase in the assay is because xylanase has a starch binding that is removed by the α-amylase freeing it up to interact with the substrate (beechwood xylan). (We note that this is simulating the natural addition of amylase (as provided in the saliva and pancreatic secretion of poultry and swine, to assist with the digestion of starch)).

Beechwood xylan is the substrate with which xylanase will interact in the product assay, the purpose of using beechwood xylan is to provide a controlled quantity of the substrate upon which xylanase will work yielding the release of xylose (reducing sugar) that interacts with Dinitrosalicylic acid (DNS) to form a red complex that can be spectrophotometrically measured at wavelength 540 nm, and therefore, the quantification of xylanase activity.

XylX6 is the substrate with which xylanase will interact in the in-feed assay, the activity that needs to be detected for xylanase activity in feed is at a lower level compared to the activity in the product, furthermore, the matrix within which the enzyme is existing is different between product and feed therefore, different substrates were used for each assay (product and in-feed).

Pages 145 and 171 of 171 in the notice were intentionally left blank.

Sections to be replaced/updated in the dossier:

Please refer to "Attachment A. Revised section to add to Appendix 21" and "Attachment A. Revised section to add to Appendix 22" for revised sections to be added to each Appendix.

LIST OF REVISED AND NEW APPENDECIES RELATED TO CMC

NEW: Attachment A Appendix 24

REVISED: Attachment A-Revised Appendix 7 (CONFIDENTIAL)

REVISED: Attachment A-Revised Attachment 23

REVISED: Attachment A. Addendum to Appendix 21 (CONFIDENTIAL)

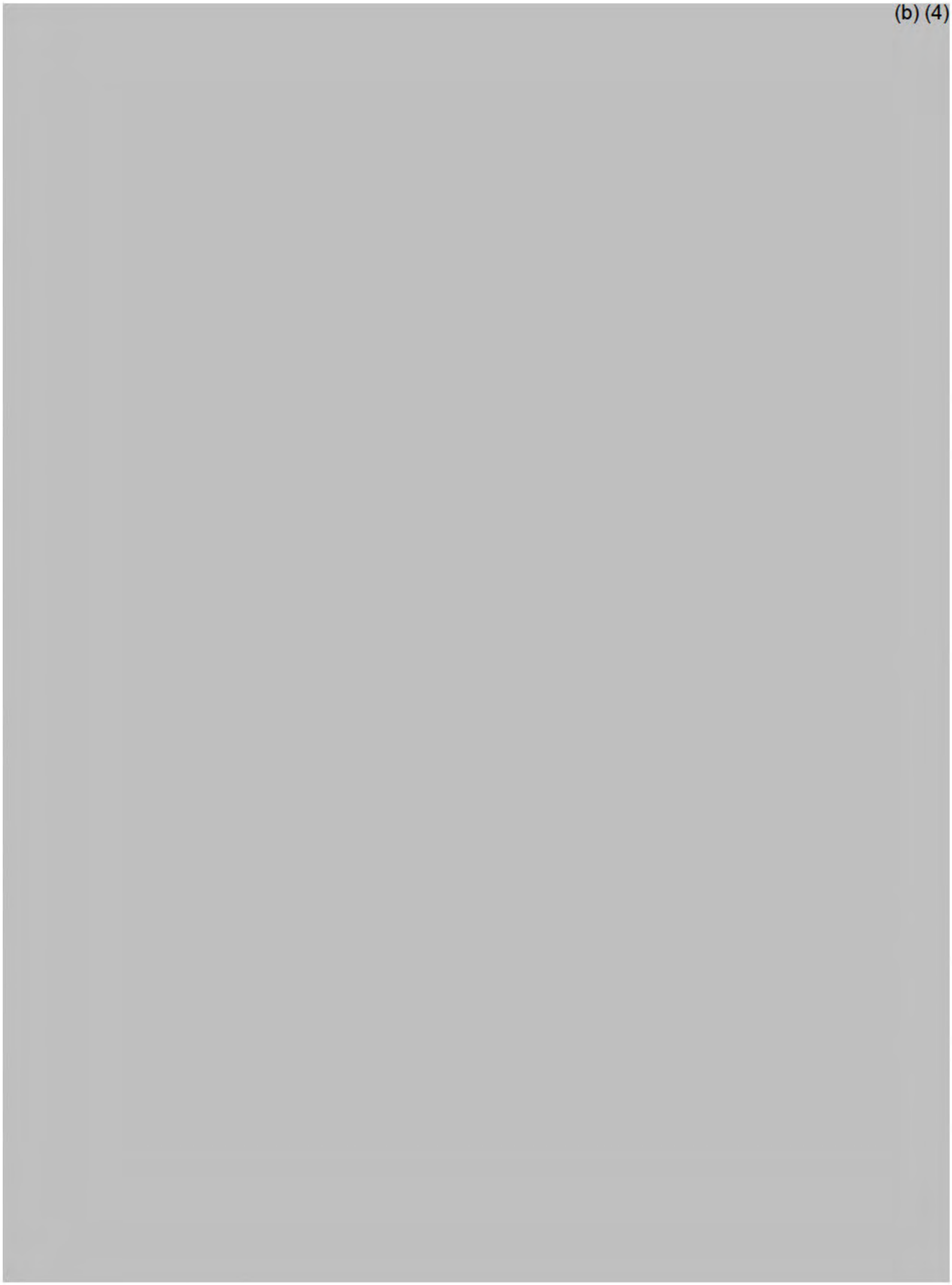
REVISED: Attachment A. Addendum to Appendix 22 (CONFIDENTIAL)

Attachment A. Revised Appendix 7

Revised Appendix 7. Raw Material Used in Fermentation and Downstream Processing (CONFIDENTIAL)

(b) (4)

[redacted]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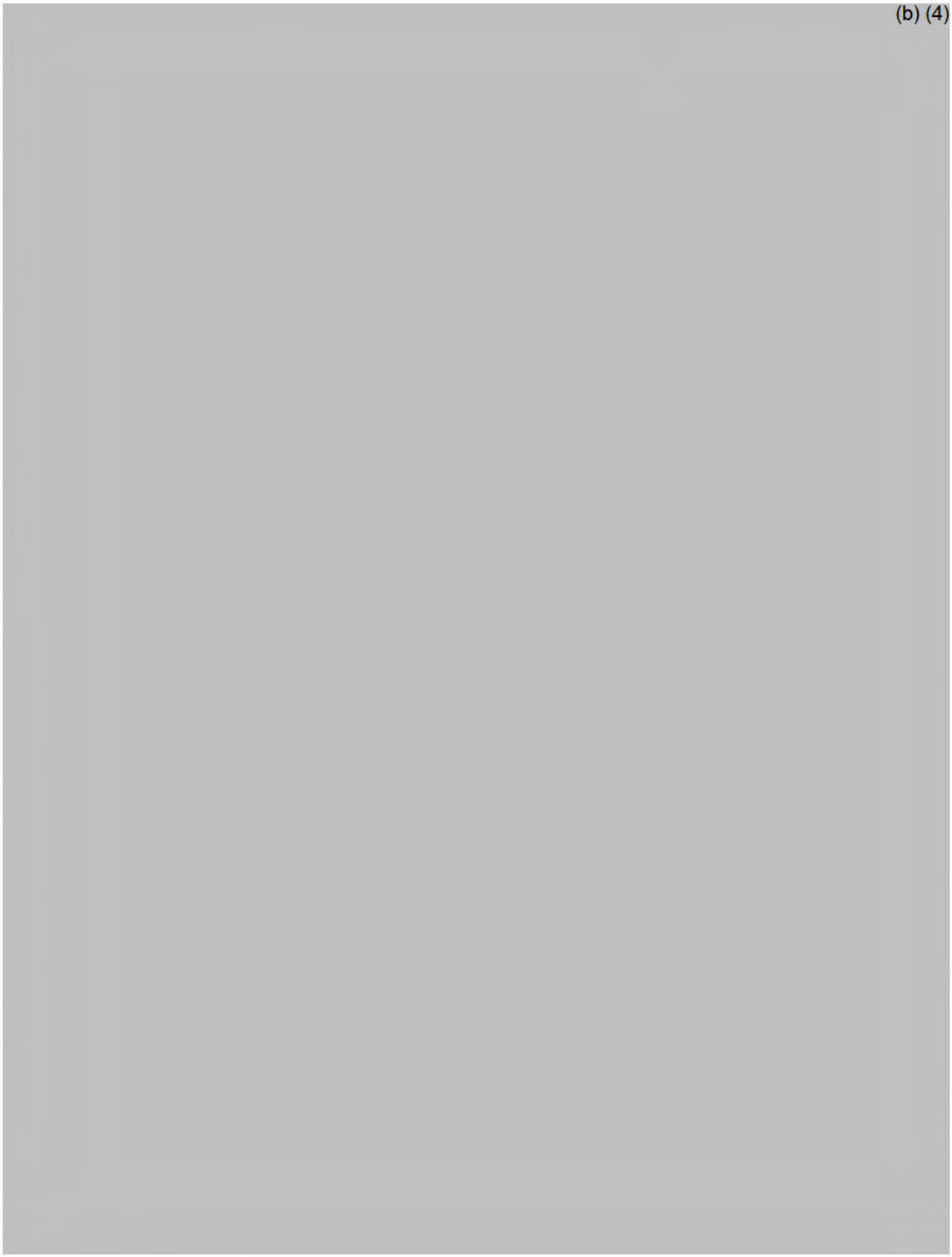
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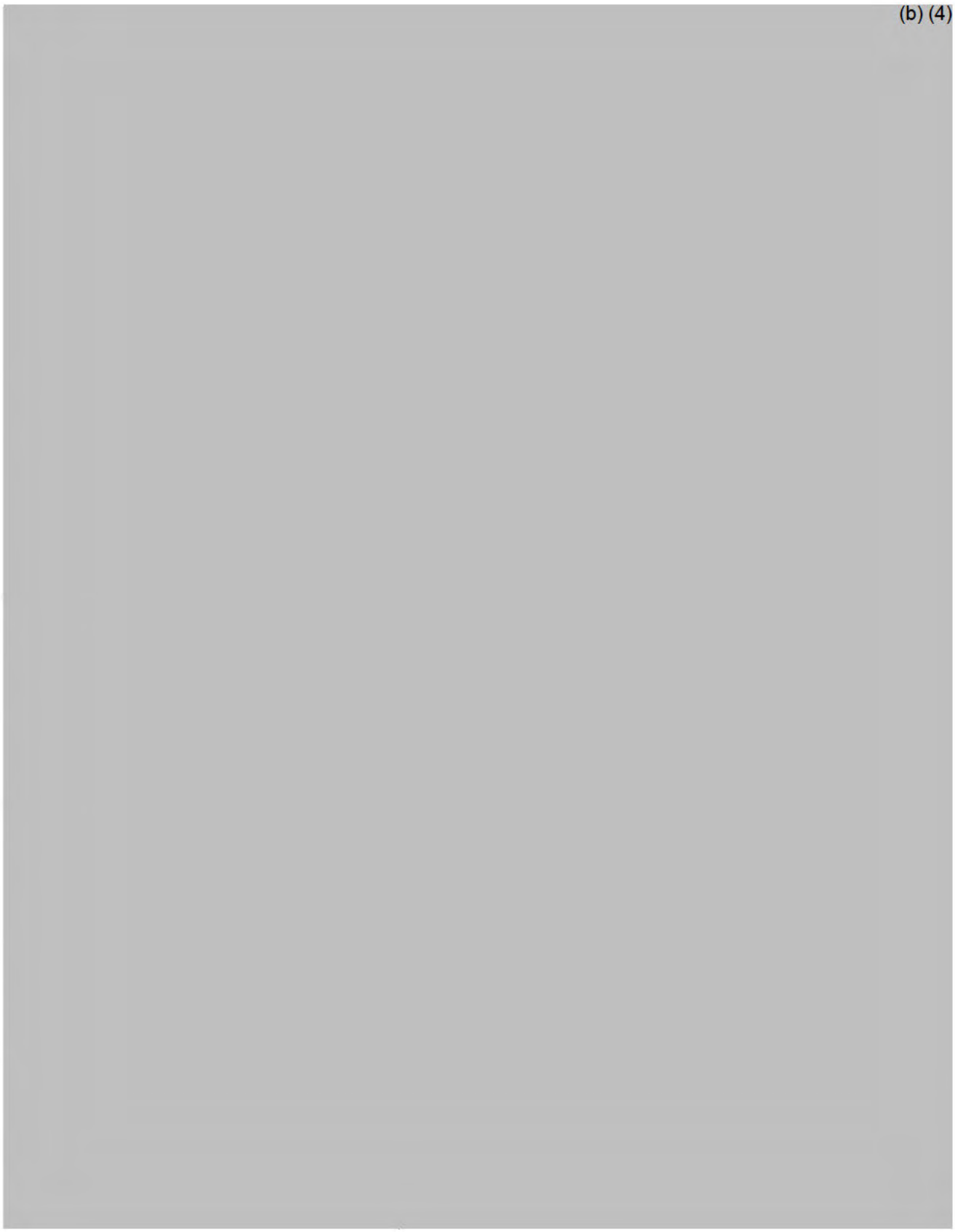
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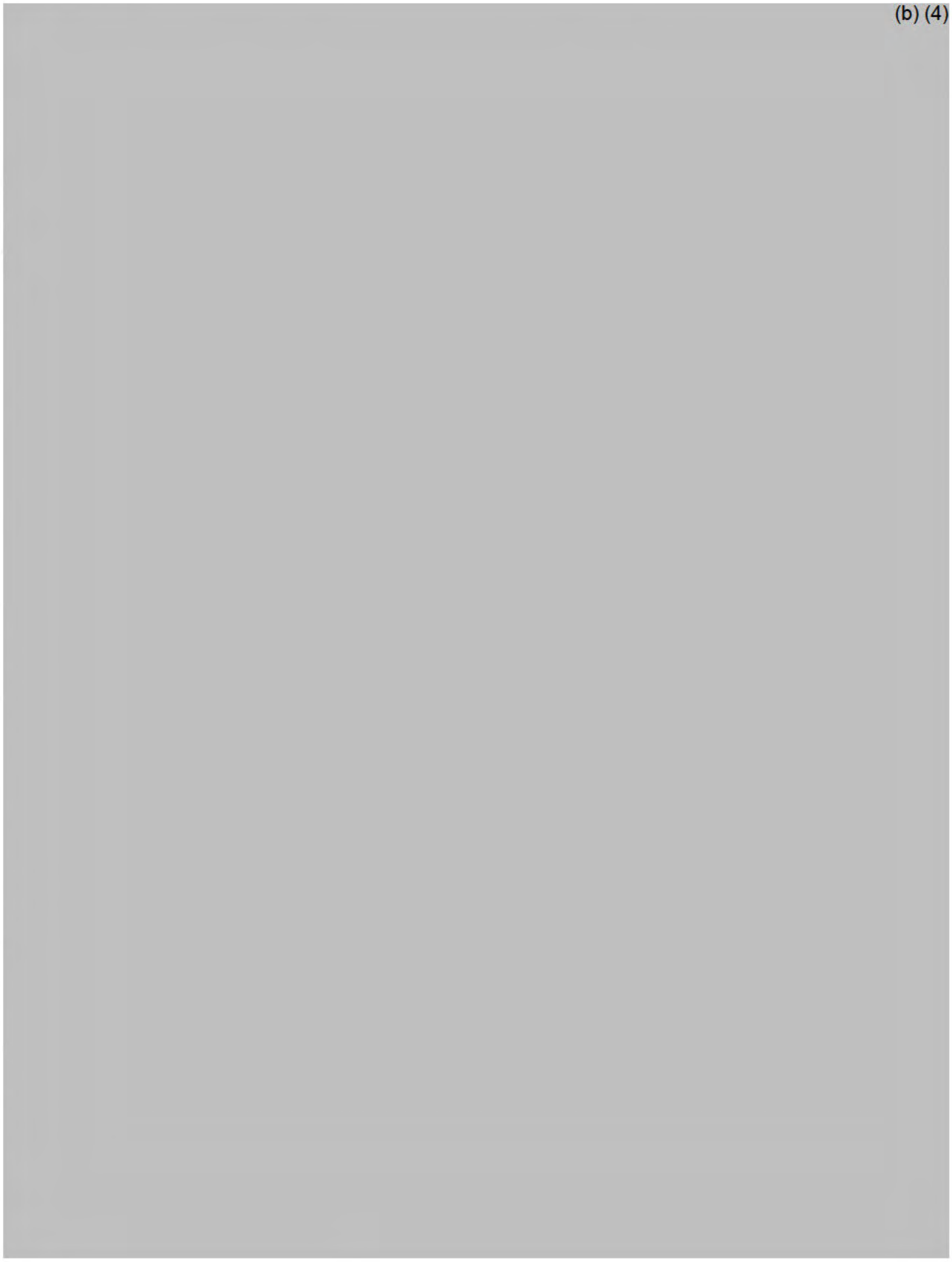
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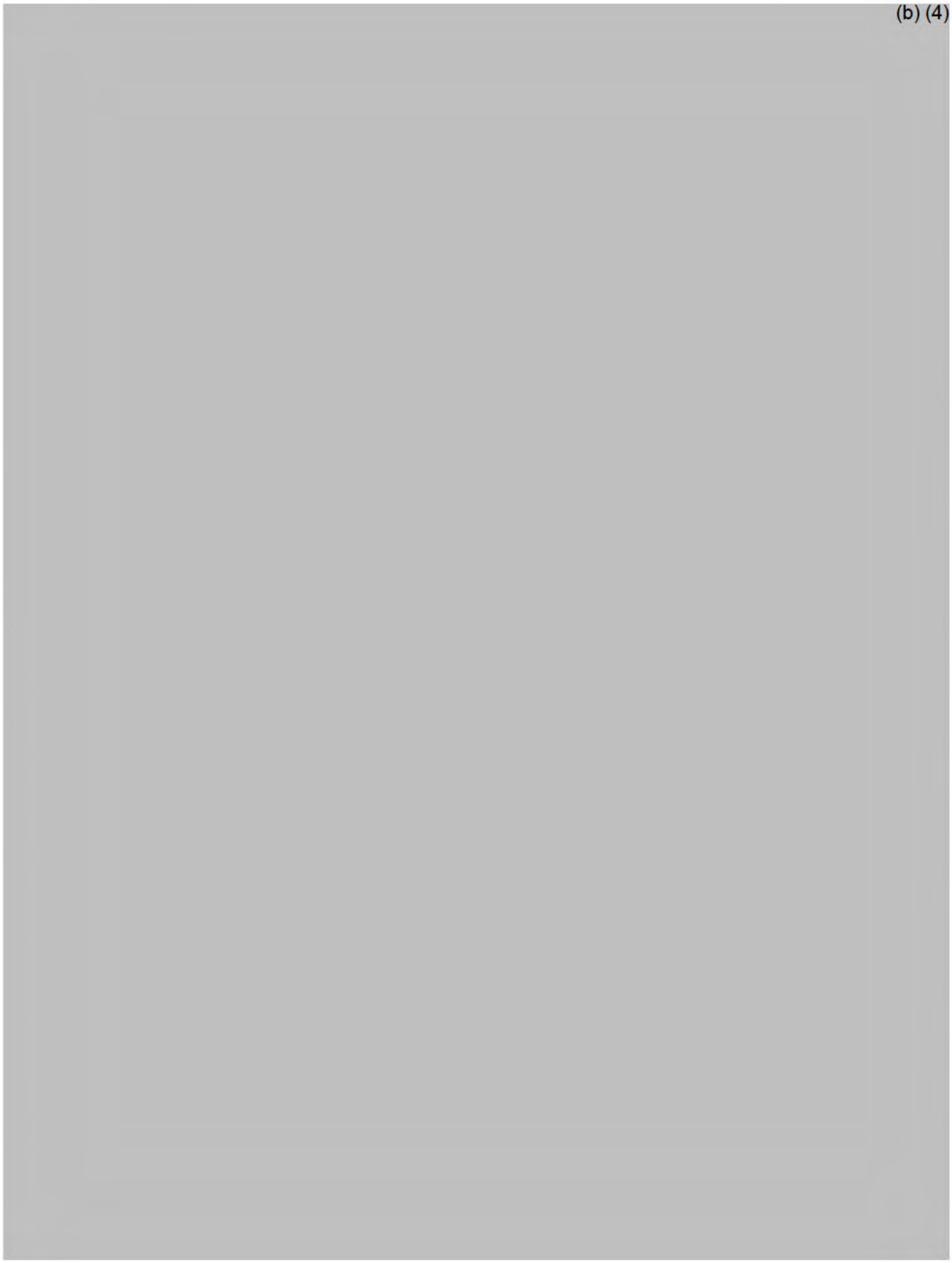
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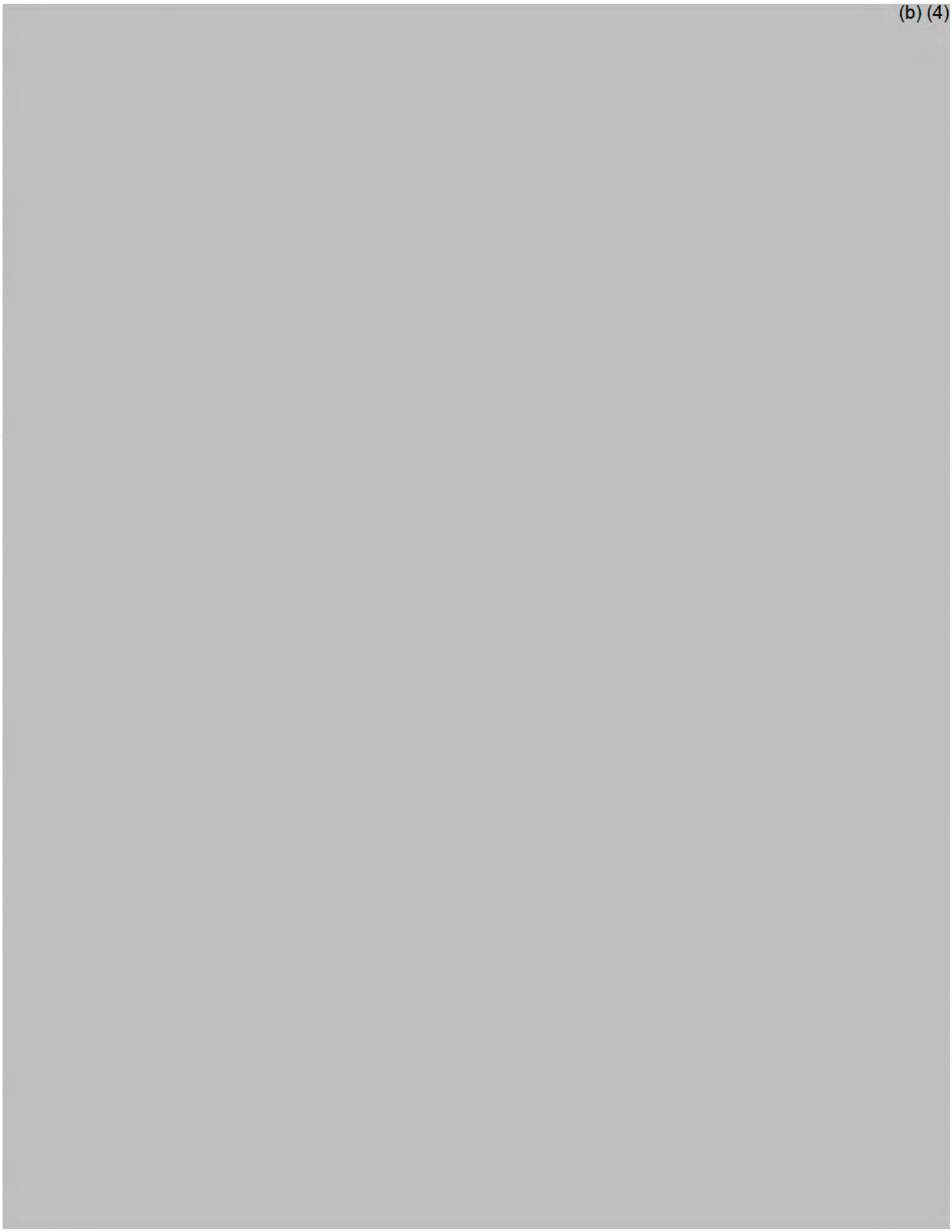
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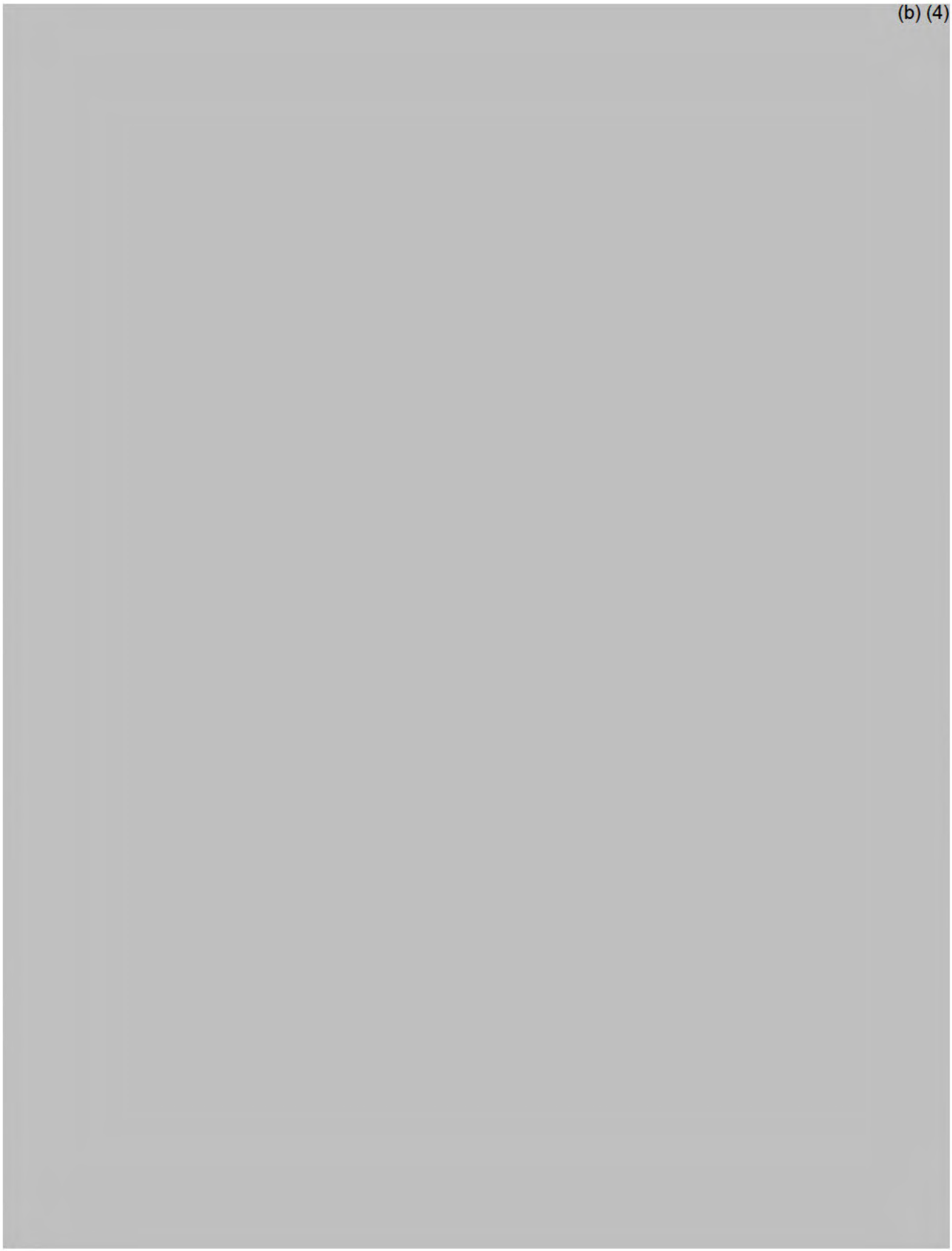
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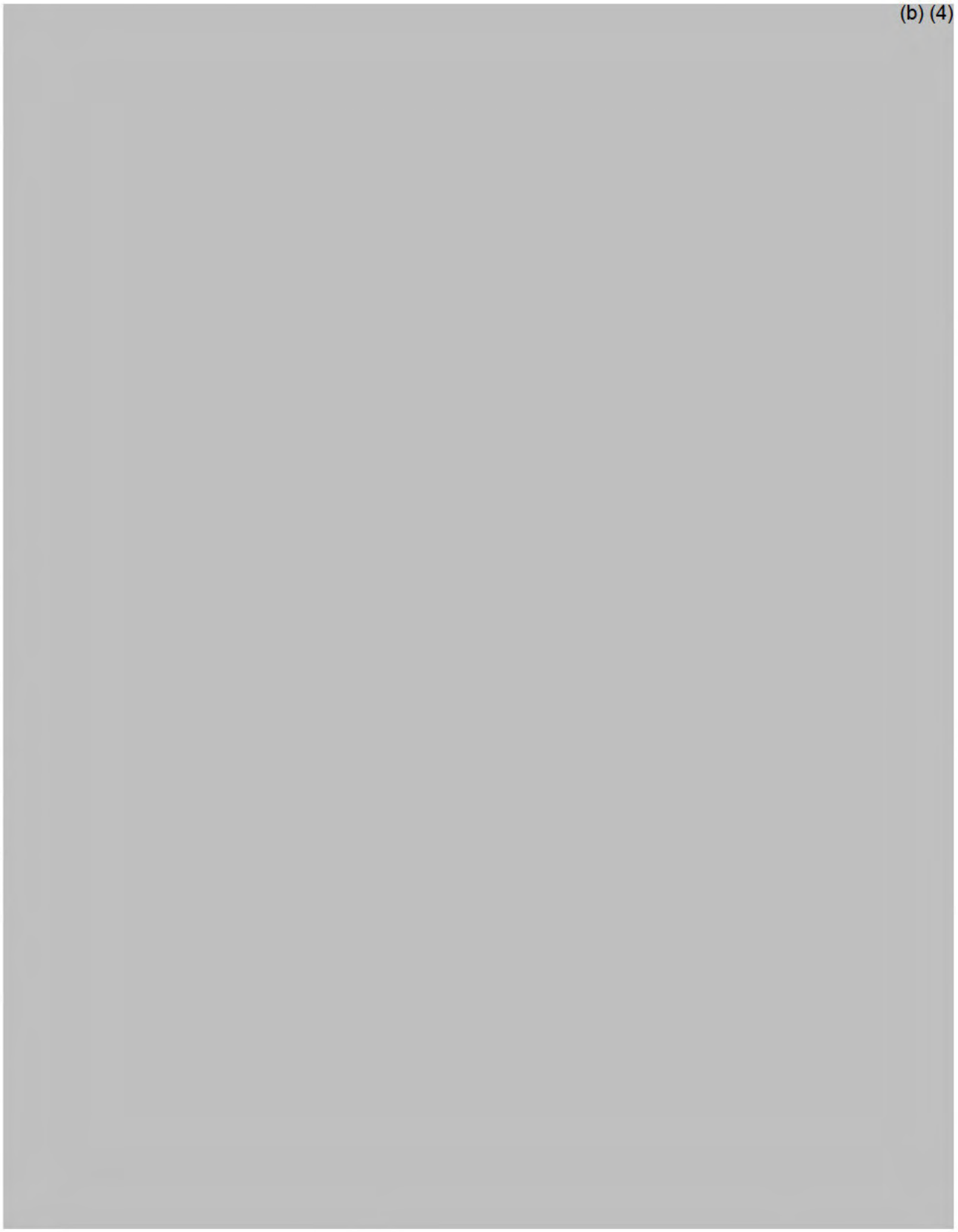
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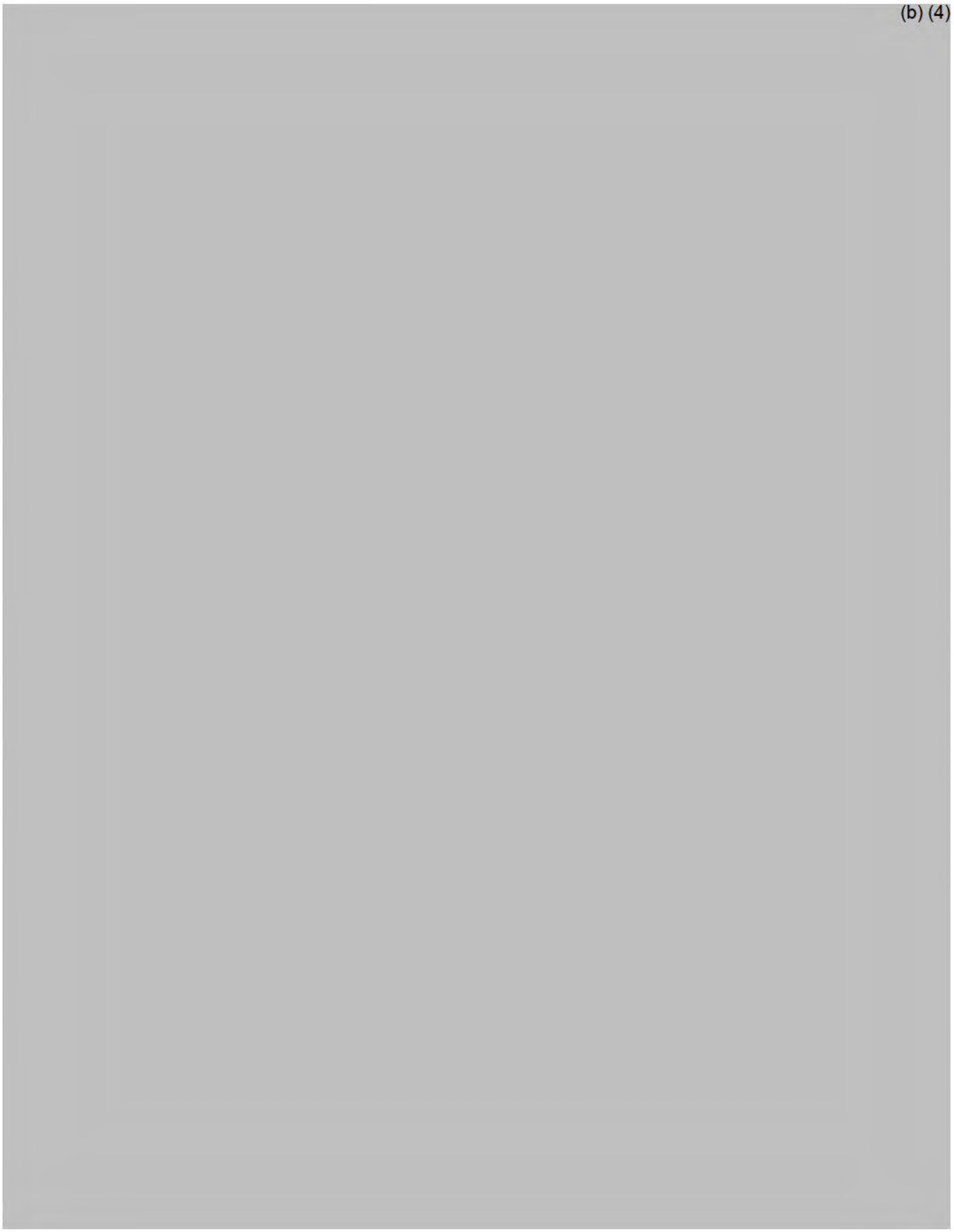
(b) (4)

Appendix 21. Assay validation and verification for xylanase in product activity assay. **CONFIDENTIAL**

*****Also add the verification report below at end of Appendix 21******

(b) (4)

(b) (4)

VERIFICATION OF ANALYTICAL METHOD FOR MEASURING XYLANASE ACTIVITY IN XYLAMAX FEED ADDITIVE

Experiment number:(b) (4)

FINAL REPORT

Revision number: 0

Date: 15th March 2021

RESEARCH ACTIVITY CONTRACTED WITH:

BioResources International
4222 Emperor Boulevard, Suite 460,
Durham NC 27703 (USA)

Study monitors: Rasha Qudsieh and Ching-Sung Tsai

Study director: (b) (6)

CONFIDENTIAL

(b) (4)

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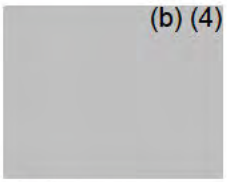
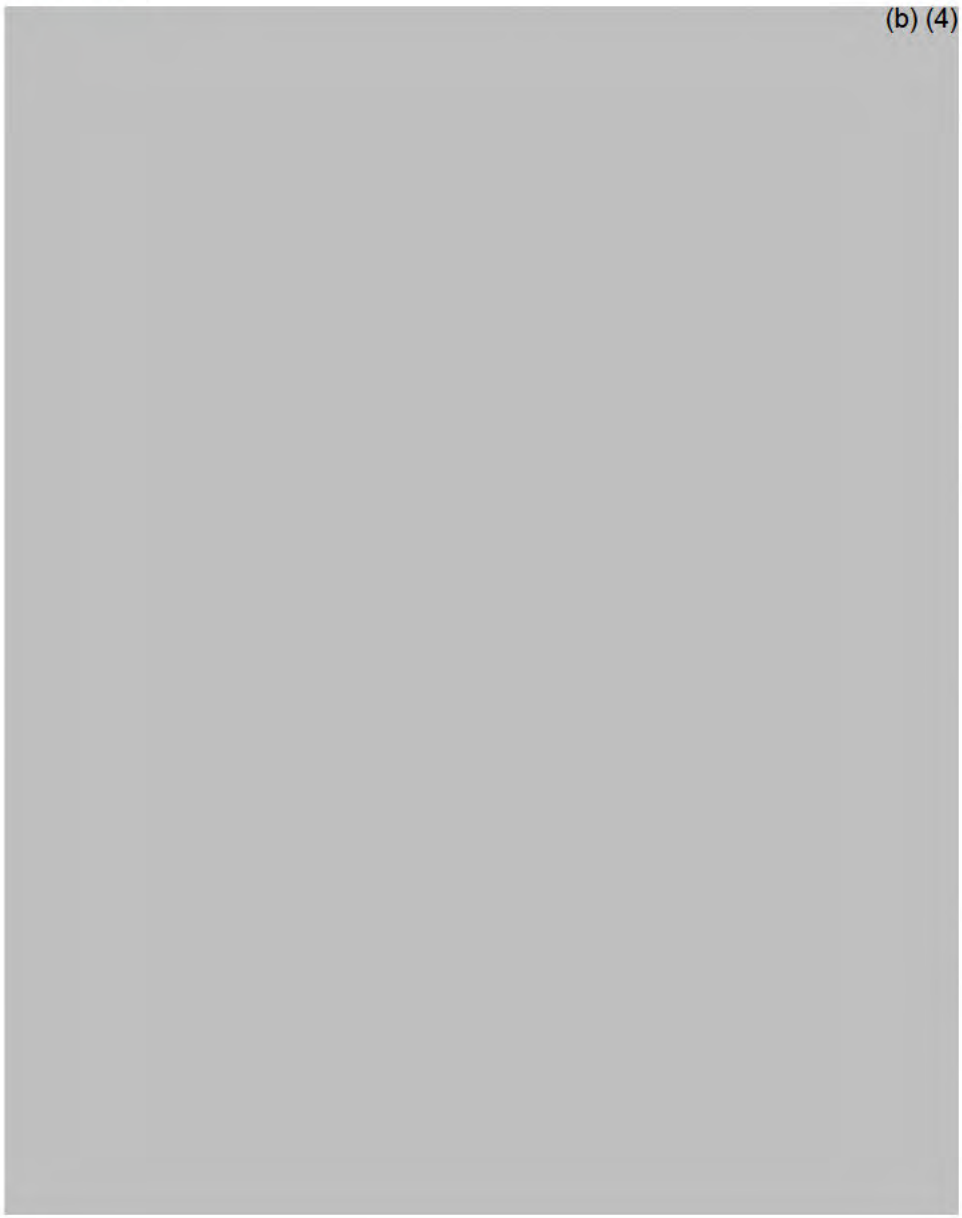


SUMMARY



(b) (4)

RESPONSIBILITIES

R&D

Study Director

(b) (4), (b) (6)

Study monitors

Rasha Qudsieh and Ching-Sung Tsai
BioResources International
4222 Emperor Boulevard, Suite 460,
Durham NC 27703 (USA)
(b) (4)

Analytical Laboratory

(b) (4), (b) (6)

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(b) (4)

OBJECTIVE

(b) (4)

QUALITY ASSURANCE

(b) (4)

(b) (4)

(b) (4)

DEFINITIONS

(b) (4)

(b) (4)

(b) (4)

(b) (4)

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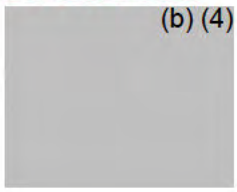

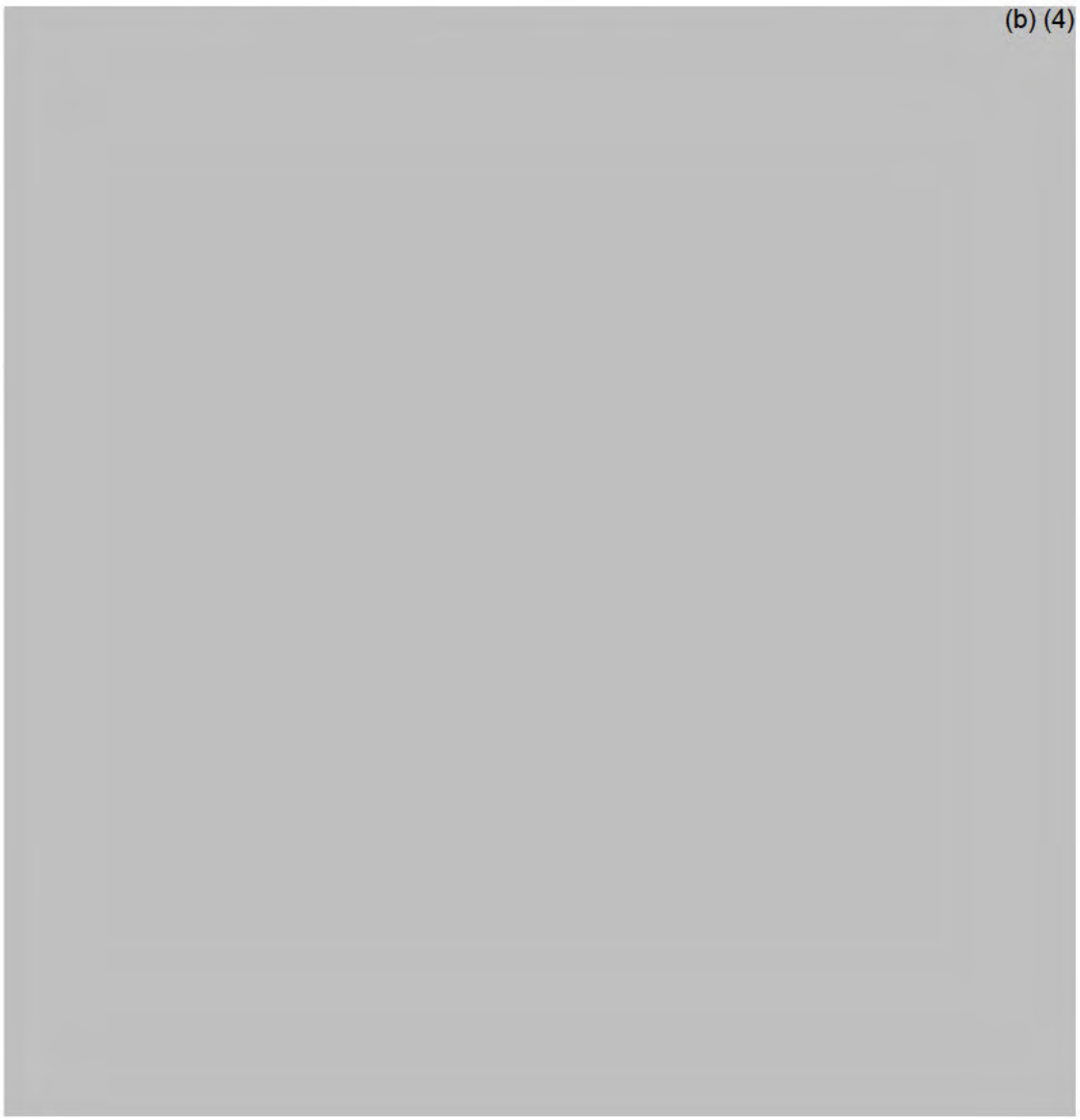

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

RESULTS AND DISCUSSION

(b) (4)

Parameter	Results of verification test
Repeatability (known sample)	(b) (4)
Intermediate precision (known sample)	
Reproducibility (unknown sample)	
Quadratic curve regression coefficient	
Range of applicability	
Recovery (%)	
LOD (limit of detection)	
LOQ (limit of quantification)	

Repeatability, intermediate precision, and reproducibility

(b) (4)

(b) (4)

(b) (4)

Table 2: Results of xylanase activity found in the evaluation of repeatability and intermediate precision of the analytical method under study.

Sample:	(b) (4)	
Curve:	(b) (4)	(b) (4)
Subsample	Results day 1 (XU/g)	Results day 2 (XU/g)
1	(b) (4)	(b) (4)
2		
3		
4		
5		
6		
Mean		
Std.dev.		
RSD_r (%)		
RSD_R (%)		

Table 3: Results of xylanase activity found in the blind sample of feed additive for evaluation of reproducibility.

Sample	(b) (4)
Curve:	(b) (4)
Subsample	Results (XU/g)
1	(b) (4)
2	
3	
Mean	
Std.dev.	
RSD_r (%)	

(b) (4)

(b) (4)

(b) (4)

(b) (4)

Range, linearity, and recovery

(b) (4)

Limits of detection (LOD) and quantification (LOQ)

(b) (4)

(b) (4)

Table 4: Results of xylanase activity found in the blank sample of feed additive for LOD and LOQ calculation.

Sample	(b) (4)	
Curve:	(b) (4)	(b) (4)
Subsample	Results day 1 (XU/g)	Results day 2 (XU/g)
1	(b) (4)	(b) (4)
2		
3		
Mean		
Std.dev.		
Average Std.dev.	(b) (4)	
LOD (XU/g)		
LOQ (XU/g)		

CONFIDENTIAL

(b) (4)

CONCLUSION

(b) (4)

REFERENCES

(b) (4)

Date: Date:

Attachment A--Addendum to Appendix 22

Appendix 22. Assay validation and verification for xylanase in-feed activity assay **CONFIDENTIAL**

***** Add the verification report below at end of Appendix 22*****

(b) (4)

VERIFICATION OF ANALYTICAL METHOD FOR MEASURING XYLANASE ACTIVITY IN FEEDS

Experiment number: (b) (4)

FINAL REPORT
Revision number: 0
Date: 19th March 2021

RESEARCH ACTIVITY CONTRACTED WITH:

BioResources International
4222 Emperor Boulevard, Suite 460,
Durham NC 27703 (USA)

Study monitors: Rasha Qudsieh and Ching-Sung Tsai

Study director: (b) (6)

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(b) (4)

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(b) (4)

SUMMARY

(b) (4)

(b) (4)

(b) (4)

(b) (4)

RESPONSIBILITIES

R&D

Study Director

(b) (4), (b) (6)

Study monitors

BioResources International
Rasha Qudsieh and Ching-Sung Tsai
4222 Emperor Boulevard, Suite 460,
Durham NC 27703 (USA)
(b) (4)

Analytical Laboratory

(b) (4), (b) (6)

CONFIDENTIAL

(b) (4)

OBJECTIVE

(b) (4)

QUALITY ASSURANCE

(b) (4)

(b) (4)

CONFIDENTIAL

(b) (4)

DEFINITIONS

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

RESULTS AND DISCUSSION

(b) (4)

Parameter	Results of verification test
Repeatability (known sample)	(b) (4)
Intermediate precision (known sample)	
Reproducibility (unknown sample)	
Quadratic curve regression coefficient	
Range of applicability	
Recovery (%)	
LOD (limit of detection)	
LOQ (limit of quantification)	

(b) (4)

Repeatability, intermediate precision, and reproducibility

(b) (4)

(b) (4)

Table 2. Results of xylanase activity found in the evaluation of repeatability and intermediate precision of the analytical method under study.

Sample:	(b) (4))	
Curve:	(b) (4)	(b) (4)
Subsample	Results day 1 (XU/g)	Results day 2 (XU/g)
1		(b) (4)
2		
3		
4		
5		
6		
Mean		
Std.dev.		
RSD_r (%)		
RSD_R (%)		

(b) (4)

Table 3. Results of xylanase activity found in the blind sample of feed for evaluation of reproducibility.

(b) (4)		
Curve:	(b) (4)	
Subsample	Results (XU/g)	
1	(b) (4)	
2		
3		
Mean		
Std.dev.		
RSD$_r$ (%)		

(b) (4)

(b) (4)

(b) (4)

(b) (4)

Table 4. Results of xylanase activity found in the blank sample of feed sample for LOD and LOQ calculation.

Sample:	(b) (4)	
Curve:	(b) (4)	(b) (4)
Subsample	Results day 1 (XU/g)	Results day 2 (XU/g)
1		(b) (4)
2		
3		
Mean		
Std.dev.		
Average Std.dev.		
LOD (XU/g)		
LOQ (XU/g)		

CONCLUSION

(b) (4)

REFERENCES

(b) (4)

CONFIDENTIAL

(b) (4)

(b) (4)

Signatures:

(b) (4), (b) (6)

(b) (4)

BioResource International Inc. - QC Department

(b) (4), (b) (6)

ANALYTICAL REPORT

(b) (4)

Report Supersedes (b) (4)

Client Code: (b) (4)
PO#: 5358

Received On: 10Aug2020
Reported On: 27Jul2021

(b) (4)

Client Sample Code: (b) (4)

Sample Registration Date: 10Aug2020
Condition Upon Receipt: acceptable, 22.7°C

Sample Description: Feed Enzyme-XYc

Sample Reference: Composite Lot#1

FSMRB - Mycotoxins

Reference
Anal.Bioanal.Chem. (2012) 402:2675-2686

Accreditation
ISO/IEC 17025:2017
A2LA 2918.01

Completed
26Aug2020

Sub
5

Parameter	Result
Aflatoxin B1	(b) (4)
Aflatoxin B2	
Aflatoxin G1	
Aflatoxin G2	
Aflatoxin M1	
Aflatoxin M2	
Deoxynivalenol	
Fumonisin B1	
Fumonisin B2	
HT-2 Toxin	
Ochratoxin A	
T-2 Toxin	
Zearalenone	

GU027 - PCR Qualitative-FMV 34S Promoter

Reference
Internal, real time PCR

Accreditation
ISO/IEC 17025:2017
A2LA 1940.01

Completed
28Aug2020

Sub
1

Parameter	Result
PCR Qualitative-FMV 34S Promoter	(b) (4)

GU103 - PCR Qualitative-CaMV 35S Promoter

Reference
Internal, real time PCR

Accreditation
ISO/IEC 17025:2017
A2LA 1940.01

Completed
28Aug2020

Sub
1

Parameter	Result
PCR Qualitative-CaMV 35S Promoter	(b) (4)

7/27/21 2:02 pm

BioResource International Inc. - QC Department

(b) (4), (b) (6)

ANALYTICAL REPORT

(b) (4)

Report Supersedes (b) (4)

Client Code: (b) (4)
PO#: 5358

Received On: 10Aug2020
Reported On: 27Jul2021

(b) (4)

Client Sample Code: (b) (4)

Sample Description: Feed Enzyme-XYc

Sample Registration Date: 10Aug2020
Condition Upon Receipt: acceptable, 22.7°C

Sample Reference: Composite Lot#1

GU105 - PCR Qualitative-NOS Terminator

Reference
Internal, real time PCR

Accreditation
ISO/IEC 17025:2017
A2LA 1940.01

Completed
28Aug2020

Sub
1

Parameter	Result
PCR Qualitative-NOS Terminator	(b) (4)

QA101 - Aflatoxin B1 B2 G1 G2 (LC-MSMS)

Reference
AOAC 999.07 Modified

Accreditation
ISO/IEC 17025:2017
A2LA 2993.01

Completed
27Aug2020

Sub
2

Parameter	Result
Aflatoxin B1	(b) (4)
Adjusted LOQ for this matrix.	
Aflatoxin B2	
Aflatoxin G1	
Aflatoxin G2	
Aflatoxins total	
Adjusted LOQ for this matrix.	

QAA07 - Vomitoxin (Deoxynivalenol, DON) LC-MSMS

Reference
Food Addit Contam Part A, 2013:30(3),541-9.

Accreditation
A2LA ISO/IEC 17025:2005 2993-01

Completed
27Aug2020

Sub
2

Parameter	Result
Vomitoxin (Deoxynivalenol)	(b) (4)

QL005 - Dioxins and Furans: PCDD/F (17 Congeners)

Reference
EPA 1613B October 1994

Completed
19Jul2021

Sub
3

Parameter	Result
2,3,7,8-TetraCDD	(b) (4)
2,3,7,8-TetraCDF	
1,2,3,7,8-PentaCDD	
1,2,3,7,8-PentaCDF	
2,3,4,7,8-PentaCDF	
1,2,3,4,7,8-HexaCDD	
1,2,3,6,7,8-HexaCDD	
1,2,3,7,8,9-HexaCDD	
1,2,3,4,7,8-HexaCDF	
1,2,3,6,7,8-HexaCDF	
1,2,3,7,8,9-HexaCDF	
2,3,4,6,7,8-HexaCDF	
1,2,3,4,6,7,8-HeptaCDD	

7/27/21 2:02 pm

BioResource International Inc. - QC Department

(b) (4), (b) (6)

ANALYTICAL REPORT

(b) (4)

Report Supersedes (b) (4)

Client Code: (b) (4)
PO#: 5358

Received On: 10Aug2020
Reported On: 27Jul2021

(b) (4)

Client Sample Code: (b) (4)

Sample Description: Feed Enzyme-XYc

Sample Registration Date: 10Aug2020
Condition Upon Receipt: acceptable, 22.7°C

Sample Reference: Composite Lot#1

QL005 - Dioxins and Furans: PCDD/F (17 Congeners)

Reference: EPA 1613B October 1994

Completed: 19Jul2021 Sub: 3

Parameter	Result
1,2,3,4,6,7,8-HeptaCDF	(b) (4)
1,2,3,4,7,8,9-HeptaCDF	
OctaCDD	
OctaCDF	
WHO(2005)-PCDD/F TEQ (lower-bound)	
WHO(2005)-PCDD/F TEQ (upper-bound)	

QL006 - Dioxin-like PCBs (12 WHO-PCBs)

Reference: EPA 1668 mod.

Completed: 19Jul2021 Sub: 3

Parameter	Result
PCB 77	(b) (4)
PCB 81	
PCB 105	
PCB 114	
PCB 118	
PCB 123	
PCB 126	
PCB 156	
PCB 157	
PCB 167	
PCB 169	
PCB 189	
WHO(2005)-PCB TEQ (lower-bound)	
WHO(2005)-PCB TEQ (upper-bound)	

QL007 - WHO-PCDD/F+PCB TEQ

Reference: EPA 1613B Modified

Completed: 19Jul2021 Sub: 3

Parameter	Result
WHO(2005)-PCDD/F+PCB TEQ (lower-bound)	(b) (4)
WHO(2005)-PCDD/F+PCB TEQ (upper-bound)	

QL008 - PCB ~6 ICES

Reference: EPA 1668 mod.

Completed: 05Sep2020 Sub: 3

Parameter	Result
PCB 28	(b) (4)
PCB 52	

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BioResource International Inc. - QC Department

(b) (4), (b) (6)

ANALYTICAL REPORT

(b) (4)

Report Supersedes (b) (4)

Client Code: (b) (4)
PO#: 5358

Received On: 10Aug2020
Reported On: 27Jul2021

(b) (4)

Client Sample Code: (b) (4)

Sample Description: Feed Enzyme-XYc

Sample Registration Date: 10Aug2020
Condition Upon Receipt: acceptable, 22.7°C

Sample Reference: Composite Lot#1

QL008 - PCB ~6 ICES

Reference EPA 1668 mod.

Completed 05Sep2020 **Sub** 3

Parameter	Result
PCB 101	(b) (4)
PCB 138	
PCB 153	
PCB 180	

TK015 - Arsenic (As) in Foods by ICP-MS

Reference AOAC 2013.06

Accreditation ISO/IEC 17025:2017 A2LA 4204.01

Completed 21Aug2020 **Sub** 4

Parameter	Result
Arsenic (As)	(b) (4)

TK024 - Cadmium (Cd) in Foods by ICP-MS

Reference AOAC 2013.06

Accreditation ISO/IEC 17025:2017 A2LA 4204.01

Completed 21Aug2020 **Sub** 4

Parameter	Result
Cadmium (Cd)	(b) (4)

TK048 - Mercury (Hg) in Foods by ICP-MS

Reference AOAC 2013.06

Accreditation ISO/IEC 17025:2017 A2LA 4204.01

Completed 21Aug2020 **Sub** 4

Parameter	Result
Mercury	(b) (4)

TK082 - Lead (Pb) in Foods by ICP-MS

Reference AOAC 2013.06

Accreditation ISO/IEC 17025:2017 A2LA 4204.01

Completed 21Aug2020 **Sub** 4

Parameter	Result
Lead (Pb)	(b) (4)

UM4BV - Yeast - FDA BAM Chapter 18 mod.

Reference FDA BAM Chapter 18 mod.

Accreditation A2LA ISO/IEC 17025:2005 3329.04

Completed 15Aug2020

Parameter	Result
Yeast	(b) (4)
Moulds	(b) (4)

7/27/21 2:02 pm

BioResource International Inc. - QC Department

(b) (4), (b) (6)

ANALYTICAL REPORT

(b) (4)

Report Supersedes (b) (4)

Client Code: (b) (4)
PO#: 5358

Received On: 10Aug2020
Reported On: 27Jul2021

(b) (4)
Client Sample Code: (b) (4)

Sample Description: Feed Enzyme-XYc

Sample Registration Date: 10Aug2020
Condition Upon Receipt: acceptable, 22.7°C

Sample Reference: Composite Lot#1

UMDTC - Salmonella spp. - AOAC-RI 121501

Reference
AOAC-RI 121501

Accreditation
ISO/IEC 17025:2017
A2LA 3329.04

Completed
11Aug2020

Parameter	Result
Salmonella	Not Detected per 25 g

UMJC3 - Total Coliforms - BAM Chapter 4

Reference
FDA BAM Chapter 4

Completed
11Aug2020

Parameter	Result
Coliforms	(b) (4)
Escherichia coli	(b) (4)

UMPD7 - Aerobic Plate Count - BAM Chapter 3

Reference
FDA BAM Chapter 3

Completed
12Aug2020

Parameter	Result
Aerobic Plate Count	(b) (4)

Comments:
Third version created to update WHO values (b) (4)

(b) (4)

Respectfully Submitted,

(b) (6)

Quality Specialist

7/27/21 2:02 pm

BioResource International Inc. - QC Department

(b) (4), (b) (6)

ANALYTICAL REPORT

(b) (4)

Report Supersedes (b) (4)

Client Code: (b) (4)
PO#: 5358

Received On: 10Aug2020
Reported On: 27Jul2021

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7/27/21 2:02 pm

(b) (4)

(b) (4)

BioResource International Inc. - QC Department

(b) (4), (b) (6)

ANALYTICAL REPORT

(b) (4)

Report Supersedes (b) (4)

Client Code: (b) (4)
PO#: 5358

Received On: 10Aug2020
Reported On: 27Jul2021

(b) (4)

Client Sample Code: (b) (4)

Sample Description: Feed Enzyme-XYc

Sample Registration Date: 10Aug2020
Condition Upon Receipt: acceptable, 22.7°C

Sample Reference: Composite Lot#2

FSMRB - Mycotoxins

Reference
Anal.Bioanal.Chem. (2012) 402:2675-2686

Accreditation
ISO/IEC 17025:2017
A2LA 2918.01

Completed 26Aug2020

Sub 5

Parameter	Result
Aflatoxin B1	(b) (4)
Aflatoxin B2	
Aflatoxin G1	
Aflatoxin G2	
Aflatoxin M1	
Aflatoxin M2	
Deoxynivalenol	
Fumonisin B1	
Fumonisin B2	
HT-2 Toxin	
Ochratoxin A	
T-2 Toxin	
Zearalenone	

GU027 - PCR Qualitative-FMV 34S Promoter

Reference
Internal, real time PCR

Accreditation
ISO/IEC 17025:2017
A2LA 1940.01

Completed 28Aug2020

Sub 1

Parameter	Result
PCR Qualitative-FMV 34S Promoter	(b) (4)

GU103 - PCR Qualitative-CaMV 35S Promoter

Reference
Internal, real time PCR

Accreditation
ISO/IEC 17025:2017
A2LA 1940.01

Completed 28Aug2020

Sub 1

Parameter	Result
PCR Qualitative-CaMV 35S Promoter	(b) (4)

7/27/21 2:01 pm

BioResource International Inc. - QC Department

(b) (4), (b) (6)

ANALYTICAL REPORT

(b) (4)

Report Supersedes (b) (4)

Client Code: (b) (4)
PO#: 5358

Received On: 10Aug2020
Reported On: 27Jul2021

(b) (4)

Client Sample Code: (b) (4)

Sample Description: Feed Enzyme-XYc

Sample Registration Date: 10Aug2020
Condition Upon Receipt: acceptable, 22.7°C

Sample Reference: Composite Lot#2

GU105 - PCR Qualitative-NOS Terminator

Reference	Accreditation	Completed	Sub
Internal, real time PCR	ISO/IEC 17025:2017 A2LA 1940.01	28Aug2020	1

Parameter	Result
PCR Qualitative-NOS Terminator	Negative

QA101 - Aflatoxin B1 B2 G1 G2 (LC-MSMS)

Reference	Accreditation	Completed	Sub
AOAC 999.07 Modified	ISO/IEC 17025:2017 A2LA 2993.01	27Aug2020	2

Parameter	Result
Aflatoxin B1	(b) (4)
Adjusted LOQ for this matrix.	
Aflatoxin B2	
Aflatoxin G1	
Aflatoxin G2	
Aflatoxins total	
Adjusted LOQ for this matrix.	

QAA07 - Vomitoxin (Deoxynivalenol, DON) LC-MSMS

Reference	Accreditation	Completed	Sub
Food Addit Contam Part A, 2013:30(3),541-9.	A2LA ISO/IEC 17025:2005 2993-01	27Aug2020	2

Parameter	Result
Vomitoxin (Deoxynivalenol)	(b) (4)

QL005 - Dioxins and Furans: PCDD/F (17 Congeners)

Reference	Completed	Sub
EPA 1613B October 1994	19Jul2021	3

Parameter	Result
2,3,7,8-TetraCDD	(b) (4)
2,3,7,8-TetraCDF	
1,2,3,7,8-PentaCDD	
1,2,3,7,8-PentaCDF	
2,3,4,7,8-PentaCDF	
1,2,3,4,7,8-HexaCDD	
1,2,3,6,7,8-HexaCDD	
1,2,3,7,8,9-HexaCDD	
1,2,3,4,7,8-HexaCDF	
1,2,3,6,7,8-HexaCDF	
1,2,3,7,8,9-HexaCDF	
2,3,4,6,7,8-HexaCDF	
1,2,3,4,6,7,8-HeptaCDD	

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BioResource International Inc. - QC Department

(b) (4), (b) (6)

ANALYTICAL REPORT

(b) (4)

Report Supersedes (b) (4)

Client Code: (b) (4)4
PO#: 5358

Received On: 10Aug2020
Reported On: 27Jul2021

(b) (4)

Client Sample Code: (b) (4)

Sample Registration Date: 10Aug2020
Condition Upon Receipt: acceptable, 22.7°C

Sample Description: Feed Enzyme-XYc

Sample Reference: Composite Lot#2

QL005 - Dioxins and Furans: PCDD/F (17 Congeners)

Reference: EPA 1613B October 1994

Completed: 19Jul2021 Sub: 3

Parameter	Result
1,2,3,4,6,7,8-HeptaCDF	(b) (4)
1,2,3,4,7,8,9-HeptaCDF	
OctaCDD	
OctaCDF	
WHO(2005)-PCDD/F TEQ (lower-bound)	
WHO(2005)-PCDD/F TEQ (upper-bound)	

QL006 - Dioxin-like PCBs (12 WHO-PCBs)

Reference: EPA 1668 mod.

Completed: 19Jul2021 Sub: 3

Parameter	Result
PCB 77	(b) (4)
PCB 81	
PCB 105	
PCB 114	
PCB 118	
PCB 123	
PCB 126	
PCB 156	
PCB 157	
PCB 167	
PCB 169	
PCB 189	
WHO(2005)-PCB TEQ (lower-bound)	
WHO(2005)-PCB TEQ (upper-bound)	

QL007 - WHO-PCDD/F+PCB TEQ

Reference: EPA 1613B Modified

Completed: 19Jul2021 Sub: 3

Parameter	Result
WHO(2005)-PCDD/F+PCB TEQ (lower-bound)	(b) (4)
WHO(2005)-PCDD/F+PCB TEQ (upper-bound)	

QL008 - PCB ~6 ICES

Reference: EPA 1668 mod.

Completed: 05Sep2020 Sub: 3

Parameter	Result
PCB 28	(b) (4)
PCB 52	

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BioResource International Inc. - QC Department

(b) (4), (b) (6)

ANALYTICAL REPORT

(b) (4)

Report Supersedes (b) (4)

Client Code: (b) (4)
PO#: 5358

Received On: 10Aug2020
Reported On: 27Jul2021

(b) (4)

Client Sample Code: (b) (4)

Sample Description: Feed Enzyme-XYc

Sample Registration Date: 10Aug2020
Condition Upon Receipt: acceptable, 22.7°C

Sample Reference: Composite Lot#2

QL008 - PCB ~6 ICES

Reference: EPA 1668 mod.

Completed: 05Sep2020
Sub: 3

Parameter	Result
PCB 101	(b) (4)
PCB 138	
PCB 153	
PCB 180	

TK015 - Arsenic (As) in Foods by ICP-MS

Reference: AOAC 2013.06

Accreditation: ISO/IEC 17025:2017 A2LA 4204.01

Completed: 21Aug2020
Sub: 4

Parameter	Result
Arsenic (As)	0.292 mg/kg

TK024 - Cadmium (Cd) in Foods by ICP-MS

Reference: AOAC 2013.06

Accreditation: ISO/IEC 17025:2017 A2LA 4204.01

Completed: 21Aug2020
Sub: 4

Parameter	Result
Cadmium (Cd)	(b) (4)

TK048 - Mercury (Hg) in Foods by ICP-MS

Reference: AOAC 2013.06

Accreditation: ISO/IEC 17025:2017 A2LA 4204.01

Completed: 21Aug2020
Sub: 4

Parameter	Result
Mercury	(b) (4)

TK082 - Lead (Pb) in Foods by ICP-MS

Reference: AOAC 2013.06

Accreditation: ISO/IEC 17025:2017 A2LA 4204.01

Completed: 21Aug2020
Sub: 4

Parameter	Result
Lead (Pb)	(b) (4)

UM4BV - Yeast - FDA BAM Chapter 18 mod.

Reference: FDA BAM Chapter 18 mod.

Accreditation: A2LA ISO/IEC 17025:2005 3329.04

Completed: 15Aug2020

Parameter	Result
Yeast	(b) (4)
Moulds	(b) (4)

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(b) (4), (b) (6)

ANALYTICAL REPORT

(b) (4)

Report Supersedes (b) (4)

Client Code: (b) (4)

Received On: 10Aug2020
Reported On: 27Jul2021

(b) (4)
Client Sample Code: (b) (4)

Sample Description: Feed Enzyme-XYc

Sample Registration Date: 10Aug2020
Condition Upon Receipt: acceptable, 22.7°C

Sample Reference: Composite Lot#2

UMDTC - Salmonella spp. - AOAC-RI 121501

Reference
AOAC-RI 121501

Accreditation
ISO/IEC 17025:2017
A2LA 3329.04

Completed
11Aug2020

Parameter	Result
Salmonella	Not Detected per 25 g

UMJC3 - Total Coliforms - BAM Chapter 4

Reference
FDA BAM Chapter 4

Completed
11Aug2020

Parameter	Result
Coliforms	(b) (4)/g
Escherichia coli	(b) (4)

UMPD7 - Aerobic Plate Count - BAM Chapter 3

Reference
FDA BAM Chapter 3

Completed
12Aug2020

Parameter	Result
Aerobic Plate Count	(b) (4)

Comments:
Third version created to update WHO values. (b) (4)

(b) (4)

Respectfully Submitted,

(b) (6)

Quality Specialist

7/27/21 2:01 pm

BioResource International Inc. - QC Department

(b) (4), (b) (6)

ANALYTICAL REPORT

(b) (4)

Report Supersedes (b) (4)

Client Code: (b) (4)
PO#: 5358

Received On: 10Aug2020
Reported On: 27Jul2021

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(b) (4)

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ANALYTICAL REPORT

(b) (4)

Report Supersedes (b) (4)

Client Code: (b) (4)
PO#: 5358

Received On: 10Aug2020
Reported On: 27Jul2021

(b) (4)
Client Sample Code: (b) (4)
Sample Description: Feed Enzyme-XYc

Sample Registration Date: 10Aug2020
Condition Upon Receipt: acceptable, 22.7°C
Sample Reference: Composite Lot#3

FSMRB - Mycotoxins

Reference
Anal.Bioanal.Chem. (2012) 402:2675-2686

Accreditation
ISO/IEC 17025:2017 A2LA 2918.01

Completed 26Aug2020

Sub 5

Parameter	Result
Aflatoxin B1	(b) (4)
Aflatoxin B2	
Aflatoxin G1	
Aflatoxin G2	
Aflatoxin M1	
Aflatoxin M2	
Deoxynivalenol	
Fumonisin B1	
Fumonisin B2	
HT-2 Toxin	
Ochratoxin A	
T-2 Toxin	
Zearalenone	

GU027 - PCR Qualitative-FMV 34S Promoter

Reference
Internal, real time PCR

Accreditation
ISO/IEC 17025:2017 A2LA 1940.01

Completed 28Aug2020

Sub 1

Parameter	Result
PCR Qualitative-FMV 34S Promoter	(b) (4)

GU103 - PCR Qualitative-CaMV 35S Promoter

Reference
Internal, real time PCR

Accreditation
ISO/IEC 17025:2017 A2LA 1940.01

Completed 28Aug2020

Sub 1

Parameter	Result
PCR Qualitative-CaMV 35S Promoter	(b) (4)

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BioResource International Inc. - QC Department

(b) (4), (b) (6)

ANALYTICAL REPORT

(b) (4)

Report Supersedes (b) (4)

Client Code: (b) (4)
PO#: 5358

Received On: 10Aug2020
Reported On: 27Jul2021

(b) (4)
Client Sample Code: (b) (4)

Sample Description: Feed Enzyme-XYc

Sample Registration Date: 10Aug2020
Condition Upon Receipt: acceptable, 22.7°C

Sample Reference: Composite Lot#3

GU105 - PCR Qualitative-NOS Terminator	**Reference** Internal, real time PCR	**Accreditation** ISO/IEC 17025:2017 A2LA 1940.01	**Completed** 28Aug2020	**Sub** 1

Parameter	**Result**
PCR Qualitative-NOS Terminator	Negative

QA101 - Aflatoxin B1 B2 G1 G2 (LC-MSMS)	**Reference** AOAC 999.07 Modified	**Accreditation** ISO/IEC 17025:2017 A2LA 2993.01	**Completed** 27Aug2020	**Sub** 2

Parameter	**Result**
Aflatoxin B1	(b) (4)
Aflatoxin B2	
Aflatoxin G1	
Aflatoxin G2	
Aflatoxins total	

QAA07 - Vomitoxin (Deoxynivalenol, DON) LC-MSMS	**Reference** Food Addit Contam Part A, 2013:30(3),541-9.	**Accreditation** A2LA ISO/IEC 17025:2005 2993-01	**Completed** 27Aug2020	**Sub** 2

Parameter	**Result**
Vomitoxin (Deoxynivalenol)	(b) (4)

QL005 - Dioxins and Furans: PCDD/F (17 Congeners)	**Reference** EPA 1613B October 1994		**Completed** 19Jul2021	**Sub** 3

Parameter	**Result**
2,3,7,8-TetraCDD	(b) (4)
2,3,7,8-TetraCDF	
1,2,3,7,8-PentaCDD	
1,2,3,7,8-PentaCDF	
2,3,4,7,8-PentaCDF	
1,2,3,4,7,8-HexaCDD	
1,2,3,6,7,8-HexaCDD	
1,2,3,7,8,9-HexaCDD	
1,2,3,4,7,8-HexaCDF	
1,2,3,6,7,8-HexaCDF	
1,2,3,7,8,9-HexaCDF	
2,3,4,6,7,8-HexaCDF	
1,2,3,4,6,7,8-HeptaCDD	
1,2,3,4,6,7,8-HeptaCDF	
1,2,3,4,7,8,9-HeptaCDF	

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(b) (4), (b) (6)

ANALYTICAL REPORT

(b) (4)

Report Supersedes (b) (4)

Client Code: (b) (4)
PO#: 5358

Received On: 10Aug2020
Reported On: 27Jul2021

(b) (4)
Client Sample Code: (b) (4)
Sample Description: Feed Enzyme-XYc

Sample Registration Date: 10Aug2020
Condition Upon Receipt: acceptable, 22.7°C
Sample Reference: Composite Lot#3

QL005 - Dioxins and Furans: PCDD/F (17 Congeners)

Reference
EPA 1613B October 1994

Completed 19Jul2021
Sub 3

Parameter	Result
OctaCDD	(b) (4)
OctaCDF	
WHO(2005)-PCDD/F TEQ (lower-bound)	
WHO(2005)-PCDD/F TEQ (upper-bound)	

QL006 - Dioxin-like PCBs (12 WHO-PCBs)

Reference
EPA 1668 mod.

Completed 19Jul2021
Sub 3

Parameter	Result
PCB 77	(b) (4)
PCB 81	
PCB 105	
PCB 114	
PCB 118	
PCB 123	
PCB 126	
PCB 156	
PCB 157	
PCB 167	
PCB 169	
PCB 189	
WHO(2005)-PCB TEQ (lower-bound)	
WHO(2005)-PCB TEQ (upper-bound)	

QL007 - WHO-PCDD/F+PCB TEQ

Reference
EPA 1613B Modified

Completed 19Jul2021
Sub 3

Parameter	Result
WHO(2005)-PCDD/F+PCB TEQ (lower-bound)	(b) (4)
WHO(2005)-PCDD/F+PCB TEQ (upper-bound)	

QL008 - PCB ~6 ICES

Reference
EPA 1668 mod.

Completed 05Sep2020
Sub 3

Parameter	Result
PCB 28	(b) (4)
PCB 52	
PCB 101	
PCB 138	

BioResource International Inc. - QC Department

(b) (4), (b) (6)

ANALYTICAL REPORT

(b) (4)

Report Supersedes (b) (4)

Client Code: (b) (4)
PO#: 5358

Received On: 10Aug2020
Reported On: 27Jul2021

(b) (4)
Client Sample Code: (b) (4)
Sample Description: Feed Enzyme-XYc

Sample Registration Date: 10Aug2020
Condition Upon Receipt: acceptable, 22.7°C
Sample Reference: Composite Lot#3

QL008 - PCB ~6 ICES	**Reference** EPA 1668 mod.		**Completed** 05Sep2020	**Sub** 3
Parameter PCB 153 PCB 180	**Result** (b) (4)			
TK015 - Arsenic (As) in Foods by ICP-MS	**Reference** AOAC 2013.06	**Accreditation** ISO/IEC 17025:2017 A2LA 4204.01	**Completed** 21Aug2020	**Sub** 4
Parameter Arsenic (As)	**Result** (b) (4)			
TK024 - Cadmium (Cd) in Foods by ICP-MS	**Reference** AOAC 2013.06	**Accreditation** ISO/IEC 17025:2017 A2LA 4204.01	**Completed** 21Aug2020	**Sub** 4
Parameter Cadmium (Cd)	**Result** (b) (4)			
TK048 - Mercury (Hg) in Foods by ICP-MS	**Reference** AOAC 2013.06	**Accreditation** ISO/IEC 17025:2017 A2LA 4204.01	**Completed** 21Aug2020	**Sub** 4
Parameter Mercury	**Result** (b) (4)			
TK082 - Lead (Pb) in Foods by ICP-MS	**Reference** AOAC 2013.06	**Accreditation** ISO/IEC 17025:2017 A2LA 4204.01	**Completed** 21Aug2020	**Sub** 4
Parameter Lead (Pb)	**Result** (b) (4)			
UM4BV - Yeast - FDA BAM Chapter 18 mod.	**Reference** FDA BAM Chapter 18 mod.	**Accreditation** A2LA ISO/IEC 17025:2005 3329.04	**Completed** 15Aug2020	
Parameter Yeast	**Result** (b) (4)			
Parameter Moulds	**Result** (b) (4)			
UMDTC - Salmonella spp. - AOAC-RI 121501	**Reference** AOAC-RI 121501	**Accreditation** ISO/IEC 17025:2017 A2LA 3329.04	**Completed** 11Aug2020	

BioResource International Inc. - QC Department

(b) (4), (b) (6)

ANALYTICAL REPORT

(b) (4)

Report Supersedes (b) (4)

Client Code: (b) (4)
PO#: 5358

Received On: 10Aug2020
Reported On: 27Jul2021

(b) (4)

Client Sample Code: (b) (4)

Sample Description: Feed Enzyme-XYc

Sample Registration Date: 10Aug2020
Condition Upon Receipt: acceptable, 22.7°C

Sample Reference: Composite Lot#3

UMDTC - Salmonella spp. - AOAC-RI 121501

Reference
AOAC-RI 121501

Accreditation
ISO/IEC 17025:2017
A2LA 3329.04

Completed
11Aug2020

Parameter	Result
Salmonella	Not Detected per 25 g

UMJC3 - Total Coliforms - BAM Chapter 4

Reference
FDA BAM Chapter 4

Completed
11Aug2020

Parameter	Result
Coliforms	(b) (4)
Escherichia coli	(b) (4)

UMPD7 - Aerobic Plate Count - BAM Chapter 3

Reference
FDA BAM Chapter 3

Completed
12Aug2020

Parameter	Result
Aerobic Plate Count	(b) (4)

Comments:
Third version created to update WHO values. (b) (4)

(b) (4)

Respectfully Submitted,

(b) (4)

Quality Specialist

7/27/21 2:01 pm

BioResource International Inc. - QC Department

(b) (4), (b) (6)

ANALYTICAL REPORT

(b) (4)

Report Supersedes (b) (4)

Client Code: (b) (4)
PO#: 5358

Received On: 10Aug2020
Reported On: 27Jul2021

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7/27/21 2:01 pm

(b) (4)

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BioResource International, Inc.

(b) (4), (b) (6)

ANALYTICAL REPORT

(b) (4)

Report Supersedes (b) (4)

Client Code: (b) (4)

Received On: 04Aug2021
Reported On: 03Sep2021

(b) (4)

Client Sample Code: (b) (4)

Sample Description: Feed Additive

Sample Registration Date: 04Aug2021
Condition Upon Receipt: acceptable, 21.4°C

Sample Reference:

FS087 - Residual Ethanol and Methanol

Reference
GC-FID*

Completed
11Aug2021

Sub
1

Parameter	Result
Ethanol	(b) (4)
Isopropanol	
Methanol	

UMJNL - Enterobacteriaceae - CMMEF Chapter 9.62

Reference
CMMEF Chapter 9.62

Completed
05Aug2021

Parameter	Result
Enterobacteriaceae	(b) (4)

Comments:
*Liquid samples can be analyzed directly or diluted with water prior to analysis. Solid samples should be weighed and diluted to a volume similar to that of the standards. A mass of 0.1g to 1.0g may be necessary. With increased sample mass, a matrix efffect is more likely to interfere with spike recoveries. The amount of each residual alcohol is determined by comparing the signal of the unknown sample, measured by the gas chromatograph and FID, with the signal of reference standard solutions.

This amended report was created to update the reference method from "Internal method" to "GC-FID," as well as to add a brief description of the method per the client's request.

Subcontracting partners:
(b) (4)

9/3/21 1:02 pm

BioResource International, Inc.
(b) (4), (b) (6)

ANALYTICAL REPORT

(b) (4)
Report Supersedes (b) (4)

Client Code: (b) (4)

Received On: 04Aug2021
Reported On: 03Sep2021

Respectfully Submitted,

(b) (6)

(b) (6)
Technican III Group Leader

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9/3/21 1:02 pm

(b) (4)

(b) (4)

BioResource International, Inc.

(b) (4), (b) (6)

ANALYTICAL REPORT

(b) (4)

Report Supersedes (b) (4)

Client Code: (b) (4)

Received On: 04Aug2021
Reported On: 03Sep2021

(b) (4)

Client Sample Code: (b) (4)

Sample Registration Date: 04Aug2021
Condition Upon Receipt: acceptable, 21.4°C

Sample Description: Feed Additive

Sample Reference:

FS087 - Residual Ethanol and Methanol

Reference GC-FID*

Completed 11Aug2021

Sub 1

Parameter	Result
Ethanol	(b) (4)
Isopropanol	
Methanol	

UMJNL - Enterobacteriaceae - CMMEF Chapter 9.62

Reference CMMEF Chapter 9.62

Completed 05Aug2021

Parameter	Result
Enterobacteriaceae	(b) (4)

Comments:
*Liquid samples can be analyzed directly or diluted with water prior to analysis. Solid samples should be weighed and diluted to a volume similar to that of the standards. A mass of 0.1g to 1.0g may be necessary. With increased sample mass, a matrix efffect is more likely to interfere with spike recoveries. The amount of each residual alcohol is determined by comparing the signal of the unknown sample, measured by the gas chromatograph and FID, with the signal of reference standard solutions.

This amended report was created to update the reference method from "Internal method" to "GC-FID," as well as to add a brief description of the method per the client's request.

(b) (4)

9/3/21 1:02 pm

BioResource International, Inc.

(b) (4), (b) (6)

ANALYTICAL REPORT

Report Supersedes (b) (4) (b) (4)

Client Code: (b) (4)

Received On: 04Aug2021
Reported On: 03Sep2021

Respectfully Submitted,

(b) (4)

(b) (4)
Technican III Group Leader

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9/3/21 1:02 pm

(b) (4)

(b) (4)

BioResource International, Inc.
(b) (4), (b) (6)

ANALYTICAL REPORT

(b) (4)
Report Supersedes (b) (4)

Client Code: (b) (4)

Received On: 04Aug2021
Reported On: 03Sep2021

(b) (4)

Client Sample Code: (b) (4)
Sample Description: Feed Additive

Sample Registration Date: 04Aug2021
Condition Upon Receipt: acceptable, 21.4°C
Sample Reference:

FS087 - Residual Ethanol and Methanol

Reference: GC-FID*

Completed: 11Aug2021
Sub: 1

Parameter	
Ethanol	(b) (4)
Isopropanol	
Methanol	

UMJNL - Enterobacteriaceae - CMMEF Chapter 9.62

Reference: CMMEF Chapter 9.62

Completed: 05Aug2021

Parameter	Result
Enterobacteriaceae	(b) (4)

Comments:
*Liquid samples can be analyzed directly or diluted with water prior to analysis. Solid samples should be weighed and diluted to a volume similar to that of the standards. A mass of 0.1g to 1.0g may be necessary. With increased sample mass, a matrix efffect is more likely to interfere with spike recoveries. The amount of each residual alcohol is determined by comparing the signal of the unknown sample, measured by the gas chromatograph and FID, with the signal of reference standard solutions.

This amended report was created to update the reference method from "Internal method" to "GC-FID," as well as to add a brief description of the method per the client's request.

(b) (4)

9/3/21 1:02 pm

BioResource International, Inc.

(b) (4), (b) (6)

ANALYTICAL REPORT

(b) (4)

Report Supersedes (b) (4)

Client Code: (b) (4)

Received On: 04Aug2021
Reported On: 03Sep2021

Respectfully Submitted,

(b) (4)

(b) (4)
Technican III Group Leader

Results shown in this report relate solely to the item submitted for analysis. | Any opinions/interpretations expressed on this report are given independent of the laboratory's scope of accreditation. | All results are reported on an "As Received" basis unless otherwise stated. | Reports shall not be reproduced except in full without written [illegible] (b) (4) | All work done in accordance with (b) (4) General Terms and Conditions of Sale: [illegible] | √ Indicates a subcontract test to a different lab. Lab(s) are listed at end of the report. For further details [illegible] performing labs please contact your customer service contact at (b) (4) Measurement of uncertainty can be obtained upon request.

9/3/21 1:02 pm

(b) (4)

(b) (4)

BioResource International, Inc.

(b) (4), (b) (6)

ANALYTICAL REPORT

(b) (4)

Client Code: (b) (4)

Received On: 18Feb2021
Reported On: 01Mar2021

(b) (4)

Client Sample Code: (b)
Sample Description: Feed Additive

Sample Registration Date: 18Feb2021
Condition Upon Receipt: acceptable, 20.1°C
Sample Reference: XY18138

QA05L - Glycols (GC-MS)

Reference
ECAL Internal

Completed 01Mar2021
Sub 1

Parameter	Result
Diethylene glycol	(b) (4)
Ethylene glycol	
Propylene glycol	

(b) (4)

Respectfully Submitted,

(b) (6)

(b) (6)
Quality Specialist

Results shown in this report relate solely to the item submitted for analysis. | Any opinions/interpretations expressed on this report are given independent of the laboratory's scope of accreditation. | All results are reported on an "As Received" basis unless otherwise stated. | Reports shall not be reproduced except in full without written permission of (b) (4). | All work done in accordance with (b) (4) General Terms and Conditions of Sale: (b) (4) | √ Indicates a subcontract test to a different lab. Lab(s) are listed at end of the report. For further details about the performing labs please contact your customer service contact at (b) (4) Measurement of uncertainty can be obtained upon request.

3/1/21 7:54 pm

-

Safety Data Sheet

1. Identification

Product identifier	**Xylamax ®**
Other means of identification	None
Recommended use	Enzyme Feed Additive
Recommended restrictions	None known

Manufacturer/Importer/Supplier/Distributor Information

Company name	BioResource International, Inc.
Address	4222 Emperor Blvd, Suite 460 Durham, NC 27703 United States
Telephone	+1 (919)993-3389
Email	info@briworldwide.com
Contact person	Not available.
Emergency phone number	+1 (919)993-3389

2. Hazard(s) identification

Physical hazards	Not classified		
Health hazards	Sensitization, respiratory	Category 1	H334 – May cause allergy or asthma symptoms or breathing difficulties if inhaled

OSHA defined hazards Not classified

Label elements

Signal word Danger

Hazard statement **H334** – May cause allergy or asthma symptoms or breathing difficulties if inhaled.

Precautionary statements

Prevention	**P261** – Avoid breathing dust
Response	**P304 + P340** – If inhaled: If breathing is difficult, remove person to fresh air and keep comfortable for breathing
Storage	**P402** – Store in a dry place
Disposal	**P501** – Dispose of contents/ Container in accordance with local/regional/notional/international regulations

Hazard(s) not otherwise classified (HNOC) None known.

Supplemental information None.

3. Composition/information on ingredients

Substances

Chemical name	Common name and synonyms	CAS number	%
Pulverized limestone (CaCO3)		1317-65-3	70-90
Xylanase, endo-1, 4-		9025-57-4	10-30
Starch		9005-25-8	5-10

Composition comments

4.First-aid Measures

Inhalation

Move to fresh air. Get medical attention if irritation, allergic symptoms, or other symptoms develop and persist

Skin Contact

No first aid should be required. Wash with soap and water after handling. Seek medical attention if irritation or rash develops and persists.

Eye contact

In case of eye contact, flush eyes immediately with plenty of water. Remove contact lenses, if present and easy to do after the first 5 minutes. Continue rinsing. Seek medical attention if irritation persists

Ingestion

No first aid should be needed. Not intended for human consumption but no adverse effects are expected from ingestion.

Most important symptoms/effects, acute and delayed

Mild eye and skin irritation. Inhalation of dust from dried product may cause allergic respiratory reaction in sensitized individuals with symptoms of wheezing and difficulty breathing.

Indication of immediate medical attention and special treatment needed

Immediate medical attention is required for inhalation allergic reactions

General Information

Ensure that medical personnel are aware of the material(s) involved, and use precautions to protect themselves

5. Fire-fighting measures

Suitable extinguishing media

Use water spray, water fog, carbon dioxide, foam, or dry chemical. Water is most effective.

Specific hazards arising from the chemical

Dust generated in handling this material may present an inhalation hazard if suspended in air at high concentrations. Minimize the generation and accumulation of dust.

Special Protective equipment and precautions for firefighters

Firefighters should wear positive pressure self-contained breathing apparatus and protective gear.

Fire fighting equipment/instructions

In case of fire and/or explosion do not breath fumes. Move containers from fire area if you can do so without risk clothing to avoid contact

Specific methods

Use standards firefighting procedures and consider the hazards of other involved materials

General fire hazards

Avoid generating dust; fine dust dispersed in air in sufficient concentration, and in the presence of an ignition source is a potential dust explosion hazard

6. Accidental release measures

Personal precautions, protective equipment and emergency procedures	Keep unnecessary personnel away. Keep people away from and upwind of spill/leak. Use only non-sparking tools. Dust deposits should not be allowed to accumulate on surfaces, as these may form an explosive mixture if they are released into the atmosphere in sufficient concentration. Wear appropriate protective equipment and clothing during clean-up. Avoid inhalation of dust. Use a NIOSH/MSHA approved respirator if there is a risk of exposure to dust/fume at levels exceeding the exposure limits. Do not touch damaged containers or spill material unless wearing appropriate protective clothing. Ensure adequate ventilation. Local authorities should be advised if significant spillages cannot be contained. For personal protection, see section 8 of the SDS
Methods and materials for containment and cleaning up	Eliminate all ignition sources (no smoking, flares, sparks, or flames in immediate area). Take precautionary measures against static discharge. Use only non-sparking tools. Avoid dispersal of dust in the air (i.e. cleaning dust surfaces with compressed air). Minimize dust generation and accumulation. Collect dust using a vacuum cleaner equipped with HEPA filter. Stop the flow of material if this is without risk. Large spills: Wet down with water and dike for later disposal. Shovel the material into waste container. Absorb in vermiculite, dry sand or earth and place into containers. Following product recovery flush area with water. Small spills: Sweep up or vacuum up spillage and collect in suitable container for disposal. Wipe up with absorbent material (e.g. cloth, fleece). Clean surface thoroughly to remove residual contamination.
Environmental precaution	Never return spills to original containers for re-use. For waste disposal, see section 13 of the SDS. Avoid discharge into drains, water courses or onto the ground.

8. Exposure controls/Personal protection

Occupational exposure limits

US. OSHA Table Z-1 Limits for Air Contaminants (29 DFR 1910.1000)

Components	Type	Value	Form
Limestone (CAS 1317-65-3)	PEL/TWA as PNOC	5 mg/m3 to 15 mg/m3	Respirable fraction to Total dust
Xylanase, endo-1, 4- (CAS 9025-57-4)	PEL/TWA as PNOC	5 mg/m3 to 15 mg/m3	Respirable fraction to Total dust
Starch	PEL/TWA as PNOC	5 mg/m3 to 15 mg/m3	Respirable fraction to Total dust

Biological limit values	No biological exposure limits noted for the ingredient(s).
Exposure guidelines	If exposure limits have not been established, maintain airborne levels to an acceptable level.
Appropriate engineering controls	Explosion-proof general and local exhaust ventilation. Good general ventilation should be used. Ventilation rates should be matched to conditions. If applicable, use process enclosure, local exhaust ventilation, or other engineering controls to maintain airborne levels below recommended exposure limits. Eye wash facilities and emergency shower must be available when handling this product.

Individual protection measures, such as personal protective equipment	
Eye/face protection	Follow facility requirements. Safety glasses or dust goggles recommended to avoid eye contact.
Skin Protection **Hand protection**	Wear appropriate chemical resistant gloves. Suitable gloves can be recommended by the glove supplier.
Skin Protection **Other**	Wear appropriate chemical resistant clothing
Respiratory protection	Chemical respirator with organic vapor cartridge, full facepiece, dust and mist filter
Thermal hazards	Wear appropriate thermal protective clothing, when necessary
General hygiene considerations	When using, do not eat, drink, or smoke. Always observe good personal hygiene measures, such as washing after handling the material and before eating, drinking, and/or smoking. Routinely wash work clothing and protective equipment to remove contaminants.

9. Physical and chemical properties

Appearance	
Physical state	Solid.
Form	Powder.
Color	Grey
Odor	Neutral.
Odor Threshold	Not Available.
pH	Not Available.
Melting point/freezing point	Not Available.
Initial boiling point and boiling range	
Flash point	Not Available.
Evaporation rate	Not Available.
Flammability (solid, gas)	Not Available.
Upper/lower flammability or explosive limits	
Flammability Limit – lower (%)	Not Available.
Flammability Limit – upper (%)	Not Available.
Explosive limit – lower (%)	~100 g/m3 (fine dust
Explosive limit – upper (%)	Not Available.
Vapor pressure	Not Available.
Vapor density	Not Available.
Relative density	Not Available.
Solubility(ies)	
Solubility (water)	Not Available.
Partition coefficient (n-octanol/water)	Not Available.

Auto-ignition temperature	Not Available.
Decomposition temperature	Not Available.
Viscosity	Not Available.
Other information	
Explosive properties	No-data- Available.
Oxidizing properties	No-data- Available.

10. Stability and reactivity

Reactivity	The product is stable and non-reactive under normal condition of use, storage, anc transport.
Chemical stability	Stable under normal storage and handling conditions.
Possibility of hazardous reactions	No dangerous reaction known under conditions of normal use.
Conditions to avoid	Keep away from heat, sparks, and open flame. Minimize dust generation and accumulation. Contact with incompatible materials.
Incompatible materials	Strong oxidizing agents. Sensitive to moisture.
Hazardous decomposition products	Thermal decomposition will release oxides of carbon and nitrogen.

11. Toxicological information

Information on likely routes of exposure

Inhalation	May cause allergy or asthma symptoms or breathing difficulties in inhaled.
Skin contact	No adverse effects due to skin contact are expected.
Eye Contact	May cause mild irritation.
Ingestion	Not intended for human consumption but no adverse effects are expected from ingestion.
Symptoms related to the physical, chemical and toxicological characteristics	Coughing. Difficulty in breathing.

Information on toxicological effects

Acute toxicity	Not available.
Skin corrosion/irritation	Not classified.
Serious eye damage/eye irritation	Mild irritation possible.

Respiratory or skin sensitization

Respiratory sensitization	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
Skin sensitization	Not classified. Not classified.
Germ cell mutagenicity	May cause allergy or asthma symptoms or breathing difficulties if inhaled.

Carcinogenicity

IARC Monographs. Overall Evaluation of Carcinogenicity

Not listed.

NTP Report of Carcinogens

Not listed.

OSHA Specifically Regulated Substances (29 CFR 1910.1001-1050)

Not listed.

Reproductive toxicity	Not classified.
Specific target organ toxicity – single exposure	Not classified.
Specific target organ toxicity – repeat exposure	Not classified.

Aspiration hazard	Not classified.

12. Ecological information

Ecotoxicity	The product is not classified as environmentally hazardous. However, this does not exclude the possibility that large or frequent spills can have a harmful or damaging effect on the environment.
Persistence and degradability	No data is available on the degradability of this product.
Bioaccumulative Potential	No data available.
Mobility in soil	No data available.
Other adverse effects	None known.

13. Disposal considerations

Disposal Instructions	This product, if disposed as purchased would not meet the criteria of a RCRA hazardous waste.
Local disposal regulations	Dispose in accordance with all applicable regulations.
Hazardous waste code	The waste code should be assigned in discussion between the user, the producer, and the waste disposal company.
Waste from residues/unused products	Dispose of in accordance with local regulations. Empty containers or liners may retain some product residues. This material and its container must be disposed of in a safe manner (see: Disposal instructions).
Contaminated packaging	Since emptied containers may retain product residue, follow label warnings even after container is emptied. Empty containers should be taken to an approved waste handling site for recycling or disposal.

14. Transport information

DOT	Not regulated as dangerous goods.
IATA	Not regulated as dangerous goods.
IMDG	Not regulated as dangerous goods.
Transport in bulk according to Annex II of MARPOL 73/78 and the IBC code	Not applicable.

15. Regulatory information

US federal regulations The product is not hazardous under the criteria of the Federal OSHA Hazard Communication Standard (29 CFR 1910.1200).

TSCA Section 12(b) Export Notification (40 CFR 707, Subpt.D)
Not regulated

OSHA Specifically Regulated Substances (29 CFR 1910.1001-1050)
Not regulated

CERCLA Hazardous Substance List (40 CFR 302.4)
Not listed

Superfund Amendments and Reauthorization Act of 1986 (SARA)

Hazard Categories
Immediate Hazard – No
Delayed Hazard – No
Fire Hazard – No
Pressure Hazard – No
Reactivity – No

SARA 302 Extremely hazardous substance
Not listed.

SARA 313 (TRI reporting)
Not regulated
Other federal regulations
Clean Air Act (CAA) Section 112 Hazardous Air Pollutants (HAPs) List
Not Regulated.
Clean Air Act (CAA) Section 112(r) Accidental Release Prevention (40 CFR 68.130)
Not regulated.
Safe Drinking Water Act (SDWA) Not regulated.
US State regulations
US. Massachusetts RTK – substance List
Limestone (CAS 1317-65-3)
US. New Jersey Worker and Community Right-to-Know act
Limestone (CAS 1317-65-3)
US. Pennsylvania Worker and Community Right-to-Know Law
Limestone (CAS 1317-65-3)
US. Rhode Island RTK
Not regulated.
US. California Proposition 65
California Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65): This material is not known to contain any chemical currently listed as carcinogens or reproductive toxins.

16. Other information, including date of preparation or last revision

Issue date 12-March-2015
Revision date 18-January-2021
Version # 06
NFPA ratings

Disclaimer BioResource International, Inc. cannot anticipate all conditions under which this information and its product, or the products of other manufacturers in combination with its product, may be used. It is the user's responsibility to ensure safe conditions for handling, storage and disposal of the product, and to assume liability for loss, injury, damage or expense due to improper use. The information in the sheet was written based on the best knowledge and experience currently available.
Xylamax ® is a trademark of BioResource International, Inc. and are registered in the United States and other countries.

***** END OF APPENDIX 23 *****

Attachment B: AGRN 44—October 6, 2021
Utility

FDA Memo :

CVM: The notifier should expand its narrative to include relevant information supporting that the expressed protein has xylanase activity. A discussion to support the identity of the notified substance as a xylanase could include several different types of data and information, such as: enzyme kinetics, substrate specificity, enzyme specific activity, and quantification of the breakdown products from substrate hydrolysis.

The notifier states that it has concluded that the functionality of the notified xylanase does not relate to safety, but presents information in its notice concerning the mode of action of xylanases. Instead, the notifier's narrative needs to clearly explain how safety is not affected if the xylanase does not accomplish the stated effect in poultry and swine diets. This issue is particularly relevant because the notifier identifies xylan (substrate of xylanase action) as an antinutritional factor. The notifier should address why, if the enzyme fails to hydrolyze the xylan in diets formulated to contain xylanase, there are no adverse effects on the animal or its nutrition. Solely addressing the presence of xylan in feed would not address safety concerns when the notified substance is not functional. The focus of the narrative should be on how the notifier reached its conclusion that if the notified substance does not have its intended effect, there is no safety concern resulting from the lack of functionality. The notifier should re-write Section 2.5 to clearly address how a lack of enzyme functionality in xylanase containing diets would not impact animal safety rather than describe and support the mode of action of xylanase enzymes. The narrative should include citations to publicly available information and the scientific literature. CVM indicated that a discussion in one of the submitted references may be useful to the notifier in addressing this point.[5]

5 Although not identified in the meeting, the reference is: Flores et al. 2017. Evaluation of a thermotolerant xylanase on broiler growth performance and dietary ileal digestible energy value. J. Appl. Poult. Res. 26:60.

If the notifier has concluded that functionality is related safety, the utility section of the notice should be re-written and include use of traditional end points, such as digesta viscosity, to support functionality relying on publicly available information for the notified substance.

RESPONSE:

Section 2.5 is specific to the intended use of Xylanase and the notifiers determination that the functionality of xylanase is not related to safety.

The confirmation of identity GRAS substance, Xylanase enzyme product, is provided in section 2.1 of the GRAS conclusion., which also discusses the specificity of the enzyme preparation

(section 2.1.1). The activity of the Xylanase enzyme product is discussed in section 2.1.3 of the GRAS conclusion and the quantification of activity. Also, conformation of that the enzyme product is nearly identical to representative endo-β-1,4-xylanases is provided in section 2.1.2 of the GRAS conclusion. The GRAS conclusion includes a study (Duarte et al., 2019) that demonstrated that the use of the Xylanase enzyme product decreased disgesta viscosity which is one of the most accepted markers of in vitro activity of effective hemicelluloses, including Xylanase (AAFCO, 2021). The information within the GRAS conclusion, provides data that unequivocally demonstrates that the BioResource International enzyme preparation is an active endo-1,4-β-xylanase.

The intended use is described section 1.4 "BioResource International's Komagataella phaffii (P. pastoris) enzyme preparation (endo-1,4-β-xylanase or xylanase) is used for the hydrolysis of xylans , a component of hemicellulose in poultry and swine feed." The description of use (as found in section 2.5) states that the product will be used in nutritionally adequate feeds. The use will increase nutritional value from these feeds. In addition, this section discussed the fact that some consider non-starch polysaccharides to reduce availability of nutrients, hence decreasing the level of available nutrients from the feed. Hence, decreasing NPS will overall increase the value of the feed. However, Flores, et al., 2017, notes that the anti—nutritional effect of xylan has consistently been demonstrated in wheat-based diets, but not in the corn-soy diets fed in the United States. As such the use of xylanase in US swine and poultry feed is specific to increasing the energy value of the feed. The published study with the Xylanase enzyme preparation (Flores et al, 2017) demonstrated this increase in ileal digestible energy, supporting the hypothesis of the increase in energy utilization with the supplementation of the Xylanase enzyme preparation, as such this is a value-added product, allowing the bird to get more energy from the nutritionally complete animal diet provided. The use of the Xylanase preparation would be the same (increase in the energy value) of completed feed.

The FDA reviewer requested that notifier address why, if the enzyme fails to hydrolyze the xylan in diets formulated to contain xylanase, there are no adverse effects on the animal or its nutrition. We note in the Flores et al, 2017 studies the control birds (that did not receive xylanase supplementation—equivalent to a diet receiving xylanase (a protein) that failed to hydrolyze xylan) had no impact on body weight at slaughter. A recent article (Bedford, 2018) indicated that the function of non-starch polysaccharidases, in general, and xylanase specifically, is dependent on diet formulation, age of animal, microbiome composition, the small intestine endosperm cell wall content, as well as the heat treatment of the feed (or pelleting). Hence with any non-starch polysaccharide, the level of function of the enzyme may vary widely, but this variation will not impact the safety of the animal, as the intent is to allow digestion of xylans, that may escape digestion without adequate levels of xylanase in feeds.

We note that in the Final Rule (Federal Register: Vol. 81 54960) of the implementation of the GRAS regulations agency response to comment 144, discusses when utility studies are required to support GRAS conclusions. The FDA response is divided into nutrients and processing aids (specifically suggesting enzymes in this case). FDA states in this discussion" However, when

the function of an enzyme in animal food is well known, it is also common to use generally available and accepted data and information about the function of the enzyme in combination with animal feeding studies and stability studies to support the function of the enzyme (see section IV in CVM's experience document (Ref. 20))." The function and use of xylanase is well established, published, and accepted in the industry and by regulators (eg note the 8 sources of xylanase authorized for use in animal feed (AAFCO, Chapter 6, section 30.1). Five published studies with BRI Xylanase are cited in this conclusion, as well as many other xylanase products.

Reference:

Bedford, M.R. 2018. The evolution and application of enzymes in the animal feed industry: the role of data interpretation. British Poultry Science volume 59:486-493.

ATTACHMENT C- AGRN 44—October 6, 2021

Target Animal Safety (TAS)

CVM: The notifier's narrative does not do a sufficient job of explaining how the pieces of information are interpreted to allow conclusion of GRAS. CVM noted the TAS portion of the narrative should be expanded to include a summary description of relevant information from the published studies. The narrative should demonstrate how the notifier reached their conclusion of safety for the intended use of the substance. CVM stated the notifier needs to include, in its expanded narrative, description of the substance used in the published studies. Did the published studies use a xylanase produced using the same / current production process or a pilot scale (i.e., "tox lot" vs scaled up "market formulation")? If the substance in the studies is not produced under current manufacturing and formulation conditions, the notifier should explain how the published data are applicable to the notified substance.

CVM explained that the notifier needs to address the differences in proposed use rate (10,000 to 50,000 XU/kg feed) and the maximum doses in the published research articles (40,000 XU/kg feed for broilers and 45,000 XU/kg feed for weanling pigs). The notifier needs to explain why the demonstrated use rates can be extrapolated to address the proposed 50,000 XU/kg feed use rate.
CVM also noted that exposure calculations provided on page 32 of the notice are inconsistent and seem to have errors.

Response and sections to amend:

BRI has modified the maximum level of use to 40,000 XU / Kg of feed for poultry and swine.

For section 2.5.2. Use Levels: please update the text to ….maximum of 40,000 XU/kg feed.

For Part 3: Target Animal and Human Exposures, section: 3.1. Target Animal: in the first paragraph, please update the text to a maximum of 40,000 XU/Kg feed (40 XU/g feed)

For the exposure calculation under Part 3, section 3.1., please change the 20 XU to 40 XU in the locations illustrated below:

For broilers,….. Assuming 1 g of feed contains the maximum of 40 XU then the Estimated Daily intake of xylanase/kg bw /day…
93 grams feed /Kg body weight x 40 XU/g feed = 3720 XU/kg BW /day. This approximates 0.025 gram of Xylanase preparation/ kg BW each day.

For swine,….. Assuming 1 g of feed contains the maximum of 40 XU then the Estimated Daily Intake of xylanase/kg bw/day…
40 grams feed/Kg BW x 40 XU/g feed = 1600 XU/kg BW g/day. This approximates 0.01 gram of Xylanase preparation/ kg BW each day.

For the elaboration on part 3 narrative: Please add the following to Part 3, section 3.1. Target animal at the end of the section:

"The xylanase (endo-1,4-β-xylanase) contained in Xylamax is produced by the genetically modified production strain *K. phaffii* (b) (4) *K. phaffii* is listed as QPS when used for production. The *K. phaffii* (b) (4) has been thoroughly characterized, furthermore, absence of production strain cells and DNA has been proved. Therefore, xylanase production strain is presumed safe for the target animal species. The BRI xylanase preparation, has been used in the market for 5 years and no adverse effects have been reported. The BRI xylanase preparation product is currently marketed in Bangladesh, Egypt, Jordan, India, Indonesia, Mexico, Brazil, Chile, Nicaragua, Costa Rica, Bolivia, Columbia, Ecuador, El Salvador, Guatemala, Honduras, Panama, and Thailand. The long history of safe use of *K. phaffii* in feed enzyme manufacture and the absence of known pathogenic or toxigenic effects in *K. phaffii* demonstrates that it is a safe production organism for animal feed enzymes. *K. phaffii* (b) (4) genetic modification does not compromise the lack of toxigenic potential of the strain. Moreover, absence of production strain cells and DNA has been proved. Therefore, the xylanase contained in Xylamax should also be consider safe for the target animals.

Also, the xylanase will be degraded or inactivated during the passage through the digestive tract. Xylanase is a processing aid and is not expected to affect safety. As noted in the specifications (and the three batch analysis) there are extremely low levels of contaminants and the animal exposure is estimated at 0.01-0.025 grams/Kg bodyweight, demonstrating a safe product. Therefore, the use of Xylamax as a feed additive will not contribute to undesirable residues in animal products.

To support this conclusion, extensive literature search has been conducted, a summary table and review are provided herein, in this notice, 40,000 XU/Kg feed was set as the max dose due cost-benefit and marketing considerations rather than safety reasons, and to align with max inclusion rate used in published research conducted using Xylamax final product.

Table B. Published studies with the BRI Xylanase preparation

Title	Journal	Author	Animal category	Active substance	Dose enzyme
Evaluation of a thermotolerant xylanase on broiler growth performance and dietary ileal digestible energy value	Poultry Science Association Inc.	Flores C.A. et al, 2017	Broilers	Xylanase	20,000 XU/kg 40,000 XU/kg
Combination of Xylanase and Bacillus Direct-fed Microbials, as an Alternative to Antibiotic Growth Promoters, Improves Live Performance and Gut Health in Subclinical Challenged Broilers	International Journal of Poultry Science	Nusairat B. et al 2018	Broilers	Xylanase (alone and in combination with *Bacillus* spp)	15,000 XU/kg
Dietary supplementation of xylanase and protease on growth performance, digesta viscosity, nutrient digestibility, immune and oxidative stress status, and gut health of newly weaned pigs	Animal Nutrition	Duarte et al 2019	Pigs	Xylanase (alone and in combination with a protease)	45,000 XU/kg
Synbiotic Effects of Enzyme and Probiotics on Intestinal Health and Growth of Newly Weaned Pigs Challenged With Enterotoxigenic F18+ Escherichia coli	Frontiers in Veterinary Science	Duarte et al 2020	Pigs	Xylanase (alone and in combination with *Bacillus* spp)	10,000 XU/kg
Xylanase and Direct-Fed Microbials (DFM) Potential for Improvement of Live Performance, Energy Digestibility, and Reduction of Environmental Microbial Load of Broilers"	Frontiers in Veterinary Science	Nusairat and Wang 2020	Broilers	Xylanase (alone and in combination with *Bacillus* spp)	10,000 XU/kg

BRI has published five articles focused on testing the xylanase enzyme produced by the genetically modified strain *K. phaffii* (b) (4) using same production process (market formulation), in collaboration with multiple external research entities. These articles were published in several journals within the last five years. These published articles investigated xylanase activity ranging between 10,000 to 45,000 XU/Kg feed in both broiler chickens and pigs. In the article published by Flores et al., (2016), broilers raised for fattening were investigated in two trials; a total of 1260 broiler chickens were used in trial 1 and 1760 were used in trial 2, the maximum enzyme activity tested was 40,000 XU/kg feed, In both trials, birds consuming xylanase performed similar or superior to birds on control feed indicating that there were no adverse effects due to enzyme supplementation. Thus, it can be concluded that xylanase enzyme can be safely included in feed based on results of this published article.

In another article published on broiler chickens by Nusairat et al. (2018), researchers investigated the influence of xylanase supplemented at 15,000 XU/Kg feed when supplemented alone or in combination with *Bacillus spp.* probiotics. In this trial, 2,496 were used. Birds were subjected to a mild, subclinical challenge with two *Eimeria* species and *Clostridium perfringens* then necropsied for lesion score and general gut health evaluation. Mortality in birds receiving only 15,000 XU/Kg feed was significantly ($P<0.05$) lower than birds in negative control group indicating that adding xylanase to broiler feed even under disease challenge did not have any adverse effect on bird health. Lesion scores, *Salmonella* incidence, and counts for *E. coli*, aerobic plate count (APC), and *Clostridium perfringens* were estimated, birds consuming feed with xylanase enzyme had significantly ($P<0.05$) lower values compared to the negative control, and there were not any anomalies noted while performing the necropsy indicating that xylanase enzyme does not have any safety concerns when used in feed. Another study was published in broilers investigating xylanase enzyme supplementation at 10,000 XU/Kg feed either alone or in combination with *Bacillus spp* probiotic in broilers under a mild environmental challenge with *Clostridium perfringens* (Nusairat et al., 2020). A total of 2,496 broilers were used in this study. Results of this study did not show any negative effects of xylanase on bird performance and health, mortality was comparable among treatments which indirectly indicates that there were not any safety concerns due to xylanase inclusion in feed.

Xylanase was also investigated in pigs; Duarte et al. (2019) investigated the effect of supplementing xylanase at 45,000 XU/Kg feed. A total of 48 pigs were used, results showed that supplementing xylanase at this level did not result in any health concerns while performance and intestinal measurements were comparable to control. Furthermore, in another study on pigs published by Duarte et al. (2020), the effect of disease challenge was investigated on intestinal health and growth of newly weaned pigs. A total of 64 pigs were used in this study, xylanase inclusion was at 10,000 XU/Kg feed, results showed that there was no adverse effects observed in pigs receiving diets with xylanase which indicates that xylanase does not raise any safety concerns when used in feed. Moreover, outcomes of these published articles can indirectly indicate safety of Xylamax for use in all poultry and swine including broiler, layers, breeders for poultry, and nursery, fattening, finishing, lactating for swine.

In addition to the published research articles which clearly demonstrates the safety of using Xylamax final market product, the purity of the Xylamax final product has been demonstrated by analyzing for multiple contaminants in several batches of Xylamax, results of the analyses

demonstrated that Xylamax final product did not contain any chemical, mycotoxins, heavy metals, Dioxins/Furans and PCBs, or biological contaminants, indicating that the raw material going into the product formulation was also free of contaminants therefore, no safety concerns are raised due to impurities.

One of the main safety components in assessing an enzyme safety is the safety of the enzyme production organism, which has been demonstrated throughout this notice that *K. phaffii* meets the criteria for determining the safety of enzymes used in animal feed by Pariza and Cook (2010). Furthermore, the xylanase produced by the genetically modified *K. phaffii* is considered safe because the genetic modifications are well characterized and specific, and the incorporated DNA does not encode and express any known harmful or toxic substances.

Therefore, it can be concluded that xylanase produced by *K. phaffii* is safe for the use in poultry and swine. This conclusion is supported by the safety of the organism (*K. phaffii*) used for xylanase production, the safety of the genetic modification performed on *K. phaffii*, the history of safe xylanase use in poultry and swine feed of both Bioresource and other marketed xylanases, the safe nature of xylanase as an enzyme (protein) that functions as a digestion aid and is degraded or inactivated in the digestive tract, as well as the safety of the other ingredients going into the Xylamax final market product formulation. Altogether, support the safe use of the xylanase in poultry and swine."

Copies of Additional References in Attachment F

Nusairat, B., and J.-J., Wang. Xylanase and Direct-Fed Microbials (DFM) Potential for Improvement of Live Performance, Energy Digestibility, and Reduction of Environmental Microbial Load of Broilers. Front. Vet. Sci., 07 December 2020. DOI: https://doi.org/10.3389/fvets.2020.606415

Duarte, M., J. Tyus, and S. W. Kim. Synbiotic Effects of Enzyme and Probiotics on Intestinal Health and Growth of Newly Weaned Pigs Challenged With Enterotoxigenic F18+ Escherichia coli. Front. Vet. Sci., 09 September 2020. DOI: https://doi.org/10.3389/fvets.2020.00573

Nusairat, B., J. McNaughton, J. Tyus, and J.-J. Wang. 2018. Combination of Xylanase and Bacillus Direct-fed Microbials, as an Alternative to Antibiotic Growth Promoters, Improves Live Performance and Gut Health in Subclinical Challenged Broilers. International Journal of Poultry Science, 17: 362-366. DOI: 10.3923/ijps.2018.362.366

ATTACHMENT D: AGRN 44—October 6, 2021

Molecular Biology

CVM: CVM stated it is unclear whether the shift in molecular weight was due to a decrease in glycosylation or the reduction in the size of the secreted protein since the notifier deleted the 5′ and 3′ ends of the protein and added the starch binding domain. CVM used the amino acids sequence that was provided on page 9 and calculated that the secreted protein would have a molecular mass of 36.7 kilodaltons (kDa), which is slightly less than the value reported by the notifier (37.4 kDa). It is possible that the notifier and CVM used different formulas to calculate the molecular mass. The notifier should describe any other data that it has which demonstrates a reduction in glycosylation of the expressed protein.

Response:

BRI uses the sequences on page 9, which starts with the (b) (4) domain of the starch binding domain to the end of xylanase, with the sequences of

(b) (4)

", to do molecular weight analysis.

BRI originally used the DNAStar Lassergene 17 software to perform the molecular weight calculation. The result is (b) (4) Daltons.

Besides DNAStar, BRI also uses two websites to get the molecular weight calculation results:

The first website BRI uses is (b) (4).

The molecular weight result is (b) (4)4 dalton.

The second website BRI used is https://www.bioinformatics.org/sms/prot_mw.html

The molecular weight result we got is (b) kilodaltons.

The differences of the results with CVM's calculation may be due to the different formula used to calculate the molecular weight.

The SDS page gel shown on page 16 are the electrophoresis results of our xylanase plus the starch binding domain, which contains the above-mentioned sequences (b) (4) The left panel is the electrophoresis result of the (b) (4) protein with (b) (4) and (b) (4) in the original sequences (wild type), while in the right panel the protein contains the mutations of (b) (4) and (b) (4). The gel electrophoresis results showing that molecular weight differences, support the conclusion that the cause of molecular weight reduction is the remove of glycosylation due to the change of the (b) (4) amino acids, because no other sequences change occurred.

CVM: CVM noted the information provided in Table 5 on page 69 of the notice suggests that the promoter sequence for the (b) (4) cassette in the first plasmid insert was not incorporated into the genome. However, the information provided in Figure 4 and the nucleotide sequence that was provided in the appendix suggest that (b) (4) complete copies of (b) (4) were inserted into the genome of the host organism. The notifier should address this discrepancy.

Response:

The contents CVM mentioned are inside the whole genome analysis report of (b) (4), the production strain of Xylamax used by BRI. BRI contacted (b) (4) the company prepared the whole genome analysis of (b) (4) and found that the table was not correctly prepared. According to the results of genome sequencing, as the genetic elements drawn on Figure 4, Table 5 should have a row in the top showing the (b) (4) promoter for the (b) (4) cassette. (b) (4) has corrected the error in Table 5 and a new version of the report is attached with this answer (Corrected Appendix 2). Therefore, the promoter sequence for the (b) (4) cassette in the first plasmid insert was incorporated.

CVM: The notifier provides a study report for the antibiotic resistance gene transferability assay. This study report appears to be incomplete. The notifier should provide a limit of detection for the antibiotic resistance gene transferability assay.

Response:

BRI relies on the fact of production strain DNA is not present in the Xylanase product (Appendix 6), for assurances that antibiotic resistance is not a hazard. Since no DNA of the production strain can be found in the product, no DNA will be transferred to other organisms. Therefore, the purpose of proving our xylanase is not a source of antibiotic resistance, can rely on the study report found in appendix 6.

The antibiotic resistance gene transferability assay also was intended to support this portion of the safety assessment by demonstrating that the antibiotic resistance genes encoded inside the genome of the production strain are stable and not be able to transfer into other organisms, such as E. coli. However, we agree the study report is incomplete and we do not have the ability to rectify the missing information.

It is BRI's conclusion that the fact that the of production strain DNA is not present in the Xylanase product, assures that Xylanase product will not be a source of antibiotic resistance.

CVM: The notifier states that plasmid (b) (4) was integrated (b) (4) (b) (4) The mode of insertion is unclear since the plasmid does not contain homology regions that are upstream and downstream of the (b) (4) expression cassettes (b) (4)). The notifier should address this issue.

Response:

(b) (4)

(b) (4)

CVM: In addition, CVM stated it is unclear what the notifier means on page 85 of the notice when it states "there are too many copies of tandem repeat integration" to determine if the (b) (4) promoter was intact or not. This statement suggests that more than (b) (4) copies of the (b) (4) plasmid were inserted (b) (4). The notifier should resolve the discrepancies between what was reported for its whole genome sequencing and this Southern blot analysis.

Response:

Appendix 5 (b) (4)

Appendix:

Corrected Appendix 2

(b) (4)

ATTACHMENT E--AGRN 44—October 6, 2021

Microbial Safety

CVM: CVM explained the notifier identifies the host strain as *K. phaffii* BG10 and provides a whole genome sequencing (WGS) report in Appendix 1 to support identity. However, Appendix 1 is marked as confidential business information (CBI) and the narrative of the notice does not include any information on confirmation of the identity of the host strain except for the reference to Appendix 1. Identity of host strain is crucial for addressing microbial safety. The notifier needs to include a summary of Appendix 1 in the narrative of the notice including the methods and various approaches used in conclusively establishing the identity of the host strain. In this narrative, the notifier should also link the host strain's identity to the microbial safety information for the genus species to establish that host organism is safe for this use.

CVM noted that the notifier also includes a statement in Section 6 of the notice which reads as follows: "Data and information cited in this notification is not generally available and Part 6 contains information that is exempt from disclosure under the FOIA". The notifier does not identify any subsections in Section 6 as confidential. The notifier should provide clarification about this sentence as it calls into question the general availability of safety data which is the primary basis for a GRAS conclusion.

Response:

Add the following text to section 2.2.2. host strain after the Komagataella phaffii (Pichia pastoris) taxonomy list:

"Four different approaches were used for the taxonomic identification (Appendix 1). The production strain was unequivocally identified as Komagataella phaffii. 1) The 18S rRNA was analysed using megablast searches against the NCBI RefSeq database of curated 18S rRNA sequence data of microbial type strains. The best match was with K. phaffii NRRL Y-7556. 2) Completeness of the (b) (4) genome was assessed against the K. phaffii CBS (b) (4) by whole genome alignment using the MCM algorithm in Mauve. The production strain aligned very well with the chosen reference genome. 3) The alignment-free genome distance estimation analysis with Mash using MinHash supported K. phaffii GS115 (GCF_000027005.1) as the closest genome. 4) A multigene phylogenetic analysis grouped (b) (4) with other K. phaffii strains. The strain is not genetically modified. As a species, K. phaffii qualifies as QPS (Qualified Presumption of Safety) when used for enzyme production. Furthermore, it is a common host for enzyme production (also referred to as Pichia pastoris) as found in the listing of authorized feed enzymes (AAFCO Chapter 6, section 30)."

The Chapter 6 narrative does not contain confidential business information, and BRI vacates that statement of confidentiality in this section of the GRAS notice.

Cerrito, Chelsea

From: Kristi Smedley <smedley@cfr-services.com>
Sent: Thursday, October 07, 2021 12:55 PM
To: Animalfood-premarket
Cc: 'Rasha Qudsieh'; Conway, Charlotte
Subject: RE: [EXTERNAL] RE: GRAS Notice No. AGRN 44 - Call Regarding Xylanase enzyme to be used in swine and poultry feeds--REFERENCES
Attachments: Attachemnt F-Additional References.docx; Bedford2018The evolution and application of enzymes in the animal feed industry the role of data interpretation.pdf; Duarte_et_al_2020.pdf; Li2007 _Article_ExpressionOfRecombinantProtein.pdf; Nusairat_et_al_2018.pdf; Nusairat_et_al_2020.pdf

I am attaching the list of new references, as well as copies of the references to the AGRN 44 amendment.

Kristi O. Smedley, Ph.D.

Center for Regulatory Services, Inc.
5200 Wolf Run Shoals Rd.
Woodbridge, VA 22192

Ph. 703-590-7337
Cell (b) (6)
Fax 703-580-8637

From: Kristi Smedley [mailto:smedley@cfr-services.com]
Sent: Thursday, October 07, 2021 12:54 PM
To: 'Animalfood-premarket'
Cc: 'Rasha Qudsieh'; 'Conway, Charlotte'
Subject: RE: [EXTERNAL] RE: GRAS Notice No. AGRN 44 - Call Regarding Xylanase enzyme to be used in swine and poultry feeds

Please see attachments to this email that provides the response to concerns raised by the Division specific to AGRN 44.

Should have any problems receiving these attachments or on the provided information, please contact me.

Kristi O. Smedley, Ph.D.

Center for Regulatory Services, Inc.
5200 Wolf Run Shoals Rd.
Woodbridge, VA 22192

Ph. 703-590-7337
Cell (b) (6)
Fax 703-580-8637

From: Animalfood-premarket [mailto:Animalfood-premarket@fda.hhs.gov]
Sent: Wednesday, September 22, 2021 12:38 PM
To: Kristi Smedley
Cc: Rasha Qudsieh; Animalfood-premarket; Conway, Charlotte
Subject: RE: [EXTERNAL] RE: GRAS Notice No. AGRN 44 - Call Regarding Xylanase enzyme to be used in swine and poultry feeds

Dear Dr. Smedley,

With regards to GRAS Notice No. AGRN 44, please find attached our meeting minutes from the September 15, 2021 teleconference and response to your request for the meeting minutes. Please let us know if you have any questions.

Kind regards,
Chelsea

From: Animalfood-premarket <Animalfood-premarket@fda.hhs.gov>
Sent: Wednesday, September 15, 2021 12:44 PM
To: Kristi Smedley <smedley@cfr-services.com>
Cc: Animalfood-premarket <Animalfood-premarket@fda.hhs.gov>; Rasha Qudsieh <rQudsieh@briworldwide.com>
Subject: RE: [EXTERNAL] RE: GRAS Notice No. AGRN 44 - Call Regarding Xylanase enzyme to be used in swine and poultry feeds

Dear Kristi,

Thank you for your request for minutes. A copy will be e-mailed to you and Dr. Qudsieh as soon they are available.

Kind regards,
Chelsea

From: Kristi Smedley <smedley@cfr-services.com>
Sent: Wednesday, September 15, 2021 12:10 PM
To: Animalfood-premarket <Animalfood-premarket@fda.hhs.gov>
Cc: Rasha Qudsieh <rQudsieh@briworldwide.com>
Subject: RE: [EXTERNAL] RE: GRAS Notice No. AGRN 44 - Call Regarding Xylanase enzyme to be used in swine and poultry feeds

Chelsea:

Thank you for organizing this meeting. It was very beneficial, and we will be working on our amendment.

We are requesting the notes of this meeting. I am requesting that they be sent by email to both Rasha (Rasha Qudsieh (rQudsieh@briworldwide.com)) and I.

Kristi O. Smedley, Ph.D.

Center for Regulatory Services, Inc.
5200 Wolf Run Shoals Rd.
Woodbridge, VA 22192

Ph. 703-590-7337
Cell (b) (6)
Fax 703-580-8637

From: Animalfood-premarket [mailto:Animalfood-premarket@fda.hhs.gov]
Sent: Wednesday, September 08, 2021 9:08 AM
To: Kristi Smedley
Cc: Animalfood-premarket
Subject: RE: [EXTERNAL] RE: GRAS Notice No. AGRN 44 - Call Regarding Xylanase enzyme to be used in swine and poultry feeds

Dear Dr. Smedley,

I have scheduled the call for Wednesday, September 15 from 10-11 am US Eastern Time.

Below are the Zoom details for the call. Once you click on the hyperlink "Join Zoom Meeting", you will be prompted to connect your audio either by using your computer audio or dialing in by phone (will require entering the meeting ID and passcode (b) (6)).

Please let me know if you have any questions.

Kind regards,
Chelsea

Join Zoom Meeting

One tap mobile: (b) (6)

Meeting URL:
Meeting ID:
Passcode:

Join by Telephone

For higher quality, dial a number based on your current location.

Dial: (b) (6)

Meeting ID:

Passcode:

International numbers

From: Kristi Smedley <smedley@cfr-services.com>
Sent: Tuesday, September 07, 2021 11:51 AM
To: Animalfood-premarket <Animalfood-premarket@fda.hhs.gov>
Subject: [EXTERNAL] RE: GRAS Notice No. AGRN 44 - Call Regarding Xylanase enzyme to be used in swine and poultry feeds

Thank you, we prefer Wednesday, September 15 from 10 – 11 am.

Kristi O. Smedley, Ph.D.

Center for Regulatory Services, Inc.
5200 Wolf Run Shoals Rd.
Woodbridge, VA 22192

Ph. 703-590-7337
Cel (b) (6)
Fax 703-580-8637

From: Animalfood-premarket [mailto:Animalfood-premarket@fda.hhs.gov]
Sent: Tuesday, September 07, 2021 9:01 AM
To: Kristi Smedley (smedley@cfr-services.com)
Cc: Animalfood-premarket
Subject: GRAS Notice No. AGRN 44 - Call Regarding Xylanase enzyme to be used in swine and poultry feeds

Dear Dr. Smedley,

I hope this e-mail finds you well. We would like to schedule a call with you, as well as any others from BioResource International, Inc., to discuss the GRAS notice. We are available during the following dates and times (US Eastern):

1. Wednesday, September 15 from 10 – 11 am
2. Thursday, September 16 from 12 – 1 pm

Please let me know if one of these options works or if I should look for more options. I will send Zoom information for the call once it has been scheduled.

Kind regards,
Chelsea

Chelsea Cerrito, MAS
Animal Scientist, Division of Animal Feeds (DAF)
Center for Veterinary Medicine

Office of Surveillance and Compliance
U.S. Food and Drug Administration
Tel: 240-402-6729
Personal e-mail address: Chelsea.Cerrito@fda.hhs.gov
To schedule a meeting with DAF, please e-mail: animalfood-premarket@fda.hhs.gov

ATTACHMENT F

Additional References

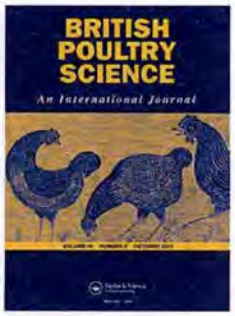
Bedford, M.R. 2018. The evolution and application of enzymes in the animal feed industry: the role of data interpretation. British Poultry Science volume 59:486-493.

Li P, Anumanthan A, Gao XG, Ilangovan K, Suzara VV, Düzgüneş N, Renugopalakrishnan V. Expression of recombinant proteins in Pichia pastoris. Appl Biochem Biotechnol. 2007 Aug;142(2):105-24. doi: 10.1007/s12010-007-0003-x. PMID: 18025573.

Nusairat, B., and J.-J., Wang. Xylanase and Direct-Fed Microbials (DFM) Potential for Improvement of Live Performance, Energy Digestibility, and Reduction of Environmental Microbial Load of Broilers. Front. Vet. Sci., 07 December 2020. DOI: https://doi.org/10.3389/fvets.2020.606415

Duarte, M., J. Tyus, and S. W. Kim. Synbiotic Effects of Enzyme and Probiotics on Intestinal Health and Growth of Newly Weaned Pigs Challenged With Enterotoxigenic F18+ Escherichia coli. Front. Vet. Sci., 09 September 2020. DOI: https://doi.org/10.3389/fvets.2020.00573

Nusairat, B., J. McNaughton, J. Tyus, and J.-J. Wang. 2018. Combination of Xylanase and Bacillus Direct-fed Microbials, as an Alternative to Antibiotic Growth Promoters, Improves Live Performance and Gut Health in Subclinical Challenged Broilers. International Journal of Poultry Science, 17: 362-366. DOI: 10.3923/ijps.2018.362.366



British Poultry Science

ISSN: 0007-1668 (Print) 1466-1799 (Online) Journal homepage: https://www.tandfonline.com/loi/cbps20

The evolution and application of enzymes in the animal feed industry: the role of data interpretation

Michael R. Bedford

To cite this article: Michael R. Bedford (2018) The evolution and application of enzymes in the animal feed industry: the role of data interpretation, British Poultry Science, 59:5, 486-493, DOI: 10.1080/00071668.2018.1484074

To link to this article: https://doi.org/10.1080/00071668.2018.1484074

Published online: 27 Jul 2018.

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2018, VOL. 59, NO. 5, 486–493
https://doi.org/10.1080/00071668.2018.1484074

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GORDON MEMORIAL LECTURE

The evolution and application of enzymes in the animal feed industry: the role of data interpretation

Michael R. Bedford

R&D, AB Vista, Marlborough, UK

ABSTRACT

1. Enzymes have been used commercially for nearly 40 years and save significant costs through sparing of expensive nutrients but the mechanism by which this is achieved is still debated.
2. The research focused on non-starch polysaccharidase (NSPase) enzymes is used as an example of where greater progress could have been made if the details of the work had been described more fully and the analysis of the data generated had been broader in scope and more critical.
3. Lack of standardisation of the details presented in the materials and methods has been identified as a significant barrier to meaningful retrospective analysis and thus limits advances in the understanding of the mode of action of these enzymes.
4. The identity of the enzyme employed and its activity is often lacking, and more importantly the purity is rarely disclosed. Contaminant activities which are neither listed nor assayed could play a significant role in the responses observed.
5. The dose optimum of most enzymes is often considerably higher than that employed in most studies. Thus studies claiming synergy between two 'activities' should ensure that the response is not related to each enzyme simply augmenting the dose of just one activity in the finished feed. This is a common problem, and coupled with the lack of factorial experiments to justify the presence of each enzyme in a multi-enzyme product, it is not surprising that there is still debate as to whether single or multi-enzymes are best suited poultry rations.
6. The three proposed mechanisms for NSPases (viscosity, cell wall and prebiotic) are discussed, and along with their strengths and weaknesses it is suggested that a re-evaluation of each is needed. Viscosity may have to be re-evaluated as being a function not only of the cereal being fed, but of the age of the animal as well. The cell wall theory as described is poorly modelled *in vitro* and hence the validity of these data is questioned. The prebiotic theory may need significant modification as it appears that the quantities of oligomers produced are insufficient to generate the additional volatile fatty acids (VFA)'s reported. It is likely that all three mechanisms play a role in the responses observed, but the prebiotic mechanism probably plays by far the most important part in low viscosity diets.
7. Future research would be improved if it considered all potential mechanisms when designing a trial. Significant failings are apparent as a result of adherence to tenets in explanation of the results. Most importantly, it should be emphasised that a hypothesis is there to be tested, not defended.

ARTICLE HISTORY
Received 1 May 2018
Accepted 10 May 2018

KEYWORDS
Cell wall theory; feed enzymes; mechanism of action; NSPase; prebiotic; viscosity

Appl Biochem Biotechnol (2007) 142:105–124
DOI 10.1007/s12010-007-0003-x

Expression of Recombinant Proteins in *Pichia Pastoris*

Pingzuo Li · Anukanth Anumanthan · Xiu-Gong Gao · Kuppusamy Ilangovan · Vincent V. Suzara · Nejat Düzgüneş · V. Renugopalakrishnan

Received: 14 April 2006 / Revised: 16 May 2006 / Accepted: 23 May 2006 /
Published online: 25 April 2007

Abstract *Pichia pastoris* has been used extensively and successfully to express recombinant proteins. In this review, we summarize the elements required for expressing heterologous proteins, and discuss various factors in applying this system for protein expression. These elements include vectors, host strains, heterologous gene integration into the genome, secretion factors, and the glycosylation profile. In particular, we discuss and evaluate the recent progress

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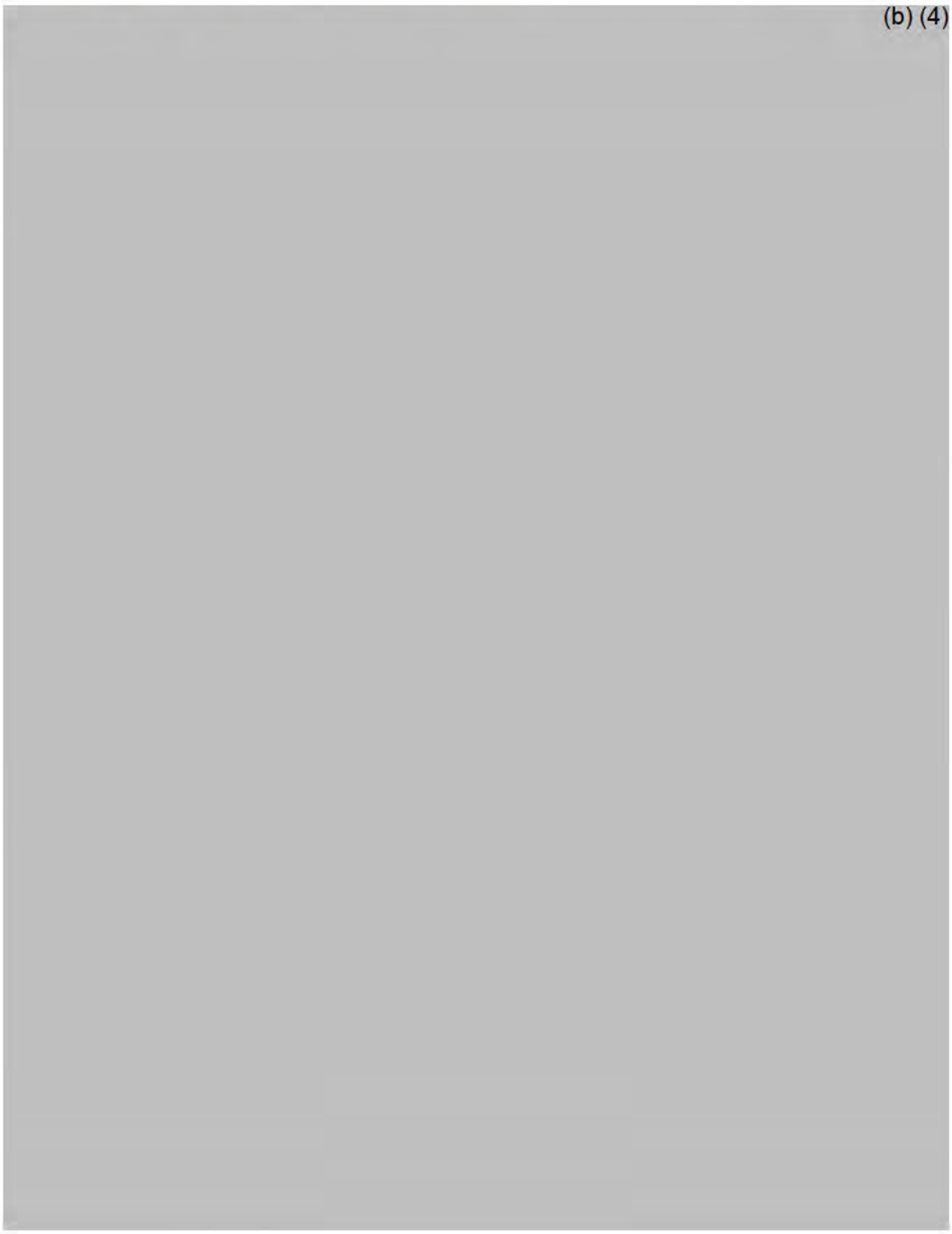
V. Renugopalakrishnan
Biophotovoltaic Group, Division of Bioengineering and Department of Mechanical Engineering, NUS Nanotechnology Initiative (NUSNNI), National University of Singapore, Singapore -117576, Singapore

in optimizing the fermentation process to improve the yield and stability of expressed proteins. Optimization can be achieved by controlling the medium composition, pH, temperature, and dissolved oxygen, as well as by methanol induction and feed mode.

Keywords *Pichia pastoris* · Protein expression · Methanol induction · Dissolved oxygen · Gene integration · Alcohol oxidase promoter · AOX1



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Research Article

Combination of Xylanase and *Bacillus* Direct-fed Microbials, as an Alternative to Antibiotic Growth Promoters, Improves Live Performance and Gut Health in Subclinical Challenged Broilers

[1]Basheer Nusairat, [2]James McNaughton, [1]James Tyus and [1]Jeng-Jie Wang

[1]BioResource International, Inc. 4222 Emperor Blvd, Suite 460 Durham, NC 27703, USA
[2]AHPharma, Inc. 27013 E Lillian St, Hebron, MD 21830, USA

Abstract

Objective: This study evaluated the effect of xylanase, *Bacillus* direct-fed microbials (DFM) and their combination on performance under a mild, subclinical challenge with two *Eimeria* species and *Clostridium perfringens* in broilers raised to 42 days. **Materials and Methods:** A total of 6 dietary treatments were used throughout the trial. Diets were supplemented with one of the following; no xylanase or *Bacillus* (control), xylanase only, *Bacillus* L. only, *Bacillus* A. only, xylanase plus *Bacillus* L. or xylanase plus *Bacillus* A. Data were analyzed as randomized complete block design. **Results:** When compared to control at 42 days, the xylanase, *Bacillus* L. and *Bacillus* A. improved ($p \leq 0.05$) BW gain by 93, 94 and 53 g, respectively and FCR by 4, 4 and 6 points, respectively. When compared to control at 42 days, the combination of xylanase and *Bacillus* L. or *Bacillus* A. improved ($p \leq 0.05$) BW gain by 142 or 147 g, respectively and FCR by 9 or 11 points, respectively. The combination of xylanase and *Bacillus* L. or *Bacillus* A. reduced ($p \leq 0.05$) BW coefficient of variation from 15.09% (control) to 8.27 or 8.22%, respectively at 42 days. The combination of xylanase and *Bacillus* L. or *Bacillus* A. reduced ($p \leq 0.05$) gross lesion scores in small intestine and *C. perfringens* count at 42 days compared to control. **Conclusion:** Results suggest that xylanase and *Bacillus* alone may improve broiler performance and reduce the severity of intestinal lesions due to *Eimeria* and *C. perfringens* challenges and that the effect of xylanase and *Bacillus* DFM are additive.

Key words: Broiler, xylanase, direct-fed microbial, *Clostridium perfringens*, *Eimeria*

Received: May 09, 2018 Accepted: June 28, 2018 Published: July 15, 2018

Citation: Basheer Nusairat, James McNaughton, James Tyus and Jeng-Jie Wang, 2018. Combination of xylanase and *Bacillus* direct-fed microbials, as an alternative to antibiotic growth promoters, improves live performance and gut health in sub-clinical challenged broilers. Int. J. Poult. Sci., 17: 362-366.

Corresponding Author: Basheer Nusairat, BioResource International, Inc. 4222 Emperor Blvd, Suite 460 Durham, NC 27703, USA

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

frontiers in Veterinary Science

ORIGINAL RESEARCH
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Synbiotic Effects of Enzyme and Probiotics on Intestinal Health and Growth of Newly Weaned Pigs Challenged With Enterotoxigenic F18$^+$ *Escherichia coli*

Marcos Elias Duarte[1], *James Tyus*[2] *and Sung Woo Kim*[1]*

[1] *Department of Animal Science, North Carolina State University, Raleigh, NC, United States,* [2] *BioResource International, Inc., Durham, NC, United States*

This study aimed to investigate the effect of dietary supplementation with xylanase and probiotics on growth performance and intestinal health of nursery pigs challenged with enterotoxigenic *Escherichia coli* (ETEC). Sixty-four newly weaned pigs (32 barrows and 32 gilts with 7.9 ± 0.4 kg BW) were allotted in a randomized complete block design (2 × 2 factorial). Two factors were ETEC challenge (oral inoculation of saline solution or *E. coli* F18$^+$ at 6 × 10^9 CFU) and synbiotics (none or a combination of xylanase 10,000 XU/kg and *Bacillus* sp. 2 × 10^8 CFU/kg). All pigs were fed experimental diets following NRC (2012) in two phases (P1 for 10 d and P2 for 11 d). The ETEC was orally inoculated on d 7 after weaning. Feed intake and BW were measured on d 7, 10, 15, and 20. On d 20, pigs were euthanized to collect samples to measure gut health parameters and microbiome. Synbiotics increased ($P < 0.05$) ADG in phase 1 and ETEC reduced ($P < 0.05$) ADG and G:F in the post-challenge period. ETEC increased ($P < 0.05$) the fecal score of pigs from d 7 to 13; however, synbiotics reduced ($P < 0.05$) it at d 9 and 11 in challenged pigs. ETEC increased ($P < 0.05$) mucosal MDA, IL-6, Ki-67$^+$, and crypt depth, whereas synbiotics tended to reduce TNFα ($P = 0.093$), protein carbonyl ($P = 0.065$), and IL-6 ($P = 0.064$); reduced ($P < 0.05$) crypt depth and Ki-67$^+$; and increased ($P < 0.05$) villus height. ETEC reduced ($P < 0.05$) the relative abundance of Bacteroidetes and Firmicutes and increased ($P < 0.05$) the relative abundance of Proteobacteria. In conclusion, ETEC challenge reduced growth performance by affecting microbiome, immune response, and oxidative stress in the jejunum. Synbiotics enhanced growth performance by reducing diarrhea, immune response, and oxidative stress in the jejunum.

Keywords: ***Escherichia coli*, growth performance, intestinal health, newly weaned pigs, probiotics, synbiotics, xylanase**

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INTRODUCTION

(b) (4)

frontiers in Veterinary Science

ORIGINAL RESEARCH
published: 07 December 2020
doi: 10.3389/fvets.2020.606415

Xylanase and Direct-Fed Microbials (DFM) Potential for Improvement of Live Performance, Energy Digestibility, and Reduction of Environmental Microbial Load of Broilers

Basheer Nusairat[1]* *and Jeng-Jie Wang*[2]

[1] Department of Animal Production, College of Agriculture, Jordan University of Science and Technology, Irbid, Jordan, [2] BioResource International, Inc., Durham, NC, United States

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Nusairat B and Wang J-J (2020) Xylanase and Direct-Fed Microbials (DFM) Potential for Improvement of Live Performance, Energy Digestibility, and Reduction of Environmental Microbial Load of Broilers. Front. Vet. Sci. 7:606415. doi: 10.3389/fvets.2020.606415

The challenge of identifying alternatives to subtherapeutic levels of antibiotic growth promoters (AGP) in animal feed has led to increased interest in feed additives such as exogenous enzymes and direct-fed microbials (DFM). Six corn soy-based dietary treatments were designed to investigate the effect of high-efficiency xylanase alone, *Bacillus* spp. probiotics alone, and their combination vs. a commonly used antibiotic growth promoter (bacitracin methylene disalicylate; BMD) on live performance and environmental *Clostridium perfringens* load of broiler chickens with eight replicate pens per treatment. Diets were as follows: standard diet (positive control; PC); 130 kcal/kg reduced-energy diet (negative control; NC); NC with xylanase (NC + Xy); NC with probiotics (NC + Pro); NC with xylanase and probiotics mix (NC + XyPro); and NC with BMD (NC + BMD). Data were analyzed as one-way ANOVA. At 35 and 42 days, birds fed with NC + XyPro and NC + BMD were heavier ($P < 0.05$) than birds fed with NC. Improvement in feed conversion ratio (FCR) ($P = 0.0001$) was observed from 1 to 42 days by ~3 points in both NC + XyPro and NC + BMD compared to NC. The NC + XyPro reduced lesion scores by 66% compared to PC and NC. Litter *C. perfringens* cell count was reduced by ~16% with supplementation of XyPro or BMD. It can be concluded that a blend of xylanase (10 XU/g feed) and *Bacillus* spp. [1×10^5 colony forming units (CFU)/g feed] can be used as an alternative to AGP in low-energy broiler diets.

Keywords: xylanase, *Bacillus* spp., DFM, antibiotic-free, energy digestibility, broiler

INTRODUCTION

(b) (4)

Cerrito, Chelsea

From: Kristi Smedley <smedley@cfr-services.com>
Sent: Tuesday, January 18, 2022 10:11 AM
To: Animalfood-premarket
Cc: Cerrito, Chelsea; 'Rasha Qudsieh'
Subject: RE: [EXTERNAL] RE: AGRN #44 Amendment Clarification
Attachments: CFR-FDA cover letter for BRI Xylanase response to concern Jan 18 2022.pdf; Response fo FDA questions GRAS notice 44 Jan 18 2022.docx; 1. Gerd et al 2005.pdf; 2. Chemical properties of Formaldehyde.pdf; 3. Chemical properties of Hydrogen peroxide.pdf; 4. Bretschger et al 1947.pdf

Megan:

We are providing the response to the request for clarification as sent on December 27, 2021. We appreciate the one week extension you provided.

Attached is the cover letter, our narrative response, and the references to support that assessment.

Should you have any questions on this response, or have trouble receiving it, please contact us.

Kristi O. Smedley, Ph.D.

Center for Regulatory Services, Inc.
5200 Wolf Run Shoals Rd.
Woodbridge, VA 22192

Ph. 703-590-7337
Cell (b) (6)
Fax 703-580-8637

From: Animalfood-premarket [mailto:Animalfood-premarket@fda.hhs.gov]
Sent: Tuesday, December 28, 2021 9:26 AM
To: Kristi Smedley; Animalfood-premarket
Cc: Cerrito, Chelsea; 'Rasha Qudsieh'
Subject: RE: [EXTERNAL] RE: AGRN #44 Amendment Clarification

Good morning,

Thank you for providing an update. We can allow for the 1-week extension, with the new due date no later than **January 18, 2022**.

Have a lovely day and New Year's holiday!

Megan

Megan Hall M.S.
Staff Fellow Animal Scientist

Center for Veterinary Medicine
WAH
U.S. Food and Drug Administration
megan.hall@fda.hhs.gov

From: Kristi Smedley <smedley@cfr-services.com>
Sent: Monday, December 27, 2021 5:21 PM
To: Animalfood-premarket <Animalfood-premarket@fda.hhs.gov>
Cc: Cerrito, Chelsea <Chelsea.Cerrito@fda.hhs.gov>; 'Rasha Qudsieh' <rQudsieh@briworldwide.com>
Subject: [EXTERNAL] RE: AGRN #44 Amendment Clarification

Ms., Hall:

The offices of BioResource International, Inc. are closed until January 4, 2021. I have communicated with their primary regulatory persons, but they have no back up or ability to complete analyses until the offices reopen.

They are requesting for a 1 week extension of the two week allotted time.

Kristi O. Smedley, Ph.D.

Center for Regulatory Services, Inc.
5200 Wolf Run Shoals Rd.
Woodbridge, VA 22192

Ph. 703-590-7337
Cell (b) (6)
Fax 703-580-8637

From: Animalfood-premarket [mailto:Animalfood-premarket@fda.hhs.gov]
Sent: Monday, December 27, 2021 4:33 PM
To: Kristi Smedley
Cc: Animalfood-premarket; Cerrito, Chelsea
Subject: AGRN #44 Amendment Clarification

Good afternoon,

We would like to clarify our question pertaining to the presence of other potential contaminants in the market formulation. The notifier stated in its amendment that "the absence of methanol contamination indirectly illustrates that formaldehyde is not a contamination concern neither in raw material nor in final manufactured

product." However, we note that methanol induces the production of alcohol oxidase, which is the first step in metabolism of methanol, and results in the production of formaldehyde and hydrogen peroxide. Thus, there is potentially an inverse relationship between methanol and formaldehyde and hydrogen peroxide concentrations. Thus, the notifier did not address CVM's concerns regarding the presence of formaldehyde and hydrogen peroxide and other potential contaminants. The notifier should provide information to illustrate that the market formulation would not contain formaldehyde and hydrogen peroxide at levels that would raise safety concerns. The notifier could, for example, use information pertaining to its current good manufacturing practices, batch records, process control information, analytical data for formaldehyde and hydrogen peroxide in the final product, and/or information in the scientific literature on intracellular metabolism of formaldehyde and hydrogen peroxidase by yeast.

We are also seeking clarification regarding our questions on the contaminant specifications. In the amendment discussion on September 15, 2021, CVM requested the notifier to "address and clarify the presence of other potential contaminants, as appropriate," and to clarify how those limits were established. However, the amendment included a replacement "Specification Table, Table 2" which omitted following specifications from the original table (both copied below): fumonisins, zearalenone, deoxynivalenol, ochratoxin, dioxins, dioxin and dioxin-like PCBs, non-dioxin like PCBs, mercury, cadmium, and absence of genetically modified organisms. Did the notifier intend to omit these specifications in the replacement Table 2 or should the original Table 2 with all specifications be maintained? If the omitted specifications are no longer monitored, should explain why this change does not affect the notifier's safety conclusion.

In your submission dated December 29, 2020, you provided the following Specification Table, Table 2:

Property	Specification
Color	Light Grey powder
Uniformity	No visible impurities
Moisture	< 3 %
Xylanase activity	≥ 150,000 XU/g
Mold	$\leq 10^3$ CFU/g
Coliforms	≤10 CFU/g
Escherichia coli	≤ 10 CFU/g
Salmonella	Not detected per 25 g
Heavy metals: Arsenic	< 2 mg/kg
Heavy metals: cadmium	< 2 mg/kg
Heavy metals: mercury	< 0.5 mg/kg
Heavy metals: lead	< 10 mg/kg
Mycotoxins: aflatoxin B1, B2, G1, G2	≤ 0.5 µg/kg
Mycotoxins: fumonisin B1 and B2	≤ 25 µg/kg
Mycotoxins: zearalenone	≤ 30 µg/kg
Mycotoxins: deoxynivalenol	≤ 100 µg/kg
Mycotoxins: Ochratoxin	≤ 1 µg/kg
Dioxins	≤ 1 ng/kg TEQ
Dioxin & Dioxin-Like PCBs	≤ 1.5 ng/kg TEQ
Non-Dioxin-Like PCBs	≤ 10 µg/kg TEQ
Genetically modified organisms	Absent

In your submission dated October 7, 2021, you provided the following replacement Specification Table, Table 2:

Property	Specification
Appearance	Light Grey powde
Moisture, %	< 3 %
Xylanase activity, XU/	≥ 150,000 XU/g
Mycotoxins, ppb	
Aflatoxin B1	<0.5
Aflatoxin B2	<0.5
Aflatoxin G1	<0.5
Aflatoxin G2	<0.5
Heavy Metals, mg/kg	
Arsenic	<3
Lead	<3
Microbial contaminants, CFU/g	
Coliforms	<10
E. Coli	<10
Salmonella	ABSENCE in 25
Molds	<10

If the notifier is able to provide this clarification within the next two weeks, **no later than January 11, 2022**, we will continue our evaluation of the notice. If the notifier is not able to provide this clarification, it may, as always, request that we cease to evaluate the GRAS notice. Please send any information to animalfood-premarket@fda.hhs.gov.

If you have questions, please do not hesitate to reach out.

Megan

Megan Hall M.S.
Staff Fellow Animal Scientist

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January 18, 2022

David Edwards, Director
Division of Animal Feeds (HFV- 220)
Center for Veterinary Medicine
Food and Drug Administration
7519 Standish Pl.
Rockville, MD 20855

Subject: Response to Division of Animal Feeds Concerns—December 27, 2021
Xylanase preparation for the use in swine and poultry feed
AGRN 44

Notifier: BioResource International, Inc.
4222 Emperor Blvd., Suite 460
Durham, NC USA 27703

Dear Dr. Edwards:

On behalf of BioResource International, Inc., I am providing clarification of issues as raised in the review of animal GRAS notice for the use of Xylanase prepared from Komagataella phaffii expressing the gene encoding xylanase from Orpinomyces spp. for use in poultry and swine diets. These issues were raised in an email of December 27, 2021.

We have provided a narrative describing our response to the two issues raised, as well as the references that support this assessment.

Should you have any questions on the filing, please contact me directly.

Sincerely,

Kristi Smedley

Digitally s gned by Kr sti Smedley
DN: cn=Kristi Smedley o=Center for Regulatory Services Inc ou email=smedley@cfr services com c=US
Date: 2022 01 18 10:04:26 05 00

Kristi O, Smedley
Consultant to BioResource International, Inc.

Cc: Rasah Qudsieh, BRI

ATTACHMENTS:

As Described in the letter

Response to FDA questions related to AGRN 44— December 27th 2021

Question #1:

FORMALDEHYDE AND HYDROGEN PEROXIDE:

The notifier should provide information to illustrate that the market formulation would not contain formaldehyde and hydrogen peroxide at levels that would raise safety concerns. The notifier could, for example, use information pertaining to its current good manufacturing practices, batch records, process control information, analytical data for formaldehyde and hydrogen peroxide in the final product, and/or information in the scientific literature on intracellular metabolism of formaldehyde and hydrogen peroxidase by yeast.

Response:

The methylotrophic yeast *Komagataella phaffii* (*K. phaffii*) has a tightly regulated system to detoxify the formaldehyde and hydrogen peroxide produced when methanol is utilized during fermentation. The metabolism of methanol in methylotrophic yeast is summarized below and illustrated in Figure 1 [1]:

Both formaldehyde and hydrogen peroxide are considered toxic for the methylotrophic yeast, therefore, the yeast has the detoxification system in place, which reduces the risk of having formaldehyde and hydrogen peroxide residual in the fermentation broth.

In the case of the manufacture of the GRAS substance, *Komagataella phaffii* enzyme preparation, any possible formaldehyde and hydrogen peroxide in the product would be eliminated due to the conditions of downstream spray drying process, which is used for producing the dried enzyme substance (in this case xylanase). This is due to the high temperature of spray drying (b) (4). Based on literature, the Heat of Vaporization (how much heat per mole that has to be added to make the compound to evaporate) of formaldehyde is 24.3 KJ/mol [2], which is much less than water (40.7 kJ/mol), indicating it's easier to evaporate. While the Heat of Vaporization for hydrogen peroxide is 48.5 KJ/mol [3], hydrogen peroxide was reported to be unstable at temperature higher than (b) (4) and can easily be decomposed into water and oxygen gas [4]

Therefore, based on literature and manufacturing process, we do not expect any residual of formaldehyde and hydrogen peroxide in the final product of active substance of xylanase enzyme.

To confirm our white paper assessment provided above, we were able to provide a sample of the *Komagataella phaffii* enzyme preparation to our cooperating laboratory. They conducted a GC-MS formaldehyde assay as validated for food. Their analysis (attached) found formaldehyde level below 5 ppm, as we had expected.

Exposure assessment

The amount of final product formulation of *Komagataella phaffii* enzyme added per ton of feed is 0.20 pound/ton, and only 20% of this product formulation is the *Komagataella phaffii* enzyme (active substance), rest 80% is a carrier (hence only 0.04 pound on the enzyme prep per ton).

Our assessment (above) would suggest that no formaldehyde or hydrogen peroxide would be found in the final product. However, in a ridiculous assumption that the product would ONLY be either formaldehyde or Hydrogen peroxide the amount of formaldehyde or hydrogen peroxide added to the feed form this source is 0.04 pounds/ton (equivalent to 18.14 ppm).

Both formaldehyde and hydrogen peroxide are authorized feed ingredients that can be added directly to feed. Formaldehyde is an approved food additive that is used in feed as an antimicrobial agent to be used at the level of 5.4 pounds/ton of feed (21 CFR 573.460(b)). The FDA has concurred with the safe use of that level approximately 1000 ppm.

Hydrogen peroxide is a GRAS General Purpose Food additive that limitation is "This substance is generally recognized as safe when used as a bleaching agent in accordance with good manufacturing or feeding practice" (no set limit) (21 CFR 582.1366). Again, the level of hydrogen peroxide that would contaminate the final product would be miniscule.

Therefore, at the extremely low level of use of this *Komagataella phaffii* enzyme preparation and the understanding that negligible levels of formaldehyde or hydrogen peroxide would contaminate the final product, supports the fact there is reasonable certainty that the substances not harmful to either the target animal or to humans consuming human food derived from food-producing animals under the conditions of its intended use.

Question #2:

CHANGE IN SPECFICATIONS:

Did the notifier intend to omit these specifications in the replacement Table 2 or should the original Table 2 with all specifications be maintained? If the omitted specifications are no longer monitored, should explain why this change does not affect the notifier's safety conclusion.

Response:

Several batches of the product has been analyzed for multiple mycotoxins, heavy metals, Dioxins, and microbial contaminants. These compounds were initially included in the specifications with an overabundance of caution. For example for the Dioxin-related compounds, but there is no real source of these compounds in the manufacturing process. The more rare mycotoxins were found below the LOD, and the aflatoxins, are more likely to be sentinel of a possible mycotoxin problem.

The analytical results of removed specified compounds were below detection limits, indicating that the final product is safe and does not contain any detectable contaminants; therefore, the list of specifications was revised to include select/representative mycotoxins, heavy metals, and microbial contaminants to be periodically tested. Furthermore, QA/QC program control on

ingredients used in manufacturing are in place to guarantee safety and wholesomeness of the ingredients used.

The fact that the removed originally listed specifications, are below the level of detection, and the very low level of the product incorporated in the feed and the close tracking of similar contaminants will assure the safety of the product.

References:

(b) (4)

[2] Chemical Properties of Formaldehyde (CAS 50-00-0) – Cheméo: https://www.chemeo.com/cid/44-234-3/Formaldehyde

[3] Chemical Properties of Hydrogen peroxide (CAS 7722-84-1): https://www.chemeo.com/cid/67-803-6/Hydrogen%20peroxide

(b) (4)

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(b) (4)

BioResource International, Inc.

(b) (6)
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St 460
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ANALYTICAL REPORT

(b) (4)

Client Code: (b) (4)

Received On: 10Jan2022
Reported On: 14Jan2022

(b) (4)

Client Sample Code: (b) (4)

Sample Registration Date: 10Jan2022
Condition Upon Receipt: acceptable, 20°C

Sample Description: Powdered Feed Additive

Sample Reference:

(b) (4) **Formaldehyde (Food, GC-MS)**

Reference
Internal Method

Completed
14Jan2022

Sub
1

Parameter	Result
Formaldehyde	(b) (4)

Subcontracting partners:
(b) (4)

Respectfully Submitted,

(b) (6)

(b) (6)
Business Unit Manager

Results shown in this report relate solely to the item submitted for analysis. | Any opinions/interpretations expressed on this report are given independent of the laboratory's scope of accreditation. | All results are reported on an "As Received" basis unless otherwise stated. | Reports shall not be reproduced except in full without written permission of (b) (4). | All work done in accordance with (b) (4) General Terms and Conditions of Sale: (b) (4) | ^ Indicates a subcontract test to a different lab. Lab(s) are listed at end of the report. For further details about the performing labs please contact your customer service contact at (b) (4) Measurement of uncertainty can be obtained upon request.

1/14/22 7:49 pm

ELSEVIER

FEMS Yeast Research 5 (2005) 1079 1096

FEMS Yeast Research

www.fems microbiology.org

MiniReview

New yeast expression platforms based on methylotrophic *Hansenula polymorpha* and *Pichia pastoris* and on dimorphic *Arxula adeninivorans* and *Yarrowia lipolytica* A comparison

Gerd Gellissen [a], Gotthard Kunze [b,*], Claude Gaillardin [c], James M. Cregg [d], Enrico Berardi [e], Marten Veenhuis [f], Ida van der Klei [f]

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[b] *Institut für Pflanzengenetik und Kulturpflanzenforschung, Correnstr. 3, 06466 Gatersleben, Germany*
[c] *Génétique Moleculaire et Cellulaire, UMR1238 INAPG INRA CNRS, Institut National Agronomique, Paris Grignon, 78850 Thiverval Grignon, France*
[d] *Keck Graduate Institute of Applied Sciences, 535 Watson Drive, Claremont, CA 91711, USA*
[e] *Università degli Studi, Via Brecce Bianche, 60131 Ancona, Italy*
[f] *Eukaryotic Microbiology, RUG, Kerklaan 30, 9751 Haren, The Netherlands*

Received 25 April 2005; received in revised form 9 June 2005; accepted 9 June 2005

First published online 24 August 2005

Abstract

Yeasts combine the ease of genetic manipulation and fermentation of a microbial organism with the capability to secrete and to modify proteins according to a general eukaryotic scheme. Yeasts thus provide attractive platforms for the production of recombi nant proteins. Here, four important species are presented and compared: the methylotrophic *Hansenula polymorpha* and *Pichia pas toris*, distinguished by an increasingly large track record as industrial platforms, and the dimorphic species *Arxula adeninivorans* and *Yarrrowia lipolytica*, not yet established as industrial platforms, but demonstrating promising technological potential, as discussed in this article.

Keywords: *Hansenula polymorpha*; *Pichia pastoris*; *Arxula adeninivorans*; *Yarrrowia lipolytica*; Yeast expression platforms

(b) (4)

1567 1356/$22.00
doi:10.1016/j.femsyr.2005.06.004

Formaldehyde

Other names: Aldehyd mravenci; Aldehyde formique; Aldeide formica; BFV; CH2O; Durine; Fannoform; Fordor; Formaldehyd; Formaldehyde, gas; Formalin; Formalin 40; Formalin-loesungen; Formalina; Formaline; Formalith; Formic aldehyde; Formol; Fyde; H2CO; Hercules 37M6-8; Karsan; Lysoform; Methaldehyde; Methanal; Methyl aldehyde; Methylene oxide; Morbicid; NCI-C02799; NSC 298885; Oplossingen; Oxomethane; Oxymethylene; Paraform; Rcra waste number U122; Superlysoform; UN 1198; UN 2209.

InChI: InChI=1S/CH2O/c1-2/h1H2

InChI Key: WSFSSNUMVMOOMR-UHFFFAOYSA-N

Formula: CH2O

SMILES: C=O

Molecular Weight: 30.03

CAS: 50-00-0

Physical Properties

Property	Value	Unit	Source
PAff	712.90	kJ/mol	NIST Webbook
PAff	711.50 ± 2.10	kJ/mol	NIST Webbook
BasG	683.30	kJ/mol	NIST Webbook
BasG	681.50 ± 0.70	kJ/mol	NIST Webbook
$\Delta_c H°_{gas}$	-570.78 ± 0.42	kJ/mol	NIST Webbook
$\Delta_c H°_{gas}$	-561.10	kJ/mol	NIST Webbook
$\Delta_f G°$	-89.60	kJ/mol	Joback Method
$\Delta_f H°_{gas}$	-108.60 ± 0.46	kJ/mol	NIST Webbook
$\Delta_f H°_{(+)\ ion}$	941.80	kJ/mol	NIST Webbook
$\Delta_f H_{(+)\ ion,0K}$	946.00	kJ/mol	NIST Webbook
$\Delta_{fus} H°$	2.32	kJ/mol	Joback Method
$\Delta_{vap} H°$	24.39	kJ/mol	Joback Method
IE	10.88 ± 0.01	eV	NIST Webbook
IE	10.88	eV	NIST Webbook
IE	10.89 ± 0.00	eV	NIST Webbook

Property	Value	Unit	Source
IE	10.86	eV	NIST Webbook
IE	10.88	eV	NIST Webbook
IE	10.90	eV	NIST Webbook
IE	10.88 ± 0.01	eV	NIST Webbook
IE	10.87 ± 0.00	eV	NIST Webbook
IE	10.87 ± 0.01	eV	NIST Webbook
IE	10.88 ± 0.02	eV	NIST Webbook
IE	10.87 ± 0.01	eV	NIST Webbook
IE	10.88 ± 0.02	eV	NIST Webbook
IE	10.88	eV	NIST Webbook
IE	10.86 ± 0.02	eV	NIST Webbook
IE	10.90 ± 0.03	eV	NIST Webbook
IE	10.87 ± 0.01	eV	NIST Webbook
IE	10.88 ± 0.01	eV	NIST Webbook
IE	10.10	eV	NIST Webbook
$logP_{oct/wat}$	-0.18		Crippen Method
P_c	6631.37	kPa	Joback Method
T_{boil}	254.05 ± 0.50	K	NIST Webbook
T_{boil}	253.85 ± 0.20	K	NIST Webbook
T_c	436.48	K	Joback Method
T_{fus}	156.15 ± 1.00	K	NIST Webbook
T_{fus}	181.00 ± 4.00	K	NIST Webbook
T_{fus}	181.00 ± 6.00	K	NIST Webbook
T_{triple}	155.10 ± 0.30	K	NIST Webbook
V_c	0.10	m^3/kg-mol	Joback Method

Temperature Dependent Properties

Property	Value	Unit	Temperature (K)	Source
$C_{p,gas}$	25.89	J/mol×K	270.24	Joback Method
η	0.00	Pa×s	270.24	Joback Method
$\Delta_{fus}H$	7.53	kJ/mol	155.0	NIST Webbook
$\Delta_{vap}H$	24.20	kJ/mol	212.0	NIST Webbook
$\Delta_{vap}H$	24.30	kJ/mol	217.5	NIST Webbook

Sources

Joback Method: https://en.wikipedia.org/wiki/Joback_method
NIST Webbook: http://webbook.nist.gov/cgi/inchi/InChI=1S/CH2O/c1-2/h1H2
Crippen Method: http://pubs.acs.org/doi/abs/10.1021/ci990307l

Legend

PAff: Proton affinity (kJ/mol).
BasG: Gas basicity (kJ/mol).
Δ_c**H°**$_{gas}$: Standard gas enthalpy of combustion (kJ/mol).
C$_{p,gas}$: Ideal gas heat capacity (J/mol×K).
η: Dynamic viscosity (Pa×s).
Δ_f**G°**: Standard Gibbs free energy of formation (kJ/mol).
Δ_f**H°**$_{gas}$: Enthalpy of formation at standard conditions (kJ/mol).
Δ_f**H°**$_{(+)\ ion}$: Enthalpy of formation of positive ion at standard conditions (kJ/mol).
Δ_f**H**$_{(+)\ ion,0K}$: Enthalpy of formation of positive ion at 0K (kJ/mol).
Δ_{fus}**H°**: Enthalpy of fusion at standard conditions (kJ/mol).
Δ_{fus}**H**: Enthalpy of fusion at a given temperature (kJ/mol).
Δ_{vap}**H°**: Enthalpy of vaporization at standard conditions (kJ/mol).
Δ_{vap}**H**: Enthalpy of vaporization at a given temperature (kJ/mol).
IE: Ionization energy (eV).
***log*P**$_{oct/wat}$: Octanol/Water partition coefficient .
P$_c$: Critical Pressure (kPa).
T$_{boil}$: Normal Boiling Point Temperature (K).
T$_c$: Critical Temperature (K).
T$_{fus}$: Normal melting (fusion) point (K).
T$_{triple}$: Triple Point Temperature (K).
V$_c$: Critical Volume (m^3/kg-mol).

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Hydrogen peroxide

InChI: InChI=1S/H2O2/c1-2/h1-2H
InChI Key: MHAJPDPJQMAIIY-UHFFFAOYSA-N
Formula: H2O2
SMILES: OO
Molecular Weight: 34.01
CAS: 7722-84-1

Physical Properties

Property	Value	Unit	Source
PAff	674.50	kJ/mol	NIST Webbook
BasG	643.80	kJ/mol	NIST Webbook
• $_{f}G°$	-324.52	kJ/mol	Joback Method
• $_{f}H°_{gas}$	-347.79	kJ/mol	Joback Method
• $_{fus}H°$	3.93	kJ/mol	Joback Method
• $_{vap}H°$	48.95	kJ/mol	Joback Method
IE	10.58 ± 0.04	eV	NIST Webbook
IE	10.62	eV	NIST Webbook
IE	10.54	eV	NIST Webbook
IE	10.92 ± 0.05	eV	NIST Webbook
IE	11.26 ± 0.05	eV	NIST Webbook
IE	11.70	eV	NIST Webbook
IE	11.69	eV	NIST Webbook
$logP_{oct/wat}$	0.02		Crippen Method
P_c	22000.00 ± 2000.00	kPa	NIST Webbook
T_{boil}	383.76	K	Joback Method
T_c	728.00 ± 15.00	K	NIST Webbook

Property	Value	Unit	Source
T_{fus}	272.26 ± 0.30	K	NIST Webbook
V_c	0.07	m^3/kg-mol	Joback Method

Temperature Dependent Properties

Property	Value	Unit	Temperature (K)	Source
$C_{p,gas}$	36.05	J/mol×K	383.76	Joback Method
•	0.00	Pa×s	383.76	Joback Method
• $_{vap}H$	48.50	kJ/mol	320.0	NIST Webbook

Sources

Joback Method: https://en.wikipedia.org/wiki/Joback_method
NIST Webbook: http://webbook.nist.gov/cgi/inchi/InChI=1S/H2O2/c1-2/h1-2H
Crippen Method: http://pubs.acs.org/doi/abs/10.1021/ci990307l

Legend

PAff: Proton affinity (kJ/mol).
BasG: Gas basicity (kJ/mol).
$C_{p,gas}$: Ideal gas heat capacity (J/mol×K).
• : Dynamic viscosity (Pa×s).
• **$_fG°$**: Standard Gibbs free energy of formation (kJ/mol).
• **$_fH°_{gas}$**: Enthalpy of formation at standard conditions (kJ/mol).
• **$_{fus}H°$**: Enthalpy of fusion at standard conditions (kJ/mol).
• **$_{vap}H°$**: Enthalpy of vaporization at standard conditions (kJ/mol).
• **$_{vap}H$**: Enthalpy of vaporization at a given temperature (kJ/mol).
IE: Ionization energy (eV).
***log*P$_{oct/wat}$**: Octanol/Water partition coefficient .
P_c: Critical Pressure (kPa).
T_{boil}: Normal Boiling Point Temperature (K).
T_c: Critical Temperature (K).
T_{fus}: Normal melting (fusion) point (K).
V_c: Critical Volume (m^3/kg-mol).


(b) (4)


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