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THE EVALUATION OF ENZYME ACTIVITY OF CELLULASE, LIGNINASE, AND IDENTIFICATION OF MICROBIAL MORPHOLOGY AT LOCAL BIOACTIVATOR FROM CATTLE AND BUFFALO RUMEN CONTENT WITH DIFFERENT ENERGY SOURCES

Tri Astuti^{1*}, Syahro A. Akbar¹, M. Nasir Rofiq², Novirman Jamarun³, Nurul Huda⁴, Ahmad Fudholi^{2,5}

¹ Department of Animal Science, Faculty of Agriculture University of Mahaputra

Muhammad Yamin,

Jenderal Sudirman Street No 6 Solok City, West Sumatera. Indonesia

² Department of Agribisnis, Faculty of Agriculture University of Mahaputra Muhammad

Yamin,

Jenderal Sudirman Street No 6 Solok City, West Sumatera. Indonesia

³ Agency for The Assessment and Application of Technology, Indonesia

⁴ Faculty of Animal Science, Andalas University, Padang, West Sumatera, Indonesia

⁵ Solar Energy Research Institute, Universiti Kebangsaan Malaysia, 43600, Bangi, Selangor, Malaysia

Correspondent email : adektuti@gmail.com

ABSTRACT

This study aims to determine the activity of cellulase and ligninase enzymes on local bioactivator. This was based on the rumen contents of the rumen incubated for seven days with supplies of different sources of enzyme energy. The method used was a factorial design of 2 x 4 with three replications for each treatment. Factor A was the type of Animal (Cattle Vs Buffalo), factor B is the addition of an enzyme source supply material: B1 = Molasses, B2 = Molasses + palm frond extract, B3 = Molasses + palm leaf extract, B4 = Molasses + palm frond, and palm leaf extract. Also, the parameters observed were the activity of enzymes cellulase, laccase, lignin and manganese peroxidase, as well as microbial identification. The results of statistical analysis showed that the activity of the enzymes cellulase 2,22-3,51 U/ml, laccase 10,62- 20,11 U/ml, lignin peroxidase 1,74-4,93 U/ml, and manganese peroxidase 2,40-7,06 U/ml showed insignificant differences ($p > 0.05$). The results of microbial identification found the bacteria *Lactobacillus* sp. Based on these, it was concluded that the microbes discovered in the local microorganism solution live because of the study environment, not the microbes inherited from the rumen's contents.

INTRODUCTION

Animal feeding is a major factor in determining the success of the livestock business. Furthermore, many study uses by-products of agriculture and plantation as substitutes for the field grass. These by-products mostly contain lignocellulosic bonds that consist of cellulose, hemicellulose, and lignin, which cause the low nutritional value of these ingredients when directly fed as feed. Used of palm oil fronds as animal feed barried are low crude protein about 2.11%, high crude fiber content reaching 46.75% [1](Murni et al., 2008). Astuti et al., (2019) reported the content of ADF 56,93%, NDF 78,05%, cellulose 21,91%, hemicellulose 15,34% and Lignin 15.34% [2]. Lignin is a polymer complex phenylpropanoids, heterogeneous, and 25-30% plant biomass. It is quite resistant to microbial degradation under natural conditions. Lignin and cellulose are the main components in plants enzymatically degraded by microorganisms such as enzyme cellulase and ligninase. Ligninolytic microbial systems have been used in improving digestibility and nutritional value. Primarily three enzymes such as lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase [3.4.5.6] (Glenn et al., 1983; Hatakka and Uusi-Rauva, 1983; Glenn and Gold, 1985), have been considered responsible for lignin degradation of animal feeds [7](Zadrazil, 1980). Lignin peroxidase (LiP) and manganese peroxidase (MnP) are extracellular peroxidase enzymes that use H₂O₂ to degrade lignin, while laccase is a copper-containing enzyme that uses molecular oxygen to degrade lignin [8] (Hattaka, 1994).

The use of microbes for enzymes producer has several advantages, including low production costs, short production time, high growth speed, and ease to control. Factors such as fermentation medium, temperature, aeration, agitation, pH, and strains, which play a role in producing these enzymes, need to be optimized. The Cheaped and inexpensive Fermentation media is required since it is easy to obtain and produce expected enzymes for large production (Trismilah et al., 2003).

Many rumen contents on the slaughterhouse waste pollute the environment due to the rumen's abundant availability contents. It was being seen based on the slaughterhouse's number of slaughtered cattle and buffalo. Based on BPS Indonesia (2021) data, the average of Livestock Slaughtered at Slaughtering House (Abattoir) in 2019-2020 was 1069.614 heads of cows and 27,487 heads of buffaloes. Beef rumen fluid, apart from containing rumen microbes and enzymes secreted by rumen microbes, also contains food substances resulting from overhaul rumen microbes and enzymes and vitamins and minerals that dissolve in the rumen fluid. The cow's rumen content was 9.29% water; 8.45% crude protein; 1.23% crude fat;

33.53% crude fiber; 0.20% Ca; 0.45% P; 16.19% ash; and 31.60% NFE, and vitamin B12. The rumen contents have the potential as feed additives (Abbas, 1987). This slaughtering of livestock leaves the contents of the rumen untapped, only to be thrown away.

Rumen fluid deposits are rich in protein, amino acids, vitamins, and minerals. The composition of amino acids, minerals, vitamins, and enzymes also depends on the feed treatment (Budiansyah et al., 2011). The assumed rumen microbes would produce enzymes according to the given substrate. For example, when given straw, it will produce enzymes to degrade fiber and break down tannins when given a substrate of calliandra (Wina, 2005). The fiber content of Lamtoro leaf meal decreased by 53.640% with 100 ml/kg of rumen content enzyme extract (Fitriyani, 2011). Lignin and cellulose were the main components in plants that the enzyme cellulase and ligninase enzymatically degrades. These enzymes were produced by microorganisms (Pandey et al., 2000). Molasses are the waste of sugar mills, cheapest and easily as carbon sources rich in nutrients and minerals, and have the potential for microbial growth media (Anggraini et al., 2016).

Enzymes are biopolymer molecules composed of a series of amino acids in an ordered and fixed composition and chain arrangement. Enzymes were proteins produced and used by living cells to catalyze chemical reactions with a high level of specification and an increase in reaction rates (Richana, 2002; Beilen & Li, 2002). Thus, enzymes have various advantages over conventional processes using chemicals. However, the main obstacle for applying enzymes in the industry is the high price of enzymes, and the enzyme cannot be used repeatedly (Huey, 2008; Troger & Niranjana, 2010; Wibisono, 2010).

Cellulases are complex enzymes that gradually cut the cellulose chains into glucose. Fungi, bacteria, and ruminants produce cellulase. The commercial production of enzymes usually uses fungi or bacteria. The production of cellulase enzymes from ruminants has not been much appreciated, however, cellulases originating from ruminant is being produced given their large availability. Rumen fluid enzymes as an alternative technology are used in hydrolyzing crude fiber to increase the nutritional value of local feed raw materials (Pamungkas, 2012). Hartati's (2012) stated that cellulase enzymes have the potential to be produced from beef rumen fluid compared to commercial enzymes. Therefore, this study aims to determine the activity of cellulase and ligninase enzymes in local microorganisms with several different ingredients added. Additionally, microbes are considered to produce enzymes according to the food they get.

MATERIALS AND METHODS

The material used in this study was rumens content of cattle and buffalo, molasses, soybean water immersion, oil palm frond, oil palm leaf, and some chemistry for measuring enzyme activity.

The bioactivator process: The rumen content of cattle and buffalo were collected from the abattoir and placed in tubes. All the content of rumen treatments was added with molasses and tofu water immersion. The treatments were the addition of palm leaves, palm fronds, and a mixture of both, which had high lignin content. The addition of palm fronds and leaves is intended, hence, the microorganisms that develop are microorganisms capable of producing the ligninase enzyme. The contents of the rumen, molasses, tofu soaked wastewater were mixed in a ratio of 1:1:8, and 10% of the oil palm frond and leaf. The tube is tightly closed, after which a hole is made in the middle and connected with a hose to a small bottle filled with water to catch the waste of fermentation, then incubated for as long as ten days, as shown in Figure 1.



Figure 1. Fermentation Process local bioactivator

The method for enzyme activity test:

Test Activity of Lignin Peroxidase (LiP) Enzymes

A 0.2 ml of enzyme filtrate (extract sample and buffer pospat were shaken until 1 hour and then centrifuged for as long as 10 minutes with 10000 rpm), 0.05 ml of H₂O₂ 5 mM, 0.1 ml of veratrine alcohol 8 mM, 0.2 ml of acetate buffer 0.05 M pH 3, and 0.45 ml of distilled water are added to cuvette and then shaken. The absorbance of the solution was at a wavelength of 310 nm for 0 and 30-minute intervals. One unit of enzyme activity in LiP is defined as the amount of enzyme that causes the conversion of 1 micromol (1 μ mol = 10⁻⁶ mol) veratril alcohol per minute (Syafrizal, 2007)

Test the Activity of Manganese Peroxidase (MnP) Enzymes

As much as 0.1 ml of 50 mM Na-lactate buffer pH five is added with 0.1 ml of 4 mMguaiacol, 0.2 ml of MnSO₄ 1 mM, 0.1 ml of H₂O₂ 1 mM, and distilled water 0.3 ml, as well as 0.2 filtrates are added enzyme. The solution was then checked and read at a wavelength of 465 nm for 0 and 30 minutes (Leonowicz and Grzywnowicz, 1981)

Test the Activity of Lakase Enzymes

As much as 0.4 ml of enzyme filtrate plus 0.5 ml pH five acetate buffer of 0.5 ml and 0.1 ml of 1 mM ABTS. Then it was checked, and measurements were read to 420 nm wavelength. The reading was taken for 0 and 30 minutes (Wariishi et al. 1., 1992).

Enzyme activity tests were conducted in the biotechnology laboratory of the Faculty of Animal Husbandry, Andalas University, Padang, and identification of microbial morphology was performed at the Baso veterinary center laboratory, Bukit Tinggi. The analysis was continued by identifying the morphology of fungi and bacteria present in the local bioactivator rumen contents. The identification of microbes was performed based on the results of the best enzyme activity evaluation. Samples were inoculated on Sodium Agar (NA) medium for identification of bacteria and Potato dextrose Agar (PDA) media as a medium for growing fungi/molds. The samples were diluted in 10⁻¹ - 10⁻¹⁰, and the selected isolates were further to be analyzed based on even colony distribution

Experimental design:

The factorial randomized block design 2 X 4 with three replications for each treatment used in this study. Factor A is the type of rumen content: A1 = Rumen cattle, A2 = Buffalo Rumen, Factor B supplies microbial energy: B1 = Molases, B2 = Molases + palm frond extract, B3 = Molases + palm leaf extract, B4 = Molases + extract of palm fronds and leaves. Enzyme activity data processing was performed using analysis of variance while identifying microorganisms using the described method. When the study resulted in a significant different affected ($p < 0.05$), it will be further tested using duncan's multiple range tests,

The variables to be observed were the isolation and identification of microorganisms (fungi and bacteria) by morphology and cellulase and ligninase activity tests for crude bio enzymes of rumen content. The morphology data are shown in description analysis.

RESULTS AND DISCUSSION

Enzyme Activity Test

The enzymes activity test were cellulase enzyme, Lacasse Enzymes, Enzim Lignin Peroksidase, Manganese Peroxidase Enzymes. Furthermore, these enzymes are essential for lignin degradation. The average results of the enzyme activity test for local microorganisms in the rumen are shown in Table 1.

Table 1. Average activity of local microorganisms in the rumen contents (U/ml)

Factor B	Factor A				Rataan
	B ₁	B ₂	B ₃	B ₄	
Cellulase enzymes					
A1	3,51	2,41	2,81	2,22	2,74
A2	3,44	2,52	3,31	3,64	3,23
	3,48	2,46	3,06	2,93	2,98
Lacasse Enzymes					
A1	20,11	16,40	11,70	15,91	16,03
A2	11,00	12,86	10,62	14,59	12,27
	15,55	14,63	11,16	15,25	14,15
Enzim Lignin Peroksidase					
A1	4,06	4,93	2,80	4,11	3,97
A2	2,61	1,74	4,16	3,80	3,08
	3,34	3,34	3,48	3,95	3,53
Manganese Peroxidase Enzymes					
A1	2,40	5,61	3,39	7,06	4,61
A2	6,86	4,17	3,18	5,18	4,85
	4,63	4,89	3,29	6,12	4,73

Note: A1 = contents of cow's rumen, A2 = contents of buffalo's rumen, B1 = contents of rumen only, B2 = contents of rumen and palm fronds, B3 = palm leaves, and B4 = leaves and palm fronds

The results of the analysis of statistics showed no interaction effect ($P > 0.05$) between the kind of rument resource (cattle and buffalo) with the sources of microbe energy on the activity of cellulase enzymes, laccases, Lignin Peroxidase Enzymes, and Manganese Peroxidase Enzymes. The data shows the rumen contents were used as a source of microorganisms that were ingested and still dominant in the form of fiber from forage consumed by livestock are given the main energy source in the form of molasses and tofu

soaking water for all treatments. Therefore, it is suspected that the main energy source shown is very representative, supplying the energy needed by microbes in the rumen to grow and develop to produce high value. This study's average cellulase enzyme activity result was much higher than Murtiyaningsih and M. Hazmi's (2017), measuring the activity of cellulase enzymes from cellulolytic bacteria from soil and getting the highest enzyme activity of 0.028279 U/ml. This was due to a large number of microbes in the rumen contents, then the addition of molasses and tofu soaking wastewater further increases microbial growth. Astuti et al. study (2020) found 40×10^{12} total colonies in 1 ml of local bio activator rumen contents mixed with molasses, soybean soaking water, and palm oil fronds.

The average laccase enzyme activity ranged from 11.0 to 20.11 U/ml, the mean lignin peroxidase enzyme ranged from 1.74 to 4.93, while the manganese peroxidase enzyme ranged from 2.40 - 7.06 U/ml. Laccase enzyme activity was much higher than Dimawarnita and Panji's (2018), which measured enzyme activity in *Pleurotus ostreatus* with media containing OPEFB 0.35 U/mL and lignin peroxidase (LiP) activity of 0.269 U/mL. Astuti et al. (2021) stated lignin enzyme activity of sugarcane fermentation by 10% *Phanerochaete chrysosporium* were laccase 2.02 U/ml, LiP 1,677 U/ml, MnP 0,33 U/ml. This was because the microbes that thrive in this local activator have been added to the palm fronds and leaves high in lignin content. Rumen microbes will develop and produce enzymes based on the feed given.

Identification of Microorganism Morphology

The analysis identifies fungi and bacteria's morphology present in the local bioactivator rumen contents. The identification of microbes was performed based on the results of the best enzyme activity evaluation.

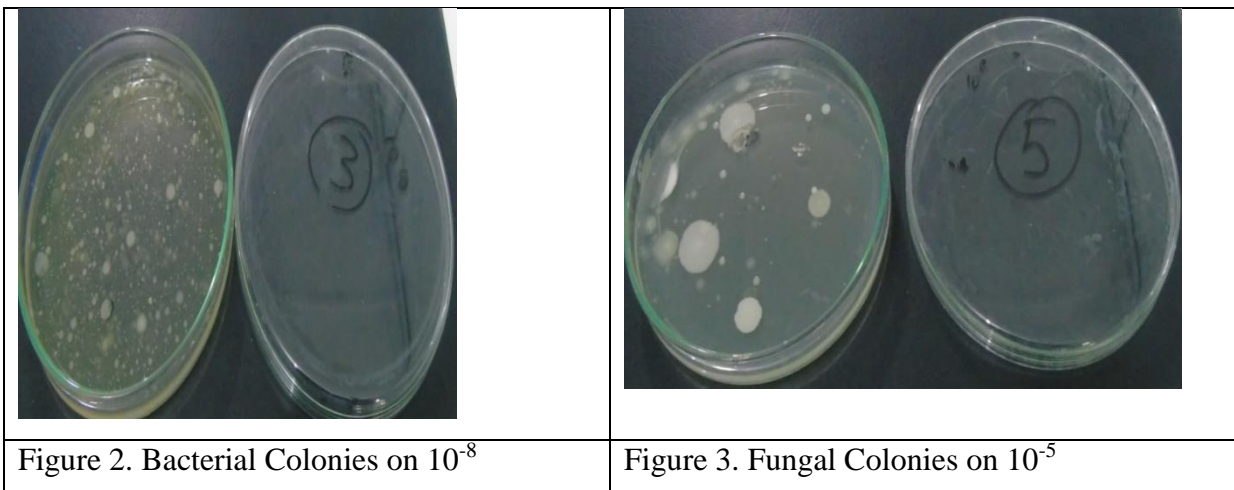


Figure 2 shows that The Bacterial colonies are circular, small spread out, thin, the edges of the settlements are flat, white in color, and the structure is transparent. Morphological observations of fungal colonies showed circular colony shape, elevation convex, uneven edges, white color, and transparent structure (Figure 3). The results of the chemical analysis are shown in table 2.

Table 2. Chemical examination results for microorganism isolates on NA media

No	Treatment	NA 1	NA 2
1.	Koloni(Warna, bentuk, Sifat)	White	White
2.	Gram (Marfologi, Spora)	+ bacteri	+ bacteri
3.	Aerobic / Anaerobic	A	A spora
4.	TSIA	M/K	M/K
5.	Gas	-	+
6.	H2S	-	-
7.	catalase	+	+
8.	Oxidase	-	-
9.	Motility	+	+
10.	Indole	-	-
11.	Urea	+	+
12.	Citrat	-	-
13.	Laktosa	-	+
14.	Glukosa	-	+
15.	Sukrosa	-	-
16.	Mannitol	-	+
17.	MR	+	+
18.	VP	+	+
19.	OF	-	+
20.	Nitrat	-	+
21.	Gelatin	+	+
22.	Genus	<i>Basillus, sp 1</i>	<i>Basillus sp 2</i>

The gram staining results on the isolates showed gram-positive bacteria, negative H2S content, positive catalase. Furthermore, in the microscopic and macroscopically observations,

bacterial isolates had similarities in all isolates bacillus shaped, and there were two genera, namely *Bacillus* sp one and *Bacillus* sp 2, Table 2).

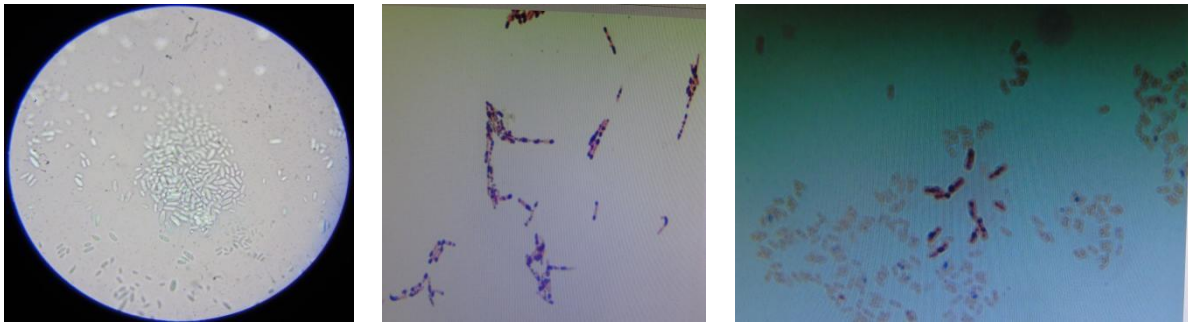


Figure 4. The image of local microorganisms staining the contents of the rumen in the form of bacilli on NA media with the genus *Bacillus* sp

The bacterial identification results showed *Bacillus* sp 1 in the rumen-filled moles added with palm fronds, and bacillus sp 2 in the local bioactivator rumen content isolates with added fronds and palm leaves. In addition, the microscopic and macroscopically observations showed that bacterial isolates in Figure 3 had similarities in all bacillus shapes.

RESULT

Based on the research results, it was concluded that the best bioactivator that can be used to improve the quality of the feed was rumen content mixed with molasses, soybean soaking water, leaves, and oil palm frond.

REFERENCES

- A. Hattaka, Modifying Enzymes from Selected White-Rot Fungi: Production and Role in Lignin Degradation, *Microbiology*, Vol. 13(1994) 125-135
- Abbas, M.H., 1987. Determination of nutrients in cow's rumen content and its utilization. In the feed of medium-type egg-laying hens during growth and production. Dissertation. Postgraduate Faculty, IPB. Bogor.
- Anggraeni F, Isnaeni, and Achmad Toto Poernomo. 2016. Jurnal Pengaruh Konsentrasi Molase Terhadap Aktivitas Enzim Fibrinolitik Dari *Bacillus Subtilis* Atcc 6633 Berkala Ilmiah Kimia Farmasi, Vo.5 No. 1 Juni 2016
- D.S. Arora, M. Chander, and P. Gill, "Involvement of lignin peroxidase, manganese peroxidase and laccase in degradation and selective

- ligninolysis of wheat straw, *International Biodeterioration & Biodegradation*. Vol. 50 (2002) 115 – 120.
- Astuti, T, M. Nasir Rofiq, Nurhaita, and U. Santoso. 2019. Analysis of fiber fraction of palm oil frond fermented with different microbes and soluble carbohydrates addition as ruminant feeding. *IOP Conference Series: Earth and Environmental Science* 347 (2019) 012059, 6th International Conference on Sustainable Agriculture, Food and Energy. Manila
- Tri Astuti , Novirman Jamarun, Arief, and Gusri Yanti. 2021. Effect Fermentation of Sugarcane Shoots With *Phanerochaete chrysosporium* on the Activity of Lacasse Enzymes, Lignin Peroxidase and Manganese Peroxidase. *IOP Conf. Series: Earth and Environmental Science* 709 (2021). 7th International Conference on Sustainable Agriculture, Food, and Energy. Phuket.
- Bernadus, J. B. B., 2007, Respon Serologi protein dan mannoprotein membran sel *Candida albicans*, *BIK Biomed.*, Vol. 3 (4): 34-9
- Statistic Indonesia. 2020.
<https://www.bps.go.id/indicator/24/214/1/livestock-slaughtered-at-slaughtering-house-abattoir-by-province-and-kind-of-livestock.html>
- Budiansyah, A., Resmi, K., Wiryawan, K.G., Soehartono, M.T., Widyastuti, Y., Ramli, N., 2010. Isolation and Characterization of Carbohydrases in Beef Cattle Rumen Liquor from Abatoir, *Media Peternakan*, 33 (1): 36-43
- Beilen, J.V., Li, Z., 2002. Enzyme Technology: an overview, *Current Opinion in Biotechnology*, 13:338-344
- Glenn, J.K., Morgan, M.A., May/eld, M.B., Kuwahara, M., Gold, M.H., 1983. An extracellular H₂O₂ is requiring enzyme preparation involved in lignin biodegradation in white-rot basidiomycete *Phanerochaete chrysosporium*. *Biochemical and Biophysical study Communications* 114, 1077–1083.
- Fogarty WC, Weshoff DC. 1983. *Microbial enzymes and Biotechnology*. Applied Science Pub., London
- Hartati I. 2012. Purification of cellulase from cow rumen liquid by using expanded bed adsorption. *Techno*, Volume 13 No. 1, April 2012 Hal. 43–51
- Leonowicz, A. & Grzywnowicz, K., 1981. Quantitative estimation of Lacasse forms in some white-rot fungi using syringaldazine as a substrate. *Enzyme and Microbial Technology*, 3(1). Pp. 55-58
- Pamungkas W. 2012. The use of rumen fluids enzymes as an alternative to support the

utilization of local fish feed raw materials. Media Akuakultur Volume 7 No 1
the year 2012

- Murni, R., Suparjo, Akmal, B. L. & Ginting. 2008. Buku Ajar Teknologi Pemanfaatan Limbah untuk Pakan. Animal feeding Laboratorium. Jambi: Fakultas Peternakan animal science Faculty. Jambi University.
- Murtiyaningsih and M. Hazmi. 2017. Isolation and cellulase enzyme activities assays in cellulolytic bacteria origin from soil wast. Agitprop, Vol. 15 (2): 293-308
- Richana, N. 2002. Produksi dan Prospek Enzim Xilanase dalam Perkembangan Bioindustri di Indonesia”, Bulletin Agrobio, 5:29-36
- R. Pandey, A., Soccol, C. R., Nigam, P.; Soccol, V. T. Vandenberghe, L. P. S. Mohan. 2000. Biotechnological potential of agro-industrial residues. Cassava bagasse. Bioresour. Technol, vol. 74, no. 1, pp. 81–87,
- Tien, M & Kirk, T.K., 1984. The lignin-degrading enzyme from *Phanerochaetachrysosporium*: Purification, characterization, and catalytic properties of a unique H₂O₂-requiring oxygenase. Proceedings of the National Academy of Science of the United States of America, pp.2280-2284
- Trismilah, Deden, Sumaryanto. 2003. Produksi xilanase. J Sains dan Teknologi (2): 66-69
- Wariishi, H., Valli, K., Gold, M.H., 1992. Manganese (II) oxidation by manganese peroxidase from the Basidiomyceteskinetic mechanism and role of chelators. J. Biol. Chem. 267, 23688e23695
- Wina E. 2005. The technology of utilizing microorganisms in feed to improve ruminant productivity in Indonesia: a review. WARTAZOA Vol.15 No. 4. Page 173-186

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Authors : Tri Astuti, Syahro A. Akbar, Nasir Rofiq, Novirman Jamarun, Nurul Huda, Ahmad Fudholi

Date : June 29, 2022

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Reviewers Comments	Author Respond and Revision
<p>Reviewer #1 Recommendation: Major revisions</p> <p>Reviewer Comments: General comment: This paper is regarding a research on the activity of cellulase and ligninase enzymes in local microorganisms from cattle and buffalo rumen content However, the writing and presentation of data is not of publication quality. The script in this current form can be revised to achieve publication quality. There are some clarifications needed to understand the processes carried out in this work. To conclude, this paper needs to be revised carefully before it can be considered in journal like BAB. Hope that comments below will be able to help to further improve the paper</p> <ul style="list-style-type: none"> * Please make sure that the paper is checked by native English speaker, the language needs improvement. * Please check Guides for Authors to make sure it is followed strictly * Language: There are some language errors (tenses, singular/plural) and incomplete sentences in the script. Please check the sentence structure, tenses and language carefully in the revised manuscript. * Take note of unit spacing issue <p>Graphical abstract and highlights: * Only highlights are provided. Suggest to edit according to the GFA. Most of the points have exceeded the maximum allowable character.</p>	<p>*The manuscript is thoroughly revised, and all possible grammatical error has been corrected with improve English using proof reading service (KG support).</p> <ul style="list-style-type: none"> * Done * The manuscript was corrected with improve English using proof reading service (KG support). * Done * Done. <p>The highlights was revised</p>

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- * An abstract was presented separately from the article. Which the problem statement, aim, novelty and results of the study was included in.
- * The abstract was revised.
- * The results of statistical analysis showed insignificant differences ($P > 0.05$) amongst the parameters of the enzyme activities of cellulase (2.22–3.51 U/ml), laccase (10.62–20.11 U/ml), lignin peroxidase (1.74–4.93 U/ml) and MnP (2.40–7.06 U/ml). *Lactobacillus* sp. was identified through bacterial identification. Therefore, the live microbes ..
- * The introduction was revised, which the environmental issues together with treatment possibility and methods was included in.
- * Highlight novelty in last paragraph was added. "This research can produce complex enzymes (cellulase, MnP, LiP and laccase) from waste materials that are cheap and easy to obtain".

- * The main body was revised.

- * Little data currently exist of this study in literature to the best of the authors' knowledge.
- * Done
- * In Table 2 was revised.
- * Little data currently exist of this study in literature (English references) to the best of the authors' knowledge.

<p>Conclusion:</p> <ul style="list-style-type: none"> * This section is too short! * Kindly improve to include in more concise and significant results. * Should include some present challenges and possible routes to improve them. Describe them in more details. <p>Papers for further reading: "Two-step thermodegradation kinetics of cellulose, hemicelluloses, and lignin under isothermal torrefaction analyzed by particle swarm optimization".</p>	<p>Conclusions was revised.</p>
<p>Reviewer #2</p> <ol style="list-style-type: none"> 1. Highlights should be revised. Please check maximal no of highlights with no of word counts. 2. Why does the title meant on 'Local microorganisms'? Consider to use native or indigenous 3. Please improve the discussion as only two scopes were discussed. Much information is required. There is no discussion with comparison with the results from other literature. Discussion should be more comprehensive. So far, it could be observed that the methodology and introduction are more than the results and discussions. 4. Please amend the conclusion as currently the conclusion is only One sentence. 	<ol style="list-style-type: none"> 1. The highlights is revised. 2. The title was revised. 3. Done. The data in Table 1 show that LiP enzyme activity ranged from 2.46 U/ml to 3.48 U/ml. LiP enzyme activity in this study was higher than that in the work of Mufidatul and Kuswytasari (2013), who found that LiP from <i>Gliomastix</i> sp. T3.7 had the enzyme activity of 0.06–1.022 in corn hump waste at pH 5 and 25 °C–35 °C. 4. Conclusions was revised.

Acknowledgement

Authors also would like to thank the Reviewers and Editors due their appropriate and constructive suggestions as well as their proposed corrections, which have been utilized in improving the quality of this manuscript.

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Activity of cellulase and ligninase enzymes in local microorganisms from cattle and buffalo rumen content --Manuscript Draft--

Manuscript Number:	BAB-D-22-00223R1
Article Type:	Research Paper
Section/Category:	Industrial Enzymes
Keywords:	Enzyme activity; microbial morphology; enzyme cellulase; enzyme ligninase, rumen microbes
Corresponding Author:	Dr. Tri Astuti Universiti Mahaputra muhammad Yamin, Indonesia
First Author:	Tri Astuti, Assoc. Prof
Order of Authors:	Tri Astuti, Assoc. Prof Syahro A. Akbar Nasir Rofiq Novirman Jamarun, Prof Nurul Huda, Assoc. Prof Ahmad Fudholi, Ph.D
Abstract:	<p>This study aims to determine the activity of cellulase and ligninase enzymes on local bioactivator. This was based on the rumen contents of the rumen incubated for seven days with supplies of different sources of enzyme energy. The method used was a factorial design of 2 × 4 with three replications for each treatment. Factor A was the type of Animal (Cattle Vs Buffalo), factor B is the addition of an enzyme source supply material: B1 = Molasses, B2 = Molasses + palm frond extract, B3 = Molasses + palm leaf extract, B4 = Molasses + palm frond, and palm leaf extract. Also, the parameters observed were the activity of enzymes cellulase, laccase, lignin and manganese peroxidase, as well as microbial identification. The results of statistical analysis showed that the activity of the enzymes cellulase 2.22-3.51 U/ml, laccase 10.62-20.11 U/ml, lignin peroxidase 1.74-4.93 U/ml, and manganese peroxidase 2.40-7.06 U/ml showed insignificant differences ($p > 0.05$). The results of microbial identification found the bacteria <i>Lactobacillus</i> sp. Based on these, it was concluded that the microbes discovered in the local microorganism solution live because of the study environment, not the microbes inherited from the rumen's contents.</p>
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February 24, 2022

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Dear Professor,

I wish to submit a manuscript entitled "Activity of cellulase and ligninase enzymes in local microorganisms from cattle and buffalo rumen content" for possible consideration.

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Thank you very much and I shall wait for your kind response.

Best regards,

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Universiti Mahaputra Muhammad Yamin

Reviewer comments and Author Respond

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Acknowledgement

Authors also would like to thank the Reviewers and Editors due their appropriate and constructive suggestions as well as their proposed corrections, which have been utilized in improving the quality of this manuscript.

Highlights

- The activity of cellulase and ligninase enzymes on local bioactivator is investigated.
- The activity of enzymes cellulase, laccase, lignin and manganese peroxidase, as well as microbial identification are observed.
- The enzyme activity test for local microorganisms in the rumen are presented.
- Chemical examination results for microorganism isolates on NA media are presented
- The microbes discovered in the local microorganism solution live because of the study environment, not the microbes inherited from the rumen's contents.

1 **Activity of cellulase and ligninase enzymes in local microorganisms from cattle and**
 2 **buffalo rumen content**

3 Tri Astuti^{1*}, Syahro A. Akbar¹, Nasir Rofiq², Novirman Jamarun³, Nurul Huda⁴,
 4 Ahmad Fudholi^{5,6}

5 ¹Department of Animal Science, Faculty of Agriculture University of Mahaputra Muhammad
 6 Yamin, Indonesia

7 ²Agency for the Assessment and Application of Technology, Indonesia

8 ³Faculty of Animal Science, Andalas University, Padang, West Sumatera, Indonesia

9 ⁴Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, Malaysia

10 ⁵Solar Energy Research Institute, Universiti Kebangsaan Malaysia, 43600, Bangi, Selangor,
 11 Malaysia

12 ⁶National Research and Innovation Agency (BRIN), Indonesia

13
 14 **Correspondent email :** adektuti@gmail.com

15
 16 **Abstract**

17 This study aims to determine the activity of cellulase and ligninase enzymes on local
 18 bioactivator. This was based on the rumen contents of the rumen incubated for seven days with
 19 supplies of different sources of enzyme energy. The method used was a factorial design of 2
 20 ×4 with three replications for each treatment. Factor A was the type of Animal (Cattle Vs
 21 Buffalo), factor B is the addition of an enzyme source supply material: B1 = Molasses, B2 =
 22 Molasses + palm frond extract, B3 = Molasses + palm leaf extract, B4 = Molasses + palm
 23 frond, and palm leaf extract. Also, the parameters observed were the activity of enzymes
 24 cellulase, laccase, lignin and manganese peroxidase, as well as microbial identification. The
 25 results of statistical analysis showed that the activity of the enzymes cellulase 2.22-3.51 U/ml,
 26 laccase 10.62-20.11 U/ml, lignin peroxidase 1.74-4.93 U/ml, and manganese peroxidase 2.40-
 27 7.06 U/ml showed insignificant differences (p> 0.05). The results of microbial identification
 28 found the bacteria *Lactobacillus sp.* Based on these, it was concluded that the microbes
 29 discovered in the local microorganism solution live because of the study environment, not the
 30 microbes inherited from the rumen's contents.

31
 32 **Keyword:** Enzyme activity, microbial morphology, enzyme cellulase, enzyme lignase, rumen
 33 microbes

35 **1. Introduction**

36 Animal feeding is a major factor in determining the success of the livestock business.
37 Furthermore, many study uses by-products of agriculture and plantation as substitutes for the
38 field grass. These by-products mostly contain lignocellulosic bonds that consist of cellulose,
39 hemicellulose, and lignin, which cause the low nutritional value of these ingredients when
40 directly fed as feed. Used of palm oil fronds as animal feed barried are low crude protein about
41 2.11%, high crude fiber content reaching 46.75% (Murni et al. 2008). The oil palm fronds
42 content of ADF 56,93%, NDF 78,05%, cellulose 12.91%, hemicellulose 15.34% and Lignin
43 15.34% (Astuti et al. 2019). Lignin is a polymer complex phenylpropanoids, heterogeneous,
44 and 25-30% plant biomass. It is quite resistant to microbial degradation under natural
45 conditions. Lignin and cellulose are the main components in plants enzymatically degraded by
46 microorganisms such as enzyme cellulase and ligninase. Ligninolytic microbial systems have
47 been used in improving digestibility and nutritional value. Primarily three enzymes such as
48 lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase (Glenn et al. 1983). Lignin
49 peroxidase LiP and manganese peroxidase (MnP) are extracellular peroxidase enzymes that
50 use H₂O₂ to degrade lignin, while laccase is a copper-containing enzyme that uses molecular
51 oxygen to degrade lignin (Hattaka 1994).

52 The use of microbes for enzymes producer has several advantages, including low
53 production costs, short production time, high growth speed, and ease to control. Factors such
54 as fermentation medium, temperature, aeration, agitation, pH, and strains, which play a role in
55 producing these enzymes, need to be optimized. The Cheaped and inexpensive Fermentation
56 media is required since it is easy to obtain and produce expected enzymes for large production
57 (Trismilah 2009).

58 Many rumen contents on the slaughterhouse waste pollute the environment due to the
59 rumen's abundant availability contents. It was being seen based on the slaughterhouse's number
60 of slaughtered cattle and buffalo. Based on data (Statistic 2020), the average of Livestock
61 Slaughtered at Slaughtering House (Abattoir) in 2019-2020 was 1069.614 heads of cows and
62 27,487 heads of buffaloes. Beef rumen fluid, apart from containing rumen microbes and
63 enzymes secreted by rumen microbes, also contains food substances resulting from overhaul
64 rumen microbes and enzymes and vitamins and minerals that dissolve in the rumen fluid. The
65 cow's rumen content was 9.29% water; 8.45% crude protein; 1.23% crude fat; 33.53% crude
66 fiber; 0.20% Ca; 0.45% P; 16.19% ash; and 31.60% NFE, and vitamin B12. The rumen contents
67 have the potential as feed additives (Abbas 1987). This slaughtering of livestock leaves the
68 contents of the rumen untapped, only to be thrown away.

69 Rumen fluid deposits are rich in protein, amino acids, vitamins, and minerals. The
70 composition of amino acids, minerals, vitamins, and enzymes also depends on the feed
71 treatment (Budiansyah et al. 2010). The assumed rumen microbes would produce enzymes
72 according to the given substrate. For example, when given straw, it will produce enzymes to
73 degrade fiber and break down tannins when given a substrate of calliandra (Wina 2005). Lignin
74 and cellulose were the main components in plants that the enzyme cellulase and ligninase
75 enzymatically degrades. These enzymes were produced by microorganisms (Pandey et al.
76 2000). Molasses are the waste of sugar mills, cheapest and easily as carbon sources rich in
77 nutrients and minerals, and have the potential for microbial growth media (Anggraeni, Isnaeni,
78 and Toto 2016).

79 Enzymes are biopolymer molecules composed of a series of amino acids in an ordered
80 and fixed composition and chain arrangement. Enzymes were proteins produced and used by
81 living cells to catalyze chemical reactions with a high level of specification and an increase in
82 reaction rates (van Beilen and Li 2002; Richana 2002). Thus, enzymes have various advantages
83 over conventional processes using chemicals. However, the main obstacle for applying
84 enzymes in the industry is the high price of enzymes, and the enzyme cannot be used repeatedly
85 (Huey 2008; Troger, C. and Niranjan 2010; Wibisono 2010).

86 Cellulases are complex enzymes that gradually cut the cellulose chains into glucose.
87 Fungi, bacteria, and ruminants produces cellulase. The commercial production of enzymes
88 usually uses fungi or bacteria. The production of cellulase enzymes from ruminants has not
89 been much appreciated, however, cellulases originating from ruminant is being produced given
90 their large availability. Rumen fluid enzymes as an alternative technology are used in
91 hydrolyzing crude fiber to increase the nutritional value of local feed raw materials (Pamungkas
92 2012). The cellulase enzymes have the potential to be produced from beef rumen fluid
93 compared to commercial enzymes. Therefore, this study aims to determine the activity of
94 cellulase and ligninase enzymes in local microorganisms with several different ingredients
95 added. Additionally, microbes are considered to produce enzymes according to the food they
96 get

97

98 **2. Materials and methods**

99 The material used in this study was rumens content of cattle and buffalo, molasses,
100 soybean water immersion, oil palm frond, oil palm leaf, and some chemistry for measuring
101 enzyme activity.

102

103 2.1. The bioactivator process

104 The rumen content of cattle and buffalo were collected from the abattoir and placed in
105 tubes. All the content of rumen treatments was added with molasses and tofu water immersion.
106 The treatments were the addition of palm leaves, palm fronds, and a mixture of both, which
107 had high lignin content. The addition of palm fronds and leaves is intended, hence, the
108 microorganisms that develop are microorganisms capable of producing the ligninase enzyme.
109 The contents of the rumen, molasses, tofu soaked wastewater were mixed in a ratio of 1:1:8,
110 and 10% of the oil palm frond and leaf. The tube is tightly closed, after which a hole is made
111 in the middle and connected with a hose to a small bottle filled with water to catch the waste
112 of fermentation, then incubated for as long as ten days, as shown in Figure 1.

113



Figure 1. Fermentation process local bioactivator

114

115 2.2. Test Activity of Lignin Peroxidase (LiP) enzymes

116 A 0.2 ml of enzyme filtrate (extract sample and buffer pospat were shaken until 1 hour
117 and then centrifuged for as long as 10 minutes with 10000 rpm), 0.05 ml of H₂O₂ 5 mM, 0.1
118 ml of veratrine alcohol 8 mM, 0.2 ml of acetate buffer 0.05 M pH 3, and 0.45 ml of distilled
119 water are added to cuvette and then shaken. The absorbance of the solution was at a wavelength
120 of 310 nm for 0 and 30-minute intervals. One unit of enzyme activity in LiP is defined as the
121 amount of enzyme that causes the conversion of 1 micromol (1 μ mol = 10⁻⁶ mol) veratril
122 alcohol per minute (Ming and Kent 1984).

123

124 2.3. Test the activity of Manganese Peroxidase (MnP) enzymes

125 As much as 0.1 ml of 50 mM Na-lactate buffer pH five is added with 0.1 ml of 4
126 mMguaiacol, 0.2 ml of MnSO₄ 1 mM, 0.1 ml of H₂O₂ 1 mM, and distilled water 0.3 ml, as well

127 as 0.2 filtrates are added enzyme. The solution was then checked and read at a wavelength of
128 465 nm for 0 and 30 minutes (Leonowicz and Grzywnowicz 1981).

129

130

131 2.4. Test the activity of Lakase enzymes

132 As much as 0.4 ml of enzyme filtrate plus 0.5 ml pH five acetate buffer of 0.5 ml and
133 0.1 ml of 1 mM ,2'-azinobis 3-ethylbenzothiazole-6-sulfonic acid (ABTS). Then it was
134 checked, and measurements were read to 420 nm wavelength. The reading was taken for 0 and
135 30 minutes (Wariishi, Valli, and Gold 1992).

136 Enzyme activity tests were conducted in the biotechnology laboratory of the Faculty of
137 Animal Husbandry, Andalas University, Padang, and identification of microbial morphology
138 was performed at the Baso veterinary center laboratory, Bukit Tinggi. The analysis was
139 continued by identifying the morphology of fungi and bacteria present in the local bioactivator
140 rumen contents. The identification of microbes was performed based on the results of the best
141 enzyme activity evaluation. Samples were inoculated on Sodium Agar (NA) medium for
142 identification of bacteria and Potato dextrose Agar (PDA) media as a medium for growing
143 fungi/molds. The samples were diluted in 10^{-1} - 10^{-10} , and the selected isolates were further to
144 be analyzed based on even colony distribution

145

146 2.5. Experimental design

147 The factorial randomized block design 2×4 with three replications for each treatment
148 used in this study. Factor A is the type of rumen content: A1 = Rumen cattle, A2 = Buffalo
149 Rumen, Factor B supplies microbial energy: B1 = Molases, B2 = Molases + palm frond extract,
150 B3 = Molases + palm leaf extract, B4 = Molases + extract of palm fronds and leaves. Enzyme
151 activity data processing was performed using analysis of variance while identifying
152 microorganisms using the described method. When the study resulted in a significant different
153 affected ($p < 0.05$), it will be further tested using duncan's multiple range tests,

154 The variables to be observed were the isolation and identification of microorganisms
155 (fungi and bacteria) by morphology and cellulase and ligninase activity tests for crude bio
156 enzymes of rumen content. The morphology data are shown in description analysis.

157

158 3. Results and discussion

159 3.1. Enzyme activity test

160 The enzymes activity test were cellulase enzyme, Lacasse Enzymes, Enzim Lignin
 161 Peroksidase, Manganese Peroxidase Enzymes. Furthermore, these enzymes are essential for
 162 lignin degradation. The average results of the enzyme activity test for local microorganisms in
 163 the rumen are shown in Table 1.

164

165 Table 1. Average activity of local microorganisms in the rumen contents (U/ml)

Factor B	Factor A				Average
	B ₁	B ₂	B ₃	B ₄	
Cellulase enzymes					
A1	3.51	2.41	2.81	2.22	2.74
A2	3.44	2.52	3.31	3.64	3.23
	3.48	2.46	3.06	2.93	2.98
Lacasse Enzymes					
A1	20.11	16.40	11.70	15.91	16.03
A2	11.00	12.86	10.62	14.59	12.27
	1.55	14.63	11.16	15.25	14.15
Enzim Lignin Peroksidase					
A1	4.06	4.93	2.80	4.11	3.97
A2	2.61	1.74	4.16	3.80	3.08
	3.34	3.34	3.48	3.95	3.53
Manganese Peroxidase Enzymes					
A1	2.40	5.61	3.39	7.06	4.61
A2	6.86	4.17	3.18	5.18	4.85
	4.63	4.89	3.29	6.12	4.73

166 Note: A1 = contents of cow's rumen, A2 = contents of buffalo's rumen, B1 = contents of
 167 rumen only, B2 = contents of rumen and palm fronds, B3 = palm leaves, and B4 =
 168 leaves and palm fronds

169

170 The results of the analysis of statistics showed no interaction effect ($P > 0.05$) between
 171 the kind of rument resource (cattle and buffalo) with the sources of microbe energy on the
 172 activity of cellulase enzymes, laccases, Lignin Peroxidase Enzymes, and Manganese
 173 Peroxidase Enzymes. The data shows the rumen contents were used as a source of
 174 microorganisms that were ingested and still dominant in the form of fiber from forage

175 consumed by livestock are given the main energy source in the form of molasses and tofu
176 soaking water for all treatments. Therefore, it is suspected that the main energy source shown
177 is very representative, supplying the energy needed by microbes in the rumen to grow and
178 develop to produce high value. This study's average cellulase enzyme activity result was much
179 higher than (Murtiyaningsih and Hazmi 2017), measuring the activity of cellulase enzymes
180 from cellulolytic bacteria from soil and getting the highest enzyme activity of 0.028279 U/ml.
181 This was due to a large number of microbes in the rumen contents, then the addition of molasses
182 and tofu soaking wastewater further increases microbial growth. The research (Astuti et al.
183 2020) found 40×10^{12} total colonies in 1 ml of local bio activator rumen contents mixed with
184 molasses, soybean soaking water, and palm oil fronds.

185 The average laccase enzyme activity ranged from 11.0 to 20.11 U/ml, the mean lignin
186 peroxidase enzyme ranged from 1.74 to 4.93, while the manganese peroxidase enzyme ranged
187 from 2.40 - 7.06 U/ml. Laccase enzyme activity was much higher than (Dimawarnita, Panji,
188 and Faramitha 2019) which measured enzyme activity in *Pleurotus ostreatus* with media
189 containing OPEFB 0.35 U/ml and lignin peroxidase (LiP) activity of 0.269 U/mL. The lignin
190 enzyme activity of sugarcane fermentation by 10% *Phanerochaete chrysosporium* were laccase
191 2.02 U/ml, LiP 1,677 U/ml, MnP 0.33 U/ml. This was because the microbes that thrive in this
192 local activator have been added to the palm fronds and leaves high in lignin content. Rumen
193 microbes will develop and produce enzymes based on the feed given (Astuti et al. 2021).

194

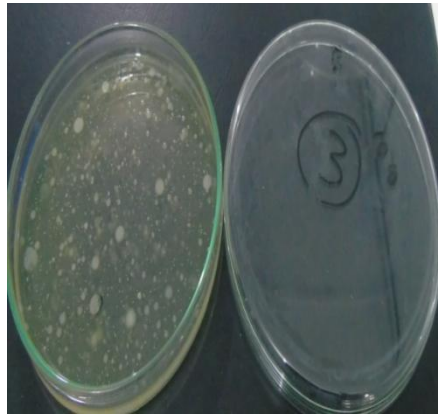
195 3.2. Identification of microorganism morphology

196 The analysis identifies fungi and bacteria's morphology present in the local bioactivator
197 rumen contents. The identification of microbes was performed based on the results of the best
198 enzyme activity evaluation. Figure 2 shows that the bacterial colonies are circular, small spread
199 out, thin, the edges of the settlements are flat, white in color, and the structure is transparent.
200 Morphological observations of fungal colonies showed circular colony shape, elevation
201 convex, uneven edges, white color, and transparent structure (Figure 2 and 3). The shape of the
202 colony in this study showed the same results with the research (Yogyaswari, Rukmi, and
203 Raharjo 2016) which found around and white colony shape from bacterial isolates rumen
204 contents of Fries Holland. Based on microscopy observations of all bacterial isolates were
205 gram-positive, and bacilli (Table 2 and Figure 4). The results of the chemical analysis are
206 shown in Table 2.

207 The gram staining results on the isolates showed gram-positive bacteria, negative H₂S
208 content, positive catalase. Furthermore, in the microscopic and macroscopically observations,

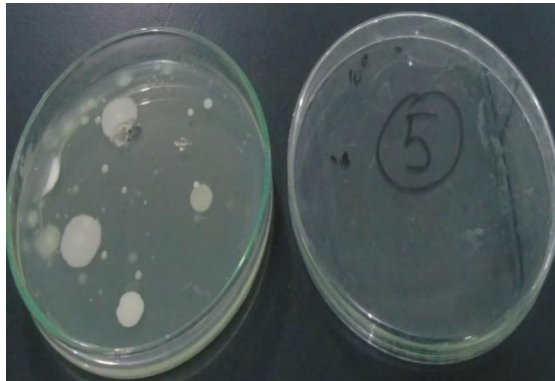
209 bacterial isolates had similarities in all isolates bacillus shaped, and there were two genera,
210 namely *Bacillus* sp one and *Bacillus* sp 2, Table 2).

211 The bacterial identification results showed *Bacillus* sp 1 in the local microorganism
212 rumen-filled added with palm fronds, and bacillus sp 2 in the local bioactivator rumen content
213 isolates with added fronds and palm leaves. In addition, the microscopic and macroscopically
214 observations showed that bacterial isolates in Figure 3 had similarities in all bacillus shapes.



215
216
217

Figure 2. Bacterial colonies on 10^{-8}



218
219
220

Figure 3. Fungal colonies on 10^{-5}

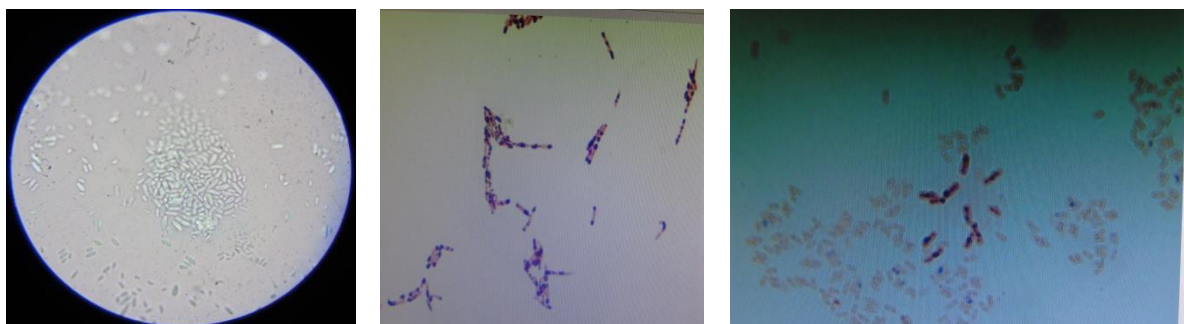


Figure 4. The image of local microorganisms staining the contents of the rumen in the form of bacilli on NA media with the genus *Bacillus* sp

221 Table 2. Chemical examination results for microorganism isolates on NA media

No	Treatment	NA 1	NA 2
1.	Colony(Color, shape, Traits)	White	White
2.	Gram (Marfologi, Spora)	+ bacteri	+ bacteri
3.	Aerobic / Anaerobic	A	A spora
4.	TSIA	M/K	M/K
5.	Gas	-	+
6.	H2S	-	-
7.	catalase	+	+
8.	Oxidase	-	-
9.	Motility	+	+
10.	Indole	-	-
11.	Urea	+	+
12.	Citrat	-	-
13.	Laktosa	-	+
14.	Glukosa	-	+
15.	Sukrosa	-	-
16.	Mannitol	-	+
17.	MR	+	+
18.	VP	+	+
19.	OF	-	+
20.	Nitrat	-	+
21.	Gelatin	+	+
22.	Genus	<i>Basillus, sp 1</i>	<i>Basillus sp 2</i>

222

223

224 **4. Conclusion**

225 Based on the research results, it was concluded that the best bioactivator that can be
 226 used to improve the quality of the feed was rumen content mixed with molasses, soybean
 227 soaking water, leaves, and oil palm frond.

228

229 **References**

230 Abbas, M. Hafil. 1987. "Penentuan Zat-Zat Makanan Dalam Isi Rumen Sapi Dan

231 Pemanfaatannya Dalam Ransum Ayam Petelur Tipe Medium Pada Masa Pertumbuhan
 232 Dan Produksi.” IPB.Bogor.

233 Anggraeni, Felita, Isnaeni, and Achmad P. Toto. 2016. “Pengaruh Konsentrasi Molase
 234 Terhadap Aktivitas Enzim Fibrinolitik Dari *Bacillus Subtilis* ATCC 6633.” *Berkala
 235 Ilmiah Kimia Farmasi* 5(1):18–24.

236 Astuti, T., Syahro Ali Akbar, Delsi Afrini, M. Nasir Rofiq, and Irna Humaira. 2020. “The
 237 Identification of Fungi Colonies Total on the Rumen Content of Cow and Buffalo with
 238 Addition of Leaves and Oil Palm Frond.” *World Journal of Advanced Research and
 239 Reviews* 02(08):314-317. doi: <https://doi.org/10.30574/wjarr.2020.8.2.0444>.

240 Astuti, T., M. Nasir Rofiq, Nurhaita, and U. Santoso. 2019. “Analysis of Fibre Fraction of Palm
 241 Oil Frond Fermented with Different Microbes and Soluble Carbohydrates Addition as
 242 Ruminant Feeding.” *IOP Conference Series: Earth and Environmental Science* 347(1).
 243 doi: 10.1088/1755-1315/347/1/012059.

244 Astuti, Tri, Novirman Jamarun, Arief, and Gusri Yanti. 2021. “Effect Fermentation of
 245 Sugarcane Shoots with *Phanerochaetechrysosporium* on the Activity of Lacase Enzymes,
 246 Lignin Peroxidase and Manganese Peroxidase.” *IOP Conference Series: Earth and
 247 Environmental Science* 709(1):3–7. doi: 10.1088/1755-1315/709/1/012065.

248 van Beilen, Jan B., and Zhi Li. 2002. “Enzyme Technology: An Overview.” *Current Opinion
 249 in Biotechnology* 13 4:338–44.

250 Budiansyah, A., Resmi, K. G. Wiryawan, M. T. Soehartono, Y. Widyastuti, and N. Ramli.
 251 2010. “Isolation and Characterization of Carbohydrases in Beef Cattle Rumen Liquor
 252 from Abattoir | Isolasi Dan Karakterisasi Enzim Karbohidrase Cairan Rumen Sapi Asal
 253 Rumah Potong Hewan.” *Media Peternakan* 33(1).

254 Dimawarnita, Firda, Tri Panji, and Yora Faramitha. 2019. “Peningkatan Kemurnian Selulosa
 255 Dan Karboksimetil Selulosa (CMC) Hasil Konversi Limbah TKKS Melalui Perlakuan
 256 NaOH 12%.” *E-Journal Menara Perkebunan* 87(2):95–103. doi:
 257 10.22302/iribb.jur.mp.v87i2.339.

258 Glenn, J. K., M. A. Morgan, M. B. Mayfield, M. Kuwahara, and M. H. Gold. 1983. “An
 259 Extracellular H₂O₂-Requiring Enzyme Preparation Involved in Lignin Biodegradation
 260 by the White Rot Basidiomycete *Phanerochaete Chrysosporium*.” *Biochemical and
 261 Biophysical Research Communications* 114(3):1077–83. doi: 10.1016/0006-
 262 291x(83)90672-1.

263 Hattaka, A. 1994. “Lignin-Modifying Enzymes from Selected White-Rot Fungi: Production
 264 and Role from in Lignin Degradation.” *FEMS Microbiology Reviews* 13:125–35. doi:

265 10.1016/j.biortech.2013.02.042.

266 Huey, H. S. 2008. "Enzymatics Enhanced Production of Gaharu Oil: Effect of Enzyme Loading
267 and Duration Time". University malaysia Pahang.

268 Leonowicz, A., and K. Grzywnowicz. 1981. "Quantitative Estimation of Laccase Forms in
269 Some White-Rot Fungi Using Syringaldazine as a Substrate." *Enzyme and Microbial
270 Technology* 3(1):55–58. doi: [https://doi.org/10.1016/0141-0229\(81\)90036-3](https://doi.org/10.1016/0141-0229(81)90036-3).

271 Ming, Tien, and Kirk T. Kent. 1984. "Lignin-Degrading Enzyme from Phanerochaete
272 Chrysosporium: Purification, Characterization, and Catalytic Properties of a Unique
273 H₂O₂-Requiring Oxygenase." *Proceedings of the National Academy of Sciences*
274 81(8):2280–84. doi: 10.1073/pnas.81.8.2280.

275 Murni, R., Suparjo, B. L. Akmal, and Ginting. 2008. *Buku Ajar Teknologi Pemanfaatan
276 Limbah Untuk Pakan*. Jambi: Animal feeding Laboratorium. Jambi University.

277 Murtiyaningsih, Hidayah, and Muhammad Hazmi. 2017. "Isolasi Dan Uji Aktivitas Enzim
278 Selulase Pada Bakteri Selulolitik Asal Tanah Sampah Isolation and Cellulase Enzyme
279 Activities Assays in Cellulolytic Bacteria Origin From Soil Waste." *Agritrop* 15(2):293–
280 308.

281 Pamungkas, Wahyu. 2012. "Penggunaan Enzim Cairan Rumen Sebagai Alternatif Untuk
282 Mendukung Pemanfaatan Bahan Baku Pakan Ikan Lokal." *Media Akuakultur* 7(1):32. doi:
283 10.15578/ma.7.1.2012.32-38.

284 Pandey, A., CR. Soccol, P. Singh - Nee Nigam, VT. Soccol, LPS. Vandenberg, and R. Mohan.
285 2000. "Biotechnological Potential of Agro-Industrial Residues. II: Cassava Bagasse." *Bioresource Technology* 74(1):81–87.

287 Richana, Nur. 2002. "Produksi Dan Prospek Enzim Xilanase Dalam Pengembangan
288 Bioindustri Di Indonesia." *Buletin AgroBio* 5(1):29–36.

289 Statistic, Indonesia. 2020. *Jumlah Ternak Yang Di Potong Di Indonesia*.

290 Trismilah, Deden Rosid; 2009. "PRODUKSI XILANASE MENGGUNAKAN MEDIA
291 LIMBAH PERTANIAN DAN PERKEBUNAN." *Jurnal Teknologi Lingkungan* (Vol. 10
292 No. 2 (2009)):137–44.

293 Troger, C., and K. Niranjana. 2010. "Sustainable Chitin Extraction and Chitosan Modification
294 for Application in the Food Industry." *International Conference on Food Innovation*.

295 Wariishi, H., K. Valli, and M. H. Gold. 1992. "Manganese(II) Oxidation by Manganese
296 Peroxidase from the Basidiomycete Phanerochaete Chrysosporium. Kinetic Mechanism
297 and Role of Chelators." *Journal of Biological Chemistry* 267(33):23688–95. doi:
298 10.1016/s0021-9258(18)35893-9.

- 299 Wibisono, Eko. 2010. "Mobilisasi Crude Enzim Papain Yang Diisolasi Dari Getah Buah
300 Pepaya (*Carica Papaya L*) Dengan Menggunakan Kappa Karagenan Dan Kitosan Serta
301 Pengujian Aktivitas Dan Stabilitasnya."
- 302 Wina, Elizabeth. 2005. "The Technology of Utilizing Microorganism in Feed To Improve
303 Ruminant Productivity in Indonesia: A Review." *Wartazoa* 15(4):173–86.
- 304 Yogyaswari, Sekar Ayoe, M. G. Isworo Rukmi, and Budi Raharjo. 2016. "Ekplorasi Bakteri
305 Selulolitik Dari Cairan Rumen Sapi Peranakan Fries Holland (PFH) Dan Limousine
306 Peranakan Ongole (Limpo)." *Jurnal Biologi* 5(4):70–80.
- 307

35 1. Introduction

36 Animal feeding is a major factor in determining the success of the livestock business.
37 Furthermore, many study uses by-products of agriculture and plantation as substitutes for the
38 field grass. These by-products mostly contain lignocellulosic bonds that consist of cellulose,
39 hemicellulose, and lignin, which cause the low nutritional value of these ingredients when
40 directly fed as feed. Used of palm oil fronds as animal feed barried are low crude protein about
41 2.11%, high crude fiber content reaching 46.75% [\[1\] \(Murni et al. 2008\)](#). The oil palm fronds
42 content of ADF 56,93%, NDF 78,05%, cellulose 12.91%, hemicellulose 15.34% and Lignin
43 15.34% [\[2\] \(Astuti et al. 2019\)](#). Lignin is a polymer complex phenylpropanoids, heterogeneous,
44 and 25-30% plant biomass. It is quite resistant to microbial degradation under natural
45 conditions. Lignin and cellulose are the main components in plants enzymatically degraded by
46 microorganisms such as enzyme cellulase and ligninase. Ligninolytic microbial systems have
47 been used in improving digestibility and nutritional value. Primarily three enzymes such as
48 lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase [\[3\] \(Glenn et al. 1983\)](#).
49 Lignin peroxidase LiP and manganese peroxidase (MnP) are extracellular peroxidase enzymes
50 that use H₂O₂ to degrade lignin, while laccase is a copper-containing enzyme that uses
51 molecular oxygen to degrade lignin [\[4\] \(Hattaka 1994\)](#).

52 The use of microbes for enzymes producer has several advantages, including low
53 production costs, short production time, high growth speed, and ease to control. Factors such
54 as fermentation medium, temperature, aeration, agitation, pH, and strains, which play a role in
55 producing these enzymes, need to be optimized. The Cheaped and inexpensive Fermentation
56 media is required since it is easy to obtain and produce expected enzymes for large production
57 [\[5\] \(Trismilah 2009\)](#).

58 Many rumen contents on the slaughterhouse waste pollute the environment due to the
59 rumen's abundant availability contents. It was being seen based on the slaughterhouse's number
60 of slaughtered cattle and buffalo. Based on data [\[6\] \(Statistic 2020\)](#), the average of Livestock
61 Slaughtered at Slaughtering House (Abattoir) in 2019-2020 was 1069.614 heads of cows and
62 27,487 heads of buffaloes. Beef rumen fluid, apart from containing rumen microbes and
63 enzymes secreted by rumen microbes, also contains food substances resulting from overhaul
64 rumen microbes and enzymes and vitamins and minerals that dissolve in the rumen fluid. The
65 cow's rumen content was 9.29% water; 8.45% crude protein; 1.23% crude fat; 33.53% crude
66 fiber; 0.20% Ca; 0.45% P; 16.19% ash; and 31.60% NFE, and vitamin B12. The rumen contents
67 have the potential as feed additives [\[7\] \(Abbas 1987\)](#). This slaughtering of livestock leaves the
68 contents of the rumen untapped, only to be thrown away.

69 Rumen fluid deposits are rich in protein, amino acids, vitamins, and minerals. The
70 composition of amino acids, minerals, vitamins, and enzymes also depends on the feed
71 treatment ~~[8]~~ (Budiansyah et al. 2010). The assumed rumen microbes would produce enzymes
72 according to the given substrate. For example, when given straw, it will produce enzymes to
73 degrade fiber and break down tannins when given a substrate of calliandra ~~[9]~~ (Wina 2005).
74 Lignin and cellulose were the main components in plants that the enzyme cellulase and
75 ligninase enzymatically degrades. These enzymes were produced by microorganisms ~~[10]~~
76 (Pandey et al. 2000). Molasses are the waste of sugar mills, cheapest and easily as carbon
77 sources rich in nutrients and minerals, and have the potential for microbial growth media ~~[11]~~
78 (Anggraeni, Isnaeni, and Toto 2016).

79 Enzymes are biopolymer molecules composed of a series of amino acids in an ordered
80 and fixed composition and chain arrangement. Enzymes were proteins produced and used by
81 living cells to catalyze chemical reactions with a high level of specification and an increase in
82 reaction rates ~~[12,13]~~ (van Beilen and Li 2002; Richana 2002). Thus, enzymes have various
83 advantages over conventional processes using chemicals. However, the main obstacle for
84 applying enzymes in the industry is the high price of enzymes, and the enzyme cannot be used
85 repeatedly ~~[14-16]~~ (Huey 2008; Troger, C. and Niranjan 2010; Wibisono 2010).

86 Cellulases are complex enzymes that gradually cut the cellulose chains into glucose.
87 Fungi, bacteria, and ruminants produces cellulase. The commercial production of enzymes
88 usually uses fungi or bacteria. The production of cellulase enzymes from ruminants has not
89 been much appreciated, however, cellulases originating from ruminant is being produced given
90 their large availability. Rumen fluid enzymes as an alternative technology are used in
91 hydrolyzing crude fiber to increase the nutritional value of local feed raw materials ~~[17]~~
92 (Pamungkas 2012). The cellulase enzymes have the potential to be produced from beef rumen
93 fluid compared to commercial enzymes. Therefore, this study aims to determine the activity of
94 cellulase and ligninase enzymes in local microorganisms with several different ingredients
95 added. Additionally, microbes are considered to produce enzymes according to the food they
96 get

98 2. Materials and methods

99 The material used in this study was rumens content of cattle and buffalo, molasses,
100 soybean water immersion, oil palm frond, oil palm leaf, and some chemistry for measuring
101 enzyme activity.

103 2.1. The bioactivator process

104 The rumen content of cattle and buffalo were collected from the abattoir and placed in
105 tubes. All the content of rumen treatments was added with molasses and tofu water immersion.
106 The treatments were the addition of palm leaves, palm fronds, and a mixture of both, which
107 had high lignin content. The addition of palm fronds and leaves is intended, hence, the
108 microorganisms that develop are microorganisms capable of producing the ligninase enzyme.
109 The contents of the rumen, molasses, tofu soaked wastewater were mixed in a ratio of 1:1:8,
110 and 10% of the oil palm frond and leaf. The tube is tightly closed, after which a hole is made
111 in the middle and connected with a hose to a small bottle filled with water to catch the waste
112 of fermentation, then incubated for as long as ten days, as shown in Figure 1.

113



Figure 1. Fermentation process local bioactivator

114

115 2.2. Test Activity of Lignin Peroxidase (LiP) enzymes

116 A 0.2 ml of enzyme filtrate (extract sample and buffer pospat were shaken until 1 hour
117 and then centrifuged for as long as 10 minutes with 10000 rpm), 0.05 ml of H₂O₂ 5 mM, 0.1
118 ml of veratrine alcohol 8 mM, 0.2 ml of acetate buffer 0.05 M pH 3, and 0.45 ml of distilled
119 water are added to cuvette and then shaken. The absorbance of the solution was at a wavelength
120 of 310 nm for 0 and 30-minute intervals. One unit of enzyme activity in LiP is defined as the
121 amount of enzyme that causes the conversion of 1 micromol (1 μmol = 10⁻⁶ mol) veratril
122 alcohol per minute-[\[19\] \(Ming and Kent 1984\)](#).

123

124 2.3. Test the activity of Manganese Peroxidase (MnP) enzymes

125 As much as 0.1 ml of 50 mM Na-lactate buffer pH five is added with 0.1 ml of 4
126 mMguaiacol, 0.2 ml of MnSO₄ 1 mM, 0.1 ml of H₂O₂ 1 mM, and distilled water 0.3 ml, as well

127 as 0.2 filtrates are added enzyme. The solution was then checked and read at a wavelength of
128 465 nm for 0 and 30 minutes ~~{20}~~ (Leonowicz and Grzywnowicz 1981).

129

130

131 2.4. Test the activity of Lakase enzymes

132 As much as 0.4 ml of enzyme filtrate plus 0.5 ml pH five acetate buffer of 0.5 ml and
133 0.1 ml of 1 mM ,2'-azinobis 3-ethylbenzothiazole-6-sulfonic acid (ABTS). Then it was
134 checked, and measurements were read to 420 nm wavelength. The reading was taken for 0 and
135 30 minutes ~~{21}~~ (Wariishi, Valli, and Gold 1992).

136 Enzyme activity tests were conducted in the biotechnology laboratory of the Faculty of
137 Animal Husbandry, Andalas University, Padang, and identification of microbial morphology
138 was performed at the Baso veterinary center laboratory, Bukit Tinggi. The analysis was
139 continued by identifying the morphology of fungi and bacteria present in the local bioactivator
140 rumen contents. The identification of microbes was performed based on the results of the best
141 enzyme activity evaluation. Samples were inoculated on Sodium Agar (NA) medium for
142 identification of bacteria and Potato dextrose Agar (PDA) media as a medium for growing
143 fungi/molds. The samples were diluted in 10^{-1} - 10^{-10} , and the selected isolates were further to
144 be analyzed based on even colony distribution

145

146 2.5. Experimental design

147 The factorial randomized block design 2×4 with three replications for each treatment
148 used in this study. Factor A is the type of rumen content: A1 = Rumen cattle, A2 = Buffalo
149 Rumen, Factor B supplies microbial energy: B1 = Molases, B2 = Molases + palm frond extract,
150 B3 = Molases + palm leaf extract, B4 = Molases + extract of palm fronds and leaves. Enzyme
151 activity data processing was performed using analysis of variance while identifying
152 microorganisms using the described method. When the study resulted in a significant different
153 affected ($p < 0.05$), it will be further tested using duncan's multiple range tests,

154 The variables to be observed were the isolation and identification of microorganisms
155 (fungi and bacteria) by morphology and cellulase and ligninase activity tests for crude bio
156 enzymes of rumen content. The morphology data are shown in description analysis.

157

158 3. Results and discussion

159 3.1. Enzyme activity test

160 The enzymes activity test were cellulase enzyme, Lacasse Enzymes, Enzim Lignin
 161 Peroksidase, Manganese Peroxidase Enzymes. Furthermore, these enzymes are essential for
 162 lignin degradation. The average results of the enzyme activity test for local microorganisms in
 163 the rumen are shown in Table 1.

164

165 Table 1. Average activity of local microorganisms in the rumen contents (U/ml)

Factor B	Factor A				Average
	B ₁	B ₂	B ₃	B ₄	
Cellulase enzymes					
A1	3.51	2.41	2.81	2.22	2.74
A2	3.44	2.52	3.31	3.64	3.23
	3.48	2.46	3.06	2.93	2.98
Lacasse Enzymes					
A1	20.11	16.40	11.70	15.91	16.03
A2	11.00	12.86	10.62	14.59	12.27
	1.55	14.63	11.16	15.25	14.15
Enzim Lignin Peroksidase					
A1	4.06	4.93	2.80	4.11	3.97
A2	2.61	1.74	4.16	3.80	3.08
	3.34	3.34	3.48	3.95	3.53
Manganese Peroxidase Enzymes					
A1	2.40	5.61	3.39	7.06	4.61
A2	6.86	4.17	3.18	5.18	4.85
	4.63	4.89	3.29	6.12	4.73

166 Note: A1 = contents of cow's rumen, A2 = contents of buffalo's rumen, B1 = contents of
 167 rumen only, B2 = contents of rumen and palm fronds, B3 = palm leaves, and B4 =
 168 leaves and palm fronds

169

170 The results of the analysis of statistics showed no interaction effect ($P > 0.05$) between
 171 the kind of rument resource (cattle and buffalo) with the sources of microbe energy on the
 172 activity of cellulase enzymes, laccases, Lignin Peroxidase Enzymes, and Manganese
 173 Peroxidase Enzymes. The data shows the rumen contents were used as a source of
 174 microorganisms that were ingested and still dominant in the form of fiber from forage

175 consumed by livestock are given the main energy source in the form of molasses and tofu
176 soaking water for all treatments. Therefore, it is suspected that the main energy source shown
177 is very representative, supplying the energy needed by microbes in the rumen to grow and
178 develop to produce high value. This study's average cellulase enzyme activity result was much
179 higher than [\[22\] \(Murtiyaningsih and Hazmi 2017\)](#), measuring the activity of cellulase enzymes
180 from cellulolytic bacteria from soil and getting the highest enzyme activity of 0.028279 U/ml.
181 This was due to a large number of microbes in the rumen contents, then the addition of molasses
182 and tofu soaking wastewater further increases microbial growth. The research [\[23\] \(Astuti et
183 al. 2020\)](#) found 40×10^{12} total colonies in 1 ml of local bio activator rumen contents mixed with
184 molasses, soybean soaking water, and palm oil fronds.

185 The average laccase enzyme activity ranged from 11.0 to 20.11 U/ml, the mean lignin
186 peroxidase enzyme ranged from 1.74 to 4.93, while the manganese peroxidase enzyme ranged
187 from 2.40 - 7.06 U/ml. Laccase enzyme activity was much higher than [\[24\] \(Dimawarnita,
188 Panji, and Faramitha 2019\)](#) which measured enzyme activity in *Pleurotus ostreatus* with media
189 containing OPEFB 0.35 U/ml and lignin peroxidase (LiP) activity of 0.269 U/mL. The lignin
190 enzyme activity of sugarcane fermentation by 10% *Phanerochaete chrysosporium* were laccase
191 2.02 U/ml, LiP 1,677 U/ml, MnP 0.33 U/ml. This was because the microbes that thrive in this
192 local activator have been added to the palm fronds and leaves high in lignin content. Rumen
193 microbes will develop and produce enzymes based on the feed given [\[25\] \(Astuti et al. 2021\)](#).

194

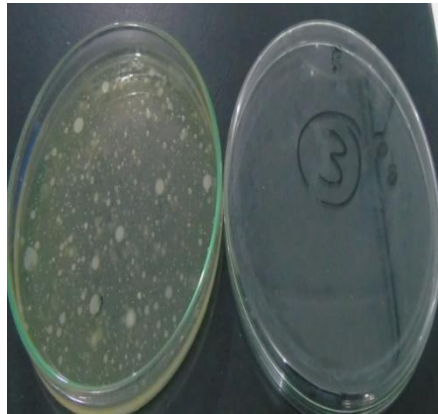
195 3.2. Identification of microorganism morphology

196 The analysis identifies fungi and bacteria's morphology present in the local bioactivator
197 rumen contents. The identification of microbes was performed based on the results of the best
198 enzyme activity evaluation. Figure 2 shows that the bacterial colonies are circular, small spread
199 out, thin, the edges of the settlements are flat, white in color, and the structure is transparent.
200 Morphological observations of fungal colonies showed circular colony shape, elevation
201 convex, uneven edges, white color, and transparent structure (Figure 2 and 3). The shape of the
202 colony in this study showed the same results with the research [\[26\] \(Yogyaswari, Rukmi, and
203 Raharjo 2016\)](#) which found around and white colony shape from bacterial isolates rumen
204 contents of Fries Holland. Based on microscopy observations of all bacterial isolates were
205 gram-positive, and bacilli (Table 2 and Figure 4). The results of the chemical analysis are
206 shown in Table 2.

207 The gram staining results on the isolates showed gram-positive bacteria, negative H₂S content,
208 positive catalase. Furthermore, in the microscopic and macroscopically observations, bacterial

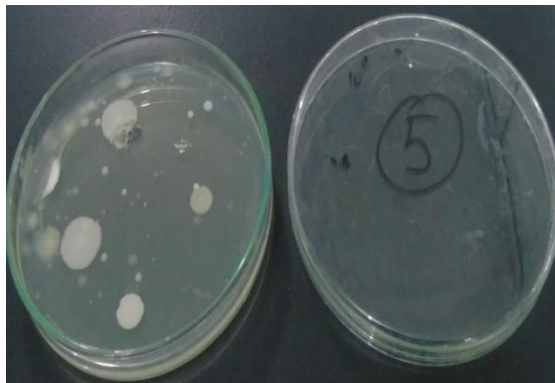
209 isolates had similarities in all isolates bacillus shaped, and there were two genera, namely
210 *Bacillus* sp one and *Bacillus* sp 2, Table 2).

211 The bacterial identification results showed *Bacillus* sp 1 in the local microorganism
212 rumen-filled added with palm fronds, and bacillus sp 2 in the local bioactivator rumen content
213 isolates with added fronds and palm leaves. In addition, the microscopic and macroscopically
214 observations showed that bacterial isolates in Figure 3 had similarities in all bacillus shapes.



215
216
217

Figure 2. Bacterial colonies on 10^{-8}



218
219
220

Figure 3. Fungal colonies on 10^{-5}

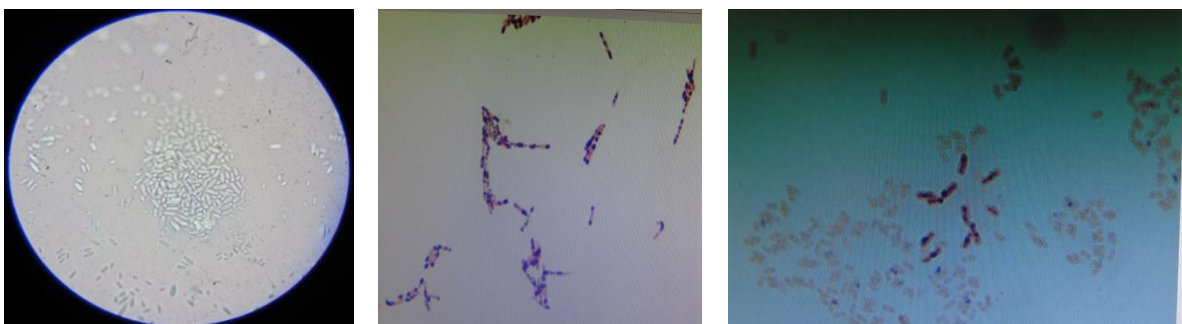


Figure 4. The image of local microorganisms staining the contents of the rumen in the form of bacilli on NA media with the genus *Bacillus* sp

221

222 Table 2. Chemical examination results for microorganism isolates on NA media

No	Treatment	NA 1	NA 2
1.	Colony(Color, shape, Traits)	White	White
2.	Gram (Morfologi, Spora)	+ bacteri	+ bacteri
3.	Aerobic / Anaerobic	A	A spora
4.	TSIA	M/K	M/K
5.	Gas	-	+
6.	H ₂ S	-	-
7.	catalase	+	+
8.	Oxidase	-	-
9.	Motility	+	+
10.	Indole	-	-
11.	Urea	+	+
12.	Citrat	-	-
13.	Laktosa	-	+
14.	Glukosa	-	+
15.	Sukrosa	-	-
16.	Mannitol	-	+
17.	MR	+	+
18.	VP	+	+
19.	OF	-	+
20.	Nitrat	-	+
21.	Gelatin	+	+
22.	Genus	<i>Basillus, sp 1</i>	<i>Basillus sp 2</i>

223

224

225 **4. Conclusion**

226 Based on the research results, it was concluded that the best bioactivator that can be
227 used to improve the quality of the feed was rumen content mixed with molasses, soybean
228 soaking water, leaves, and oil palm frond.

229

230 References

- 231 [1] Murni, R., Suparjo, Akmal, B. L. & Ginting. 2008. Buku Ajar Teknologi Pemanfaatan
232 Limbah untuk Pakan. Animal feeding Laboratorium. Jambi: Fakultas Peternakan
233 animal science Faculty. Jambi University
- 234 [2] Astuti, T., M. Nasir Rofiq, Nurhaita, and U. Santoso. 2019. Analysis of fiber fraction
235 of palm oil frond fermented with different microbes and soluble carbohydrates addition
236 as ruminant feeding. IOP Conference Series: Earth and Environmental Science 347
237 (2019) 012059, 6th International Conference on Sustainable Agriculture, Food and
238 Energy. Manila
- 239 [3] Glenn, J.K., Morgan, M.A., May/eld, M.B., Kuwahara, M., Gold, M.H., 1983. An
240 extracellular H₂O₂ is requiring enzyme preparation involved in lignin biodegradation
241 in white rot basidiomycete Phanerochaete chrysosporium. Biochemical and
242 Biophysical study Communications 114, 1077–1083.
- 243 [4] Hattaka, A. 1994. “Lignin Modifying Enzyme From Selected White Rot Fungi:
244 Production And Role In Lignin Degradation”. FEMS Microbial, Vol: 13 hlm 125–
245 135.
- 246 [5] Trismilah, Deden, Sumaryanto. 2003. Produksi xilanase. J
247 Sains dan Teknologi (2): 66-69
- 248 [6] Statistic Indonesia. 2020.
249 [https://www.bps.go.id/indicator/24/214/1/livestock-slaughtered-at-slaughtering-house-](https://www.bps.go.id/indicator/24/214/1/livestock-slaughtered-at-slaughtering-house-abattoir-by-province-and-kind-of-livestock.html)
250 [abattoir-by-province-and-kind-of-livestock.html](https://www.bps.go.id/indicator/24/214/1/livestock-slaughtered-at-slaughtering-house-abattoir-by-province-and-kind-of-livestock.html)
- 251 [7] Abbas, M.H., 1987. Determination of nutrients in cow's rumen content and its
252 utilization. In the feed of medium-type egg-laying hens during growth and production.
253 Dissertation. Postgraduate Faculty, IPB. Bogor.
- 254 [8] Budiansyah, A., Resmi, K., Wiryawan, K.G., Soehartono, M.T., Widyastuti, Y., Ramli,
255 N., 2010. Isolation and Characterization of Carbohydrases in Beef Cattle Rumen Liquor
256 from Abattoir, Media Peternakan, 33 (1): 36-43
- 257 [9] Wina, E. 2005. The technology of utilizing microorganisms in feed to improve ruminant
258 productivity in Indonesia: a review. WARTAZOA Vol.15 No. 4. Page 173-186
- 259 [10] Pandey, R., A., Soccol, C. R., Nigam, P.; Soccol, V. T. Vandenberghe, L. P. S. Mohan.

- 260 2000. — Biotechnological potential of agro industrial residues. Cassava bagasse.
261 Bioresour. Technol, vol. 74, no. 1, pp. 81–87,
- 262 [11] — Anggraeni, F., Isnaeni, and Achmad Toto Poernomo. 2016. Pengaruh Konsentrasi
263 Molase Terhadap Aktivitas Enzim Fibrinolitik Dari *Bacillus Subtilis* Atcc 6633. *Jurnal*
264 *Berkala Ilmiah Kimia Farmasi*, Vo.5 No. 1 Juni 2016.
- 265 [12] — Richana, N. 2002. Produksi dan Prospek Enzim Xilanase dalam Perkembangan
266 Bioindustri di Indonesia”, *Bulletin Agrobio*, 5:29–36
- 267 [13] — Beilen, J.V., Li, Z., 2002, ”Enzyme Technology: an overview”, *Current Opinion in*
268 *Biotechnology*, 13:338–344.
- 269 [14] — Huey, H.S., 2008, ”Enzymatics Enhanced Production of gaharu Oil: Effect of
270 Enzyme Loading and Duration Time” A thesis submitted in fulfilment of the
271 requirements for the award of the degree of Bachelor of Chemical Engineering,
272 University malaysia Pahang.
- 273 [15] — Troger, C., Niranjana, K., 2010. Sustainable Chitin Extraction and Chitosan
274 Modification for Application in the Food Industry” *International Conference on Food*
275 *Innovation*.
- 276 [16] — Wibisono, E., 2010, ”Imobilisasi Crude Enzim Papain yang Diisolasi Dari Getah Buah
277 Pepaya Dengan Menggunakan Kappa Karagenan dan Kitosan Serta Pengujian
278 Aktivitas dan Stabilitasnya”, *Skripsi pada Departemen Kimia MIPA USU*.
- 279 [17] — Pamungkas, W. 2012. The use of rumen fluids enzymes as an alternative to support
280 the utilization of local fish feed raw materials. *Media Akuakultur Volume 7, No 1*
281 *2012*.
- 282 [18] — Hartati, I. 2012. Purification of cellulase from cow rumen liquid by using expanded
283 bed adsorption. *Techno*, Volume 13 No. 1, April 2012 Hal. 43–51.
- 284 [19] — Tien, M, Kirk, T.K., 1984. Lignin-degrading enzyme from
285 *Phanerochaeta chrysosporium*: Purification, characterization and catalytic properties of
286 a unique H₂O₂-requiring oxygenase. *Proceedings of the national Academy of Science*
287 *of the United States of America*, pp.2280–2284.
- 288 [20] — Leonowicz, A., Grzywnowicz, K., 1981. Quantitative estimation of lacasse forms in
289 some white rot fungi using syringaldazine as a substrat. *Enzyme and Microbial*
290 *Tekenologi*, 3(1). Pp. 55–58.
- 291 [21] — Wariishi, H., Valli, K., Gold, M.H., 1992. Manganese (II) oxidation by manganese
292 peroxidase from the Basidiomyceteskinetic mechanism and role of chelators. *J. Biol.*
293 *Chem.* Nov 25;267(33):23688–95.

- 294 [22]—Murtiyaningsih, and M. Hazmi. 2017. Isolation and cellulase enzyme activities assays
295 in cellulolytic bacteria origin from soil wast. *Agitprop*, Vol. 15 (2): 293-308.
- 296 [23]—Astuti, T., Syahro Ali Akbar, Delsi Afrini, M. Nasir Rofiq, and Irna Humaira. 2020.
297 The identification of fungi colonies total on the rumen content of cow and buffalo
298 with addition of leaves and oil palm frond. *World Journal of Advanced Research and*
299 *Reviews*, 2020, 08(02), 314-317.
- 300 [24]—Dimawarnita, F., Tri Panji. 2019. Activity of ligninolytic enzyme of *Pleurotus*
301 *ostreatus* on media containing OPEFB and their application for dyes decolorization.
302 *Menara Perkebunan* 2019, 87(1), 31-40.
- 303 [25]—Astuti, T., Novirman Jamarun, Arief, and Gusri Yanti. 2021. Effect Fermentation of
304 Sugarcane Shoots With *Phanerochaete chrysosporium* on the Activity of Lacase
305 Enzymes, Lignin Peroxidase and Manganese Peroxidase. *IOP Conf. Series: Earth and*
306 *Environmental Science* 709 (2021). 7th International Conference on Sustainable
307 Agriculture, Food, and Energy. Phuket
- 308 [26]—Yogyaswari, S.A, M.G. Isworo Rukmi, B. Raharjo. 2016. Ekplorasi bakteri selulolitik
309 dari cairan rumen sapi Peranakan fries holland (pfh) dan limousine peranakan Ongole
310 (limpo). *Jurnal Biologi*, Volume 5 No 4, Oktober 2016, 70-80.
- 311 Abbas, M. Hafil. 1987. "Penentuan Zat-Zat Makanan Dalam Isi Rumen Sapi Dan
312 Pemanfaatannya Dalam Ransum Ayam Petelur Tipe Medium Pada Masa Pertumbuhan
313 Dan Produksi." IPB.Bogor.
- 314 Anggraeni, Felita, Isnaeni, and Achmad P. Toto. 2016. "Pengaruh Konsentrasi Molase
315 Terhadap Aktivitas Enzim Fibrinolitik Dari Bacillus Subtilis ATCC 6633." *Berkala*
316 *Ilmiah Kimia Farmasi* 5(1):18-24.
- 317 Astuti, T., Syahro Ali Akbar, Delsi Afrini, M. Nasir Rofiq, and Irna Humaira. 2020. "The
318 Identification of Fungi Colonies Total on the Rumen Content of Cow and Buffalo with
319 Addition of Leaves and Oil Palm Frond." *World Journal of Advanced Research and*
320 *Reviews* 02(08):314-317. doi: <https://doi.org/10.30574/wjarr.2020.8.2.0444>.
- 321 Astuti, T., M. Nasir Rofiq, Nurhaita, and U. Santoso. 2019. "Analysis of Fibre Fraction of Palm
322 Oil Frond Fermented with Different Microbes and Soluble Carbohydrates Addition as
323 Ruminant Feeding." *IOP Conference Series: Earth and Environmental Science* 347(1).
324 doi: 10.1088/1755-1315/347/1/012059.
- 325 Astuti, Tri, Novirman Jamarun, Arief, and Gusri Yanti. 2021. "Effect Fermentation of
326 Sugarcane Shoots with *Phanerochaete chrysosporium* on the Activity of Lacase Enzymes,
327 Lignin Peroxidase and Manganese Peroxidase." *IOP Conference Series: Earth and*
328 *Environmental Science* 709(1):3-7. doi: 10.1088/1755-1315/709/1/012065.

329 van Beilen, Jan B., and Zhi Li. 2002. "Enzyme Technology: An Overview." *Current Opinion*
330 *in Biotechnology* 13 4:338–44.

331 Budiansyah, A., Resmi, K. G. Wiryawan, M. T. Soehartono, Y. Widyastuti, and N. Ramli.
332 2010. "Isolation and Characterization of Carbohydases in Beef Cattle Rumen Liquor
333 from Abattoir | Isolasi Dan Karakterisasi Enzim Karbohidrase Cairan Rumen Sapi Asal
334 Rumah Potong Hewan." *Media Peternakan* 33(1).

335 Dimawarnita, Firda, Tri Panji, and Yora Faramitha. 2019. "Peningkatan Kemurnian Selulosa
336 Dan Karboksimetil Selulosa (CMC) Hasil Konversi Limbah TKKS Melalui Perlakuan
337 NaOH 12%." *E-Journal Menara Perkebunan* 87(2):95–103. doi:
338 10.22302/iribb.jur.mp.v87i2.339.

339 Glenn, J. K., M. A. Morgan, M. B. Mayfield, M. Kuwahara, and M. H. Gold. 1983. "An
340 Extracellular H₂O₂-Requiring Enzyme Preparation Involved in Lignin Biodegradation
341 by the White Rot Basidiomycete Phanerochaete Chrysosporium." *Biochemical and*
342 *Biophysical Research Communications* 114(3):1077–83. doi: 10.1016/0006-
343 291x(83)90672-1.

344 Hattaka, A. 1994. "Lignin-Modifying Enzymes from Selected White-Rot Fungi: Production
345 and Role from in Lignin Degradation." *FEMS Microbiology Reviews* 13:125–35. doi:
346 10.1016/j.biortech.2013.02.042.

347 Huey, H. S. 2008. "Enzymatics Enhanced Production of Gaharu Oil: Effect of Enzyme Loading
348 and Duration Time" University malaysia Pahang.

349 Leonowicz, A., and K. Grzywnowicz. 1981. "Quantitative Estimation of Laccase Forms in
350 Some White-Rot Fungi Using Syringaldazine as a Substrate." *Enzyme and Microbial*
351 *Technology* 3(1):55–58. doi: [https://doi.org/10.1016/0141-0229\(81\)90036-3](https://doi.org/10.1016/0141-0229(81)90036-3).

352 Ming, Tien, and Kirk T. Kent. 1984. "Lignin-Degrading Enzyme from Phanerochaete
353 Chrysosporium: Purification, Characterization, and Catalytic Properties of a Unique
354 H₂O₂-Requiring Oxygenase." *Proceedings of the National Academy of Sciences*
355 81(8):2280–84. doi: 10.1073/pnas.81.8.2280.

356 Murni, R., Suparjo, B. L. Akmal, and Ginting. 2008. *Buku Ajar Teknologi Pemanfaatan*
357 *Limbah Untuk Pakan*. Jambi: Animal feeding Laboratorium. Jambi University.

358 Murtiyaningsih, Hidayah, and Muhammad Hazmi. 2017. "Isolasi Dan Uji Aktivitas Enzim
359 Selulase Pada Bakteri Selulolitik Asal Tanah Sampah Isolation and Cellulase Enzyme
360 Activities Assays in Cellulolytic Bacteria Origin From Soil Waste." *Agritrop* 15(2):293–
361 308.

362 Pamungkas, Wahyu. 2012. "Penggunaan Enzim Cairan Rumen Sebagai Alternatif Untuk

363 Mendukung Pemanfaatan Bahan Baku Pakan Ikan Lokal.” *Media Akuakultur* 7(1):32. doi:
364 10.15578/ma.7.1.2012.32-38.

365 Pandey, A., CR. Soccol, P. Singh - Nee Nigam, VT. Soccol, LPS. Vandenberghe, and R. Mohan.
366 2000. “Biotechnological Potential of Agro-Industrial Residues. II: Cassava Bagasse.”
367 *Bioresource Technology* 74(1):81–87.

368 Richana, Nur. 2002. “Produksi Dan Prospek Enzim Xilanase Dalam Pengembangan
369 Bioindustri Di Indonesia.” *Buletin AgroBio* 5(1):29–36.

370 Statistic, Indonesia. 2020. *Jumlah Ternak Yang Di Potong Di Indonesia.*

371 Trismilah, Deden Rosid; 2009. “PRODUKSI XILANASE MENGGUNAKAN MEDIA
372 LIMBAH PERTANIAN DAN PERKEBUNAN.” *Jurnal Teknologi Lingkungan* (Vol. 10
373 No. 2 (2009)):137–44.

374 Troger, C., and K. Niranjana. 2010. “Sustainable Chitin Extraction and Chitosan Modification
375 for Application in the Food Industry.” *International Conference on Food Innovation.*

376 Wariishi, H., K. Valli, and M. H. Gold. 1992. “Manganese(II) Oxidation by Manganese
377 Peroxidase from the Basidiomycete *Phanerochaete Chrysosporium*. Kinetic Mechanism
378 and Role of Chelators.” *Journal of Biological Chemistry* 267(33):23688–95. doi:
379 10.1016/s0021-9258(18)35893-9.

380 Wibisono, Eko. 2010. “Mobilisasi Crude Enzim Papain Yang Diisolasi Dari Getah Buah
381 Pepaya (*Carica Papaya* L) Dengan Menggunakan Kappa Karagenan Dan Kitosan Serta
382 Pengujian Aktivitas Dan Stabilitasnya.”

383 Wina, Elizabeth. 2005. “The Technology of Utilizing Microorganism in Feed To Improve
384 Ruminant Productivity in Indonesia: A Review.” *Wartazoa* 15(4):173–86.

385 Yogyaswari, Sekar Ayoe, M. G. Isworo Rukmi, and Budi Raharjo. 2016. “Ekplorasi Bakteri
386 Selulolitik Dari Cairan Rumen Sapi Peranakan Fries Holland (PFH) Dan Limousine
387 Peranakan Ongole (Limpo).” *Jurnal Biologi* 5(4):70–80.

388

Declaration of interests

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Activity of cellulase and ligninase enzymes in local microorganisms from cattle and buffalo rumen content

Corresponding Author:

Assoc. Prof. Tri Astuti

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Activity of Cellulase and Ligninase Enzymes in a Local Bioactivator from Cattle and Buffalo
Rumen Contents

Tri Astuti^{1*}, Syahro A. Akbar¹, Muhamad Nasir Rofiq², Novirman Jamarun³, Nurul Huda⁴,
Ahmad Fudholi^{2,5}

¹Department of Animal Science, Faculty of Agriculture University of Mahaputra Muhammad
Yamin, Indonesia

²National Research and Innovation Agency (BRIN), Indonesia

³Faculty of Animal Science, Andalas University, Padang, West Sumatera, Indonesia

⁴Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, Malaysia

⁵Solar Energy Research Institute, Universiti Kebangsaan Malaysia, 43600, Bangi, Selangor,
Malaysia

Correspondent author : adektuti@gmail.com

Abstract

Lignin is the main component of agricultural and plantation wastes, such as bagasse, straw and oil palm fronds. Lignocellulosic bonds in lignin, cellulose and hemicellulose can be broken down by enzymes. Numerous studies have utilised plantation waste as feed ingredients. Lignin is the limiting factor that affects the digestibility of this material. Therefore, the lignin content of plantation waste must be reduced before it is used as a feed ingredient. The use of local bioenzymes will be effective in breaking lignocellulose bonds. Thus, finding sources of enzymes that are easy to obtain, inexpensive to produce and effective as lignocellulose-degrading enzymes is necessary. This study aims to determine the activity of cellulase and ligninase enzymes in a bioactivator from rumen contents incubated for 7 days with different enzyme energy sources. The treatments included cattle and buffalo rumen contents added with molasses, palm frond, palm leaf extract and each enzyme. The parameters observed were the enzyme activities of cellulase, laccase, lignin and manganese peroxidase (MnP). Microbial identification was also performed. The results of statistical analysis showed insignificant differences ($P > 0.05$) amongst the parameters of the enzyme activities of cellulase (2.22–3.51 U/ml), laccase (10.62–20.11 U/ml), lignin peroxidase (1.74–4.93 U/ml) and MnP (2.40–7.06 U/ml). *Lactobacillus* sp. was identified through bacterial identification. Therefore, the live

33 microbes discovered in the local microorganism solution originated from the study
34 environment and not from the rumen contents.

35 **Keyword:** Enzyme activity, microbial morphology, cellulase, lignase, rumen microbes

36 1. Introduction

37 Animal feeding is a major factor determining the success of livestock businesses. Many
38 studies have used agricultural and plantation by-products as substitutes for field grass in
39 ruminant feed. These by-products mostly contain cellulose, hemicellulose and lignin that
40 consist of lignocellulosic bonds. The digestibility of ruminant feed ingredients is influenced by
41 the contents of lignin, cellulose and soluble substances. These rumen microorganisms could not
42 for lignin degradation because they do not generate enzymes with ligninolytic activity
43 (Pollegioni et al., 2015). Oil palm fronds as animal feed berries are low crude protein about
44 1.6%, high crude fiber content reaching 56.1%, and contain 79.27% ADF, 64.25% NDF,
45 12.91% cellulose, 15.34% hemicellulose and 15.34% lignin (Astuti et al. 2019, Tafsin et al,
46 2018). Lignin is a complex, heterogeneous phenylpropanoid polymer that accounts for 25%–
47 30% of plant biomass. It is resistant to microbial degradation under natural conditions. Lignin
48 and cellulose are the main plant components that are enzymatically degraded by bioactivators,
49 such as the enzymes cellulase and ligninase. Ligninolytic microbial systems, which are
50 primarily composed of lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase
51 (Glenn et al. 1983), have been used to improve digestibility and nutritional value. LiP and MnP
52 are extracellular peroxidase enzymes that use H₂O₂ to degrade lignin, while laccase is a copper-
53 containing enzyme that uses molecular oxygen to degrade lignin (Hattaka 1994). Laccase is a
54 metalloenzyme that can degrade lignin during the bio-catalysis process (Kameshwar and Qin,
55 2016). The use of commercial enzymes for animal feeding will need additional livestock
56 operational costs. Therefore, because of it is necessary to find sources of natural enzymes that
57 are cheap and easy to produce.

58 The use of microbes for enzyme production has several advantages, including low
59 production costs, short production times, high growth speeds, and easy control. The bacteria
60 responsible for lignin degradation can be found in environments such as soil, digestive system
61 of herbivora, wood-eating insects, effluents from paper industry, sludge, etc. (Brown and
62 Chang 2014; Tian et al 2014).

63 The contents of the rumen cattle contain crude protein, about 18.52 - 19.56%, Amino acids
64 include lysine, leucine, alanine, aspartate, arginine, valine, threonine and low methionine
65 (Jovanovic et al., 1997). Rumen contents It also contains high crude fiber, calcium, phosphorus

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66 and magnesium (Agbabiaka et al., 2012; Elfaki and Abdelati 2015). ~~Rumina Rument microbes~~
67 ~~would will~~ produce enzymes in accordance with the given substrate. For example, ~~rument~~
68 ~~inants-microbes would will~~ produce enzymes that degrade fibre when given straw and
69 enzymes that break down tannins when given calliandra (Wina 2005). Lignin and cellulose are
70 the main plant components that are enzymatically degraded by cellulose and lignase. These
71 enzymes are produced by bioactivators (Pandey et al. 2000). Molasses is the waste of sugar
72 mills; that can use as a fermentation stimulant to increase the rate of silage acidification by
73 providing fermentable sugars for the growth of Lactic Acid Bacteria (Guo et al, 2014).

74 Enzymes are biopolymer molecules that are composed of a series of amino acids in an
75 ordered and fixed composition and chain arrangement. They are proteins that are produced and
76 used by living cells to catalyse chemical reactions with a high level of specificity and increased
77 reaction rates (van Beilen and Li 2002; Richana 2002). Thus, enzymes have various advantages
78 over conventional processes using chemicals. However, the main obstacle to the industrial
79 application of enzymes is the high price of enzymes, ~~;- moreover, enzymes and~~ cannot be used
80 repeatedly (Huey 2008; Troger, C. and Niranjana 2010; Wibisono 2010). ~~Lignin-degrading or~~
81 ~~ligninolytic enzymes include laccase (polyphenol oxidase), LiP and MnP; all three are~~
82 ~~extracellular multienzymes that participate in lignin depolymerisation (Sanchez, 2010).~~
83 Extracellular oxidative enzymes can attack and degrade lignin, by utilizing types of peroxidases
84 including lignin peroxidase (LiP), manganese peroxidase (MnP), versatile peroxidase (VP),
85 and dye remover, peroxidase (DyP) (Lambertz et al., 2016).

86
87 Cellulases are complex enzymes that gradually cut cellulose chains into glucose. Fungi,
88 bacteria and ruminants produce cellulase. The commercial production of enzymes usually
89 applies fungi or bacteria. Although the production of cellulases from ruminants has been
90 underappreciated, cellulases originating from ruminants are being produced given their high
91 availability. The rumen microbes are considered the most efficient microbial system in
92 degrading lignocellulosic biomass (Flint et al., 2008), Rumen microbes are very good sources
93 of fibrinolytic enzymes, carbohydrate-active enzymes that can degrade lignocellulose, because
94 they can produce enzymes from their environment and can be used in the feed and food
95 industry, cellulose biofuels, and other industrial processes (Ribeirio et al, 2016). In contrast to
96 commercial enzymes, cellulases have the potential to be produced from beef rumen fluid.
97 Therefore, this study aims to determine the activity of cellulase and ligninase as a local
98 bioactivator produced by incubating rumen contents with several different ingredients as the

99 microbe substrate. This research can produce complex enzymes (cellulase, MnP, LiP and
100 laccase) from waste materials that are cheap and easy to obtain.

101

102 **2. Materials and methods**

103 The materials used in this study were cattle and buffalo rumen contents, molasses,
104 soybean soaking wastewater, oil palm fronds, oil palm leaves and some chemicals for enzyme
105 activity measurement.

106 2.1. Bioactivator process

107 The rumen contents of cattle and buffalo were collected from an abattoir and placed in
108 tubes. All the rumen contents were added with molasses and tofu soaking water. The treatments
109 were the addition of palm leaves, palm fronds and a mixture of these materials, which had high
110 lignin contents. Palm fronds and leaves were added to induce the bioactivator to produce
111 ligninase. The rumen contents, molasses and tofu soaking wastewater were mixed at the ratio
112 of 1:1:8 with 10% oil palm fronds and leaves. The tubes were tightly closed. Then, a hole was
113 made in the middle and connected with a hose to a small bottle filled with water to catch
114 fermentation waste. The tubes were incubated for as long as 10 days as shown in Figure 1.

115



116

117 Figure 1. Fermentation with the local bioactivator

118

119 2.2. LiP activity test

120 A total of 0.2 ml of enzyme filtrate (sample extract and phosphate buffer shaken for 1
121 h and then centrifuged for 10 min at 10 000 rpm), 0.05 ml of 5 mM H₂O₂, 0.1 ml of 8 mM
122 veratrine alcohol, 0.2 ml of 0.05 M acetate buffer (pH 3) and 0.45 ml of distilled water were
123 added to a cuvette and then shaken. The absorbance of the solution at 310 nm was recorded at
124 0 and 30 min intervals. One unit of LiP enzyme activity was defined as the amount of enzyme

125 that caused the conversion of 1 μmol ($1\mu\text{mol} = 10^{-6}$ mol) veratril alcohol per minute (Ming
126 and Kent 1984).

127

128 2.3. MnP activity test

129 A total of 0.1 ml of 50 mM Na-lactate buffer (pH 5) was added with 0.1 ml of 4 mM
130 guaiacol, 0.2 ml of 1 mM MnSO_4 , 0.1 ml of 1 mM H_2O_2 and 0.3 ml of distilled water, as well
131 as 0.2 ml of enzyme filtrates. The solution was then checked and read at the wavelength of 465
132 nm at 0 and 30 min (Leonowicz and Grzywnowicz 1981).

133

134 2.4. Laccase activity test

135 A total of 0.4 ml of enzyme filtrate was added with 0.5 ml of acetate buffer (pH 5) and
136 0.1 ml of 1 mM 2'-azinobis-3-ethylbenzothiazole-6-sulphonic acid. Measurements using
137 spectrophotometer were taken at 420 nm at 0 and 30 min (Wariishi, Valli and Gold 1992).

138 Enzyme activity tests were conducted at the biotechnology laboratory of the Faculty of
139 Animal Husbandry, Andalas University, Padang. Microbial morphology identification was
140 performed at the Baso Veterinary Centre Laboratory, Bukit Tinggi. The analysis was continued
141 by identifying the morphology of the fungi and bacteria present in the local bioactivator rumen
142 contents. Microbes were identified on the basis of the results of the best enzyme activity
143 evaluation. Samples were inoculated onto sodium agar (NA) medium for the identification of
144 bacteria, and potato dextrose agar (PDA) was used as the medium for fungal/mould growth.
145 The samples were diluted to the concentration of 10^{-1} – 10^{-10} , and the selected isolates were
146 further analysed on the basis of colony distribution

147

148 2.5. Experimental design

149 A 2×4 factorial randomised block design with three replications for each treatment
150 was used in this study. Factor A was the type of rumen content: A1 = rumen cattle and A2 =
151 buffalo rumen. Factor B was the microbial energy substrate: B1 = molasses, B2 = molasses +
152 palm frond extract, B3 = molasses + palm leaf extract and B4 = molasses + palm frond and leaf
153 extract. Enzyme activity data were processed by using analysis of variance. The bioactivator
154 was identified by using the described method. Significant differences ($P < 0.05$) were further
155 tested by using Duncan's multiple range tests.

156 The variables were observed through the isolation and identification of the bioactivator
157 (fungi and bacteria) based on morphology and cellulase and ligninase activity tests on the crude
158 bioenzymes in rumen content. Morphological data were obtained through descriptive analysis.

159

160

161 **3. Results**

162 3.1. Enzyme activity test

163 The activities of cellulose, laccase, LiP and MnP were tested. These enzymes are
 164 essential for lignin degradation. The average results of the enzyme activity test for the local
 165 bioactivator in rumen content are shown in Table 1.

166

167

168

169 Table 1. Average activity (U/ml) of the local bioactivator in rumen contents

Factor B	Factor A				Average
	B ₁	B ₂	B ₃	B ₄	
Cellulase enzymes					
A1	3.51	2.41	2.81	2.22	2.74
A2	3.44	2.52	3.31	3.64	3.23
	3.48	2.46	3.06	2.93	2.98
Lacasse Enzymes					
A1	20.11	16.40	11.70	15.91	16.03
A2	11.00	12.86	10.62	14.59	12.27
	15.55	14.63	11.16	15.25	14.15
Enzim Lignin Peroksidase					
A1	4.06	4.93	2.80	4.11	3.97
A2	2.61	1.74	4.16	3.80	3.08
	3.34	3.34	3.48	3.95	3.53
Manganese Peroxidase Enzymes					
A1	2.40	5.61	3.39	7.06	4.61
A2	6.86	4.17	3.18	5.18	4.85
	4.63	4.89	3.29	6.12	4.73

170 Note: A1 = cow rumen content, A2 = buffalo rumen content, B1 = rumen content only, B2 =
 171 rumen content and palm fronds, B3 = palm leaves and B4 = leaves and palm fronds

172

173 **3.2. Identification of microbial morphology**

The results of the chemical analysis are shown in Table 2. Microscopic and macroscopic analyses revealed that the bacterial colonies in Figure 2, the fungal colonies in Figure 3 and the bacterial isolates in Figure 4 had similar bacillus shapes.

174 Table 2. Chemical examination results for microbial isolates on NA media

No	Treatment	NA 1	NA 2
1.	Colony (color, shape, traits)	White	White
2.	Grams (morphology, spora)	+ bacteri	+ bacteri
3.	Aerobic / Anaerobic	A	A spora
4.	TSIA	M/K	M/K
5.	Gas	-	+
6.	H ₂ S	-	-
7.	catalase	+	+
8.	Oxidase	-	-
9.	Motility	+	+
10.	Indole	-	-
11.	Urea	+	+
12.	Citrate	-	-
13.	Lactose	-	+
14.	Glucose	-	+
15.	Sucrose	-	-
16.	Mannitol	-	+
17.	MR	+	+
18.	VP	+	+
19.	OF	-	+
20.	Nitrat	-	+
21.	Gelatin	+	+
22.	Genus	<i>Basillus, sp 1</i>	<i>Basillus sp 2</i>

175

176

177

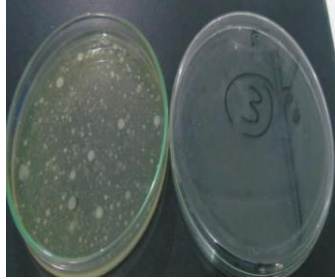


Figure 2. Bacterial colonies on 10^{-8}

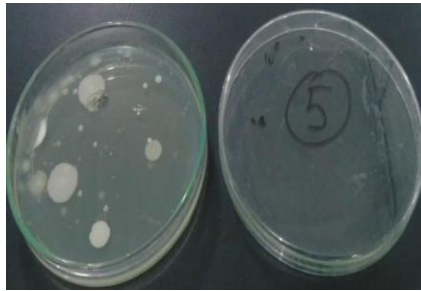


Figure 3. Fungal colonies on 10^{-5}



Figure 4. Staining of bacilli in the genus *Bacillus* in rumen contents on NA media

4. Discussion

4.1. Enzyme activity test

Statistical analysis showed that no interaction effect ($P > 0.05$) existed between the rumen source (cattle and buffalo) and microbe energy source on the activities of cellulase, laccases, LiP and MnP. The data in Table 1 show that LiP enzyme activity ranged from 2.46 U/ml to 3.48 U/ml. LiP enzyme activity in this study was higher than that in the work of Mufidatul and Kuswytasari (2013), who found that LiP from *Gliomastix* sp. T3.7 had the

193 enzyme activity of 0.06–1.022 in corn hump waste at pH 5 and 25 °C–35 °C. The data showed
194 the rumen contents were the source of the bioactivator that was ingested and remained
195 dominant in the form of fibre from forage consumed by livestock. For all treatments, the main
196 energy source was molasses and soybean soaking water. Therefore, the main energy source in
197 this work was very representative and supplied the energy needed by the microbes in the rumen
198 to grow and exhibit high production. The average cellulase activity in this study was
199 considerably higher than that in a previous work (Murtiyaningsih and Hazmi 2017) that
200 obtained the highest enzyme activity of 0.028279 U/m for cellulase from cellulolytic soil
201 bacteria. This difference was due to the large number of microbes in the rumen contents and
202 the addition of molasses and tofu soaking wastewater that further increased microbial growth.
203 Another study (Astuti et al. 2020) found 40×10^{12} total colonies in 1 ml of local bioactivator
204 rumen contents mixed with molasses, soybean soaking water and palm oil fronds.

205 In this work, the average laccase enzyme activity ranged from 11.0 U/ml to 20.11 U/ml,
206 LiP activity ranged from 1.74 U/ml to 4.93 U/ml and MnP activity ranged from 2.40 U/ml to
207 7.06 U/ml. Heterologous laccase production has been used for obtaining increased amount of
208 the enzyme (Debnath, et al., 2020). The laccase enzyme activity in this work was considerably
209 higher than the LiP activity of 0.269 U/ml and OPEFB activity of 0.35 U/ml found by a
210 previous study (Dimawarnita, Panji and Faramitha 2019) on *Pleurotus ostreatus* in media. The
211 activity of laccase and LiP enzymes in this study was much higher than the results of the study
212 of Dimawarnita, et al. (2019) who found LiP activity of 0.269 U/ml and laccase activity of 0.35
213 U/ml in *Pleurotus ostreatus* in oil palm empty fruit bunch media. Research of fithri et al; (2020)
214 Laccase could degrade lignin with apparent damage the lignocellulose substrate of corn cob
215 and rice straw. Laccase, LiP and MnP activities of 2.02, 1.677 and 0.33 U/ml, respectively,
216 were observed in sugarcane fermentation by 10% *Phanerochaete chrysosporium* because the
217 microbes that thrived in the local activator were supplied with palm fronds and leaves with
218 high lignin contents. Dhakar et al., (2014) maximum laccase production was observe as 28,2
219 U/l. Rumen microbes develop and produce enzymes on the basis of given feed (Astuti et al.
220 2021).

221

222 4.2. Identification of microbial morphology

223 The morphology of the fungi and bacteria present in the local bioactivator rumen
224 contents was analysed. The microbes were identified on the basis of the best results of the
225 enzyme activity evaluation.

226 Figure 2 shows that the bacterial colonies were circular, small, spread out and thin and
227 had flat colony edges, a white colour and transparent structure. Morphological observation
228 revealed that the fungal colonies had a circular colony shape, convex elevation, uneven edges,
229 white colour and transparent structure (Figures 2 and 3). The shape of the colonies in this study
230 was the same as that of the colonies reported by Yogyaswari et al. (2016), who found that the
231 bacterial isolates from the rumen contents of Fries Holland cows formed white colonies. The
232 microscopy observations demonstrated that all of the bacterial isolates were Gram-positive
233 bacilli (Table 2 and Figure 4).

234 The Gram staining results for the isolates showed the bacteria were Gram-positive
235 bacteria that were negative for H₂S content and positive for catalase content. Furthermore,
236 microscopic and macroscopic analyses revealed that the bacterial isolates shared similar
237 bacillus shapes and belonged to two genera, namely, *Bacillus* sp. 1 and *Bacillus* sp. 2 (Table
238 2).

239 The bacterial identification results demonstrated that *Bacillus* sp. 1 was present in the local
240 bioactivator comprising rumen content added with palm fronds and that *Bacillus* sp. 2 was
241 present in the local bioactivator comprising rumen content added with fronds and palm leaves.
242 In addition, the microscopic and macroscopic observations showed that the bacterial isolates
243 in Figure 3 all had similar bacillus shapes. Some bacterial strains could lignin-degrading such
244 as *Bacillus* sp. CS-1, *Bacillus pumilus*, *Bacillus atrophaeus*, *Bacillus* sp, ect. (Longe et al 2016;
245 Priyadarshinee et al 2016)

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247 5. Conclusion

248 On the basis of the research results, the best bioactivator that can be used to improve
249 feed quality was concluded to be rumen content mixed with molasses, soybean soaking water
250 and oil palm fronds and leaves. It had the cellulase, laccase, LiP and MnP activities of 2.2,
251 15.91, 4.11 and 7.06 U/ml, respectively. Bacterial identification revealed that *Bacillus* sp. was
252 present under the best treatment.

253

254 References

- 255 Agbabiaka, L.A.; Madubuike, F.N. and Amadi, S.A. (2012). Studies on nutrients and anti-
256 nutrients of rumen digesta from three most domesticated ruminants in Nigeria.
257 Pakistan Journal of Nutrition, 11 (7): 580- 582.
- 258 Astuti, T., Syahro Ali Akbar, Delsi Afrini, M. Nasir Rofiq, and Irma Humaira. 2020. "The
259 Identification of Fungi Colonies Total on the Rumen Content of Cow and Buffalo with

260 Addition of Leaves and Oil Palm Frond.” *World Journal of Advanced Research and*
261 *Reviews* 02(08):314-317. doi: <https://doi.org/10.30574/wjarr.2020.8.2.0444>.

262 Astuti, T., M. Nasir Rofiq, Nurhaita, and U. Santoso. 2019. “Analysis of Fibre Fraction of Palm
263 Oil Frond Fermented with Different Microbes and Soluble Carbohydrates Addition as
264 Ruminant Feeding.” *IOP Conference Series: Earth and Environmental Science* 347(1).
265 doi: [10.1088/1755-1315/347/1/012059](https://doi.org/10.1088/1755-1315/347/1/012059).

266 Astuti Tri, Novirman Jamarun, Arief, and Gusri Yanti. 2021. “Effect Fermentation of
267 Sugarcane Shoots with Phanerochaetechrysosporium on the Activity of Lacase Enzymes,
268 Lignin Peroxidase and Manganese Peroxidase.” *IOP Conference Series: Earth and*
269 *Environmental Science* 709(1):3–7. doi: [10.1088/1755-1315/709/1/012065](https://doi.org/10.1088/1755-1315/709/1/012065).

270 Beilen, Jan B.van, and Zhi Li. 2002. “Enzyme Technology: An Overview.” *Current Opinion*
271 *in Biotechnology* 13 4:338–44.

272 [Brown, Margaret E; Chang, Michelle CY. 2014. Exploring bacterial lignin degradation.](#)
273 [Current Opinion in Chemical Biology, 19\(\), 1–7. doi:10.1016/j.cbpa.2013.11.015](#)

274 [Debnath R and Tanima Saha. 2020. An insight into the production strategies and applications](#)
275 [of the ligninolytic enzyme laccase from bacteria and fungi. Biocatalysis and Agriculture](#)
276 [Biotechnology 26 \(2020\) 101645-](#)

277 Elfaki, M.O.A.; Abdelatti, K.A. and Malik, H.E.E. (2015). Effect of dietary dried rumen
278 content on broiler performance, plasma constituents and carcass characteristics. *Global*
279 *Journal of Animal Scientific Research*, 3(1): 264-270

280 [Fithri, L, Tri Puspaningsih, N N., Asmarani, O., Matuzahroh, N., Fitrah Dewi, G.D. Arizandy,](#)
281 [R.Y., Characterization of Fungal Laccase Isolated from oil palm empty fruit bunches](#)
282 [\(OPEFB\) and its degradation from the Agriculture Waste. Biocatalysis and Agricultural](#)
283 [Biotechnology. Volume 27 August 2020.](#)

284 [Flint, Harry J.; Bayer, Edward A.; Rincon, Marco T.; Lamed, Raphael; White, Bryan A. \(2008\).](#)
285 [Polysaccharide utilization by gut bacteria: potential for new insights from genomic](#)
286 [analysis. , 6\(2\), 121–131. doi:10.1038/nrmicro1817](#)

287

288 Glenn, J. K., M. A. Morgan, M. B. Mayfield, M. Kuwahara, and M. H. Gold. 1983. “An
289 Extracellular H₂O₂-Requiring Enzyme Preparation Involved in Lignin Biodegradation
290 by the White Rot Basidiomycete Phanerochaete Chrysosporium.” *Biochemical and*
291 *Biophysical Research Communications* 114(3):1077–83. doi: [10.1016/0006-](https://doi.org/10.1016/0006-291x(83)90672-1)
292 [291x\(83\)90672-1](https://doi.org/10.1016/0006-291x(83)90672-1).

293 Guo, G., Yuan, X. J., Li, L. X., Wen, A. Y. & Shao, T. Effects of fibrolytic enzymes, molasses

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294 and lactic acid bacteria on fermentation quality of mixed silage of corn and hulless-barely
295 straw in the Tibetan Plateau. *Grassl. Sci.* 60, 240–246 (2014).

296 Dimawarnita, Firda, Tri Panji, and Yora Faramitha. 2019. “Peningkatan Kemurnian Selulosa
297 Dan Karboksimetil Selulosa (CMC) Hasil Konversi Limbah TKKS Melalui Perlakuan
298 NaOH 12%.” *E-Journal Menara Perkebunan* 87(2):95–103. doi:
299 10.22302/iribb.jur.mp.v87i2.339.

300 Dhakar K, Rahul jain, Sushma, and Anita Pandey. 2014. Prolonged Laccase Production by a
301 Cold andpH Tolerant Strain of Penicillium pinophilum (MCC 1049)
302 Isolated from a Low Temperature Environment. Enzyme Research. Volume 2014, 6
303 pages

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Formatted: English (United States)

304 Glenn, J. K., M. A. Morgan, M. B. Mayfield, M. Kuwahara, and M. H. Gold. 1983. “An
305 Extracellular H₂O₂-Requiring Enzyme Preparation Involved in Lignin Biodegradation
306 by the White Rot Basidiomycete *Phanerochaete Chrysosporium*.” *Biochemical and*
307 *Biophysical Research Communications* 114(3):1077–83. doi: 10.1016/0006-
308 291x(83)90672-1.

309 Guo, G., Yuan, X. J., Li, L. X., Wen, A. Y. & Shao, T. Effects of fibrolytic enzymes, molasses
310 and lactic acid bacteria on fermentation quality of mixed silage of corn and hulless-barely
311 straw in the Tibetan Plateau. *Grassl. Sci.* 60, 240–246 (2014).

312 Hattaka, A. 1994. “Lignin-Modifying Enzymes from Selected White-Rot Fungi: Production
313 and Role from in Lignin Degradation.” *FEMS Microbiology Reviews* 13:125–35. doi:
314 10.1016/j.biortech.2013.02.042.

315 Huey, H. S. 2008. “Enzymatics Enhanced Production of Gaharu Oil: Effect of Enzyme Loading
316 and Duration Time.” University malaysia Pahang.

317 Jovanovic, M. and Cuperlovic, M. (1977). Nutritive value of rumen contents for monogastric
318 animals. *Anim. Feed Sci. Technology*, 2(4): 351-360

319 Kameshwar, A.K.S., Qin, W., 2016. Lignin degrading fungal enzymes in: Production of
320 Biofuels and Chemical from lignin. Springer. pp. 81-130. Singapore

321 Kumar M, Raj Morya, Asmita Gupta, Vivek Kumar, and I.S. Thakur. 2021. Bacterial Mediated
322 Depolymerization And Degradation of Lignin. In book : Environmental Microbiology and
323 Biotechnology. pp 83-103

324 Lambertz, Camilla; Ece, Selin; Fischer, Rainer; Commandeur, Ulrich. 2016. Progress and
325 obstacles in the production and application of recombinant lignin-degrading
326 peroxidases. Bioengineered, (), 145–154. doi:10.1080/21655979.2016.1191705

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Formatted: Indent: Left: 0 cm, First line: 0 cm

- 328 Leonowicz, A., and K. Grzywnowicz. 1981. "Quantitative Estimation of Laccase Forms in
329 Some White-Rot Fungi Using Syringaldazine as a Substrate." *Enzyme and Microbial
330 Technology* 3(1):55–58. doi: [https://doi.org/10.1016/0141-0229\(81\)90036-3](https://doi.org/10.1016/0141-0229(81)90036-3).
- 331 Ming, Tien, and Kirk T. Kent. 1984. "Lignin-Degrading Enzyme from Phanerochaete
332 Chrysosporium: Purification, Characterization, and Catalytic Properties of a Unique
333 H₂O₂-Requiring Oxygenase." *Proceedings of the National Academy of Sciences*
334 81(8):2280–84. doi: 10.1073/pnas.81.8.2280.
- 335 Mufidatul Ilmi I, Nengah Dwianita Kuswytasari. 2013. Aktifitas Enzim Lignin
336 Peroksidase oleh Gliomastix sp. T3.7 pada Limbah Bonggol Jagung dengan Berbagai pH
337 dan Suhu. JURNAL SAINS DAN SENI POMITS Vol. 2, No.1. Page 38-42
- 338 [Murtiyaningsih H. and Muhammad Hazmi, 2017. Isolation and cellulase enzyme activities
339 assays in cellulolytic bacteria origin from soil waste. Agritop. Vol 15 \(2\). 293-308](#)
- 340 Pandey, A., CR. Soccol, P. Singh - Nee Nigam, VT. Soccol, LPS. Vandenberg, and R. Mohan.
341 2000. "Biotechnological Potential of Agro-Industrial Residues. II: Cassava Bagasse." *Bioresource
342 Technology* 74(1):81–87.
- 343 Pollegioni L, Tonin F, Rosini E (2015). Lignin-degrading enzymes. *FEBS J.* 282(7): 1190-
344 1213. [https://doi.org/ org/10.1111/febs.13224](https://doi.org/org/10.1111/febs.13224).
- 345 Ribeiro G.O., R.J. Gruninger, A. Badhan, T.A. McAllister . 2016. Mining the rumen for
346 fibrolytic feed enzymes. *Animal Frontiers*, Volume 6, Issue 2, April 2016, Pages 20–26.
- 347 Richana, Nur. 2002. "Produksi Dan Prospek Enzim Xilanase Dalam Pengembangan
348 Bioindustri Di Indonesia." *Buletin AgroBio* 5(1):29–36.
- 349 Sánchez C (2010). Cultivation of *Pleurotus ostreatus* and other edible mushrooms. *Appl
350 Microbiol Biotechnol* 85, 1321–1337
- 351 Statistic, Indonesia. 2020.. Statistics, Indonesia. 2020. Number of Livestock Slaughtered in
352 Indonesia.
- 353 Tafsin M, N D Hanafi, Yunilas and R Mulianda. 2018. Nutrient quality of oil palm frond
354 fermented by local microorganism (MOL) with different dosage and incubation time. *IOP
355 Conf. Series: Earth and Environmental Science* 260 (2019) 012050 IOP Publishing.
356 doi:10.1088/1755-1315/260/1/012050
- 357 [Tian JH, Pourcher AM, Bouchez T et al \(2014\) Occurrence of lignin degradation genotypes
358 and phenotypes among prokaryotes. *Appl Microbiol Biotechnol* 98:9527-9544](#)
- 359 Trismilah, Deden Rosid; 2009. "Produksi Xilanase Menggunakan Media Limbah Pertanian
360 Dan Perkebunan." *Jurnal Teknologi Lingkungan* (Vol. 10 No. 2 (2009)):137–44.

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Formatted: English (United States)

- 361 Troger, C., and K. Niranjan. 2010. "Sustainable Chitin Extraction and Chitosan Modification
362 for Application in the Food Industry." *International Conference on Food Innovation*.
- 363 Wariishi, H., K. Valli, and M. H. Gold. 1992. "Manganese(II) Oxidation by Manganese
364 Peroxidase from the Basidiomycete *Phanerochaete Chrysosporium*. Kinetic Mechanism
365 and Role of Chelators." *Journal of Biological Chemistry* 267(33):23688–95. doi:
366 10.1016/s0021-9258(18)35893-9.
- 367 Wina, Elizabeth. 2005. "The Technology of Utilizing Microorganism in Feed To Improve
368 Ruminant Productivity in Indonesia: A Review." *Wartazoa* 15(4):173–86.
- 369 Yogyaswari, Sekar Ayoe, M. G. Isworo Rukmi, and Budi Raharjo. 2016. "Ekplorasi Bakteri
370 Selulolitik Dari Cairan Rumen Sapi Peranakan Fries Holland (PFH) Dan Limousine
371 Peranakan Ongole (Limpo)." *Jurnal Biologi* 5(4):70–80.

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Activity of Cellulase and Ligninase Enzymes in a Local Bioactivator from Cattle and Buffalo
Rumen Contents

Tri Astuti^{1*}, Syahro A. Akbar¹, Muhamad Nasir Rofiq², Novirman Jamarun³, Nurul Huda⁴,
Ahmad Fudholi^{2,5}

¹Department of Animal Science, Faculty of Agriculture University of Mahaputra Muhammad
Yamin, Indonesia

²Research Center for Energy Conversion and Conservation, National Research and
Innovation Agency (BRIN), Indonesia

³Faculty of Animal Science, Andalas University, Padang, West Sumatera, Indonesia

⁴Faculty of Food Science and Nutrition, Universiti Malaysia Sabah, Malaysia

⁵Solar Energy Research Institute, Universiti Kebangsaan Malaysia, 43600, Bangi, Selangor,
Malaysia

⁶Departemen of Economic Education. University of Mahaputra Muhammad Yamin,
Indonesia

Correspondent author: adektuti@gmail.com

Abstract

Lignin is the main component of agricultural and plantation wastes, such as bagasse, straw and oil palm fronds. Lignocellulosic bonds in lignin, cellulose and hemicellulose can be broken down by enzymes. Numerous studies have utilised plantation waste as feed ingredients. Lignin is the limiting factor that affects the digestibility of this material. Therefore, the lignin content of plantation waste must be reduced before it is used as a feed ingredient. The use of local bioenzymes will be effective in breaking lignocellulose bonds. Thus, finding sources of enzymes that are easy to obtain, inexpensive to produce and effective as lignocellulose-degrading enzymes is necessary. This study aims to determine the activity of cellulase and ligninase enzymes in a bioactivator from rumen contents incubated for 7 days with different enzyme energy sources. The treatments included cattle and buffalo rumen contents added with molasses, palm frond, palm leaf extract and each enzyme. The parameters observed were the enzyme activities of cellulase, laccase, lignin and manganese peroxidase (MnP). Microbial identification was also performed. The results of statistical analysis showed insignificant

33 differences ($P > 0.05$) amongst the parameters of the enzyme activities of cellulase (2.22–3.51
34 U/ml), laccase (10.62–20.11 U/ml), lignin peroxidase (1.74–4.93 U/ml) and MnP (2.40–7.06
35 U/ml). *Lactobacillus* sp. was identified through bacterial identification. Therefore, the live
36 microbes discovered in the local microorganism solution originated from the study
37 environment and not from the rumen contents.

38 **Keyword:** Enzyme activity, microbial morphology, cellulase, lignase, rumen microbes

39

40 1. Introduction

41 Animal feeding is a major factor determining the success of livestock businesses. Many
42 studies have used agricultural and plantation by-products as substitutes for field grass in
43 ruminant feed. These by-products mostly contain cellulose, hemicellulose and lignin that
44 consist of lignocellulosic bonds. A large amount of lignocellulose in agricultural by-products
45 that are disposed of not only causes environmental damage but also loses materials that have
46 the potential to be used in the production of paper animal feed, and other materials Sanchez,
47 2009).

48 .The digestibility of ruminant feed ingredients is influenced by the contents of lignin,
49 cellulose and soluble substances. These rumen microorganisms could not for lignin degradation
50 because they do not generate enzymes with ligninolytic activity (Pollegioni et al., 2015). Oil
51 palm fronds as animal feed berries are low crude protein about 1.6%, high crude fiber content
52 reaching 56.1%, and contain 79.27% ADF, 64.25% NDF, 12.91% cellulose, 15.34%
53 hemicellulose and 15.34% lignins (Astuti et al. 2019; Tafsir et al, 2019). Lignin is a complex,
54 heterogeneous phenylpropanoid polymer that accounts for 25%–30% of plant biomass. It is
55 resistant to microbial degradation under natural conditions. Lignin and cellulose are the main
56 plant components that are enzymatically degraded by bioactivators, such as the enzymes
57 cellulase and ligninase. Ligninolytic microbial systems, which are primarily composed of
58 lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase (Glenn et al., 1983), have
59 been used to improve digestibility and nutritional value. LiP and MnP are extracellular
60 peroxidase enzymes that use H_2O_2 to degrade lignin, while laccase is a copper-containing
61 enzyme that uses molecular oxygen to degrade lignin (Hattaka, 1994). Laccase is a
62 metalloenzyme that can degrade lignin during the bio-catalysis process (Kameshwar and Qin,
63 2016). The use of commercial enzymes for animal feeding will need additional livestock

64 operational costs. Therefore, because of it is necessary to find sources of natural enzymes that
65 are cheap and easy to produce.

66 Using microbes for enzyme production has several advantages, including low
67 production costs, short production times, high growth speeds, and easy control. The bacteria
68 responsible for lignin degradation can be found in environments such as soil, digestive system
69 of herbivora, wood-eating insects, effluents from paper industry, sludge, etc. (Brown and
70 Chang, 2014; Tian et al., 2014). The contents of the rumen cattle contain crude protein, about
71 18.52 - 19.56%, Amino acids include lysine, leucine, alanine, aspartate, arginine, valine,
72 threonine and low methionine (Jovanović and Čuperlović, 1997). Rumen contents It also
73 contains high crude fiber, calcium, phosphorus and magnesium (Agbabiaka et al., 2012; Elfaki
74 and Abdelati, 2015). Rument microbes will produce enzymes in accordance with the given
75 substrate. For example, rument microbes will produce enzymes that degrade fiber when given
76 straw and enzymes that break down tannins when given calliandra (Wina, 2005). Lignin and
77 cellulose are the main plant components that are enzymatically degraded by cellulose and
78 lignase. These enzymes are produced by bioactivators (Pandey et al., 2000). Molasses is the
79 waste of sugar mills; that can use as a fermentation stimulant to increase the rate of silage
80 acidification by providing fermentable sugars for the growth of Lactic Acid Bacteria (Guo et
81 al., 2014).

82 Enzymes are biopolymer molecules that are composed of a series of amino acids in an
83 ordered and fixed composition and chain arrangement. They are proteins that are produced and
84 used by living cells to catalyse chemical reactions with a high level of specificity and increased
85 reaction rates (Beilen and Li, 2002; Richana, 2002). Thus, enzymes have various advantages
86 over conventional processes using chemicals. However, the main obstacle to the industrial
87 application of enzymes is the high price of enzymes, and cannot be used repeatedly (Huey,
88 2008; Troger and Niranjana, 2010; Wibisono, 2010). Extracellular oxidative enzymes can attack
89 and degrade lignin, by utilizing types of peroxidases including lignin peroxidase (LiP),
90 manganese peroxidase (MnP), versatile peroxidase (VP), and dye remover, peroxidase (DyP)
91 (Lambertz et al., 2016).

92 Cellulases are complex enzymes that gradually cut cellulose chains into glucose. Fungi,
93 bacteria and ruminants produce cellulase. The commercial production of enzymes usually
94 applies fungi or bacteria. Although the production of cellulases from ruminants has been
95 underappreciated, cellulases originating from ruminants are being produced given their high
96 availability. The rumen microbes are considered the most efficient microbial system in
97 degrading lignocellulosic biomass (Flint et al., 2008), Rumen microbes are very good sources

98 of fibrinolytic enzymes, carbohydrate-active enzymes that can degrade lignocellulose. because
99 they can produce enzymes from their environment and can be used in the feed and food
100 industry, cellulose biofuels, and other industrial processes (Ribeirio et al., 2016). In contrast to
101 commercial enzymes, cellulases have the potential to be produced from beef rumen fluid.
102 Therefore, this study aims to determine the activity of cellulase and ligninase as a local
103 bioactivator produced by incubating rumen contents with several different ingredients as the
104 microbe substrate. This research can produce complex enzymes (cellulase, MnP, LiP and
105 laccase) from waste materials that are cheap and easy to obtain.

106

107 **2. Materials and methods**

108 The materials used in this study were cattle and buffalo rumen contents, molasses,
109 soybean soaking wastewater, oil palm fronds, oil palm leaves and some chemicals for enzyme
110 activity measurement.

111 2.1. Bioactivator process

112 The rumen contents of cattle and buffalo were collected from an abattoir and placed in
113 tubes. All the rumen contents were added with molasses and tofu soaking water. The treatments
114 were the addition of palm leaves, palm fronds and a mixture of these materials, which had high
115 lignin contents. Palm fronds and leaves were added to induce the bioactivator to produce
116 ligninase. The rumen contents, molasses and tofu soaking wastewater were mixed at the ratio
117 of 1:1:8 with 10% oil palm fronds and leaves. The tubes were tightly closed. Then, a hole was
118 made in the middle and connected with a hose to a small bottle filled with water to catch
119 fermentation waste. The tubes were incubated for as long as 10 days as shown in Figure 1.

120



121

122 Figure 1. Fermentation with the local bioactivator

123

124 2.2. LiP activity test

125 A total of 0.2 ml of enzyme filtrate (sample extract and phosphate buffer shaken for 1
126 h and then centrifuged for 10 min at 10 000 rpm), 0.05 ml of 5 mM H₂O₂, 0.1 ml of 8 mM
127 veratrine alcohol, 0.2 ml of 0.05 M acetate buffer (pH 3) and 0.45 ml of distilled water were
128 added to a cuvette and then shaken. The absorbance of the solution at 310 nm was recorded at
129 0 and 30 min intervals. One unit of LiP enzyme activity was defined as the amount of enzyme
130 that caused the conversion of 1 μmol (1 μmol = 10⁻⁶ mol) veratril alcohol per minute (Tien and
131 Kent, 1984).

132

133 2.3. MnP activity test

134 A total of 0.1 ml of 50 mM Na-lactate buffer (pH 5) was added with 0.1 ml of 4 mM
135 guaiacol, 0.2 ml of 1 mM MnSO₄, 0.1 ml of 1 mM H₂O₂ and 0.3 ml of distilled water, as well
136 as 0.2 ml of enzyme filtrates. The solution was then checked and read at the wavelength of 465
137 nm at 0 and 30 min (Leonowicz and Grzywnowicz, 1981).

138 2.4. Laccase activity test

139 A total of 0.4 ml of enzyme filtrate was added with 0.5 ml of acetate buffer (pH 5) and
140 0.1 ml of 1 mM 2'-azinobis-3-ethylbenzothiazole-6-sulphonic acid. Measurements using
141 spectrophotometer were taken at 420 nm at 0 and 30 min (Wariishi et al., 1992).

142 Enzyme activity tests were conducted at the biotechnology laboratory of the Faculty of
143 Animal Husbandry, Andalas University, Padang. Microbial morphology identification was
144 performed at the Baso Veterinary Centre Laboratory, Bukit Tinggi. The analysis was continued
145 by identifying the morphology of the fungi and bacteria present in the local bioactivator rumen
146 contents. Microbes were identified on the basis of the results of the best enzyme activity
147 evaluation. Samples were inoculated onto sodium agar (NA) medium for the identification of
148 bacteria, and potato dextrose agar (PDA) was used as the medium for fungal/mould growth.
149 The samples were diluted to the concentration of 10⁻¹ – 10⁻¹⁰, and the selected isolates were
150 further analysed on the basis of colony distribution

151

152 2.5. Experimental design

153 A 2 × 4 factorial randomised block design with three replications for each treatment
154 was used in this study. Factor A was the type of rumen content: A1 = rumen cattle and A2 =
155 buffalo rumen. Factor B was the microbial energy substrate: B1 = molasses, B2 = molasses +
156 palm frond extract, B3 = molasses + palm leaf extract and B4 = molasses + palm frond and leaf
157 extract. Enzyme activity data were processed by using analysis of variance. The bioactivator

158 was identified by using the described method. Significant differences ($P < 0.05$) were further
 159 tested by using Duncan's multiple range tests.

160 The variables were observed through the isolation and identification of the bioactivator
 161 (fungi and bacteria) based on morphology and cellulase and ligninase activity tests on the crude
 162 bioenzymes in rumen content. Morphological data were obtained through descriptive analysis.

163
 164

165 3. Results

166 3.1. Enzyme activity test

167 The activities of cellulose, laccase, LiP and MnP were tested. These enzymes are
 168 essential for lignin degradation. The average results of the enzyme activity test for the local
 169 bioactivator in rumen content are shown in Table 1.

170
 171
 172

173 Table 1. Average activity (U/ml) of the local bioactivator in rumen contents

Factor B	Factor A				Average
	B ₁	B ₂	B ₃	B ₄	
Cellulase enzymes					
A1	3.51	2.41	2.81	2.22	2.74
A2	3.44	2.52	3.31	3.64	3.23
	3.48	2.46	3.06	2.93	2.98
Lacasse Enzymes					
A1	20.11	16.40	11.70	15.91	16.03
A2	11.00	12.86	10.62	14.59	12.27
	15.55	14.63	11.16	15.25	14.15
Enzim Lignin Peroksidase					
A1	4.06	4.93	2.80	4.11	3.97
A2	2.61	1.74	4.16	3.80	3.08
	3.34	3.34	3.48	3.95	3.53
Manganese Peroxidase Enzymes					
A1	2.40	5.61	3.39	7.06	4.61
A2	6.86	4.17	3.18	5.18	4.85

4.63 4.89 3.29 6.12 4.73

174 Note: A1 = cow rumen content, A2 = buffalo rumen content, B1 = rumen content only, B2 =
 175 rumen content and palm fronds, B3 = palm leaves and B4 = leaves and palm fronds

176

177 **3.2. Identification of microbial morphology**

The results of the chemical analysis are shown in Table 2. Microscopic and macroscopic analyses revealed that the bacterial colonies in Figure 2, the fungal colonies in Figure 3 and the bacterial isolates in Figure 4 had similar bacillus shapes.

178 Table 2. Chemical examination results for microbial isolates on NA media

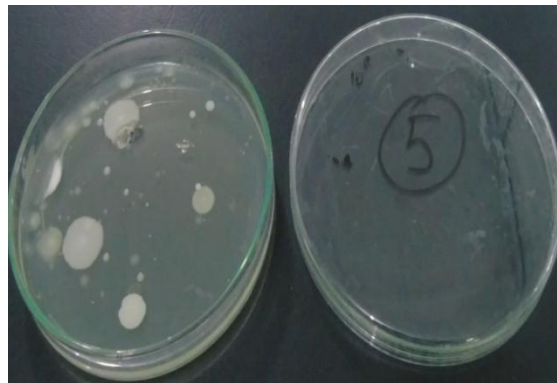
No	Treatment	NA 1	NA 2
1.	Colony (color, shape, traits)	White	White
2.	Grams (morphology, spora)	+ bacteri	+ bacteri
3.	Aerobic / Anaerobic	A	A spora
4.	TSIA	M/K	M/K
5.	Gas	-	+
6.	H ₂ S	-	-
7.	catalase	+	+
8.	Oxidase	-	-
9.	Motility	+	+
10.	Indole	-	-
11.	Urea	+	+
12.	Citrate	-	-
13.	Lactose	-	+
14.	Glucose	-	+
15.	Sucrose	-	-
16.	Mannitol	-	+
17.	MR	+	+
18.	VP	+	+
19.	OF	-	+
20.	Nitrat	-	+
21.	Gelatin	+	+
22.	Genus	<i>Basillus, sp 1</i>	<i>Basillus sp 2</i>

179
180
181



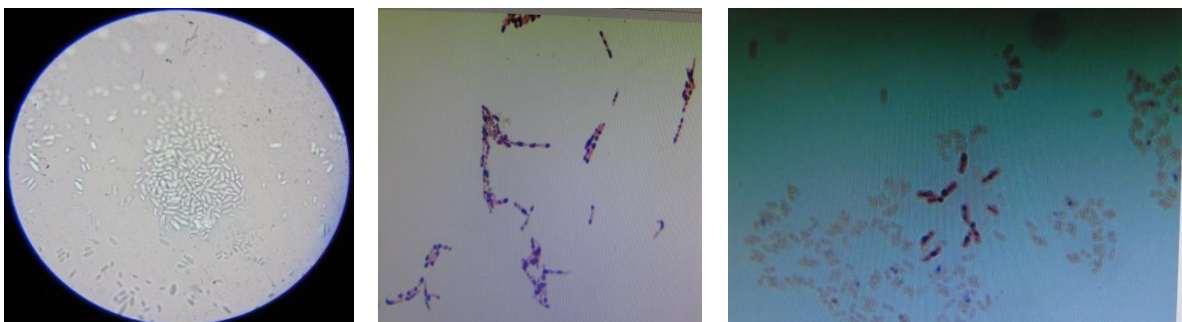
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Figure 2. Bacterial colonies on 10^{-8}



185
186
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Figure 3. Fungal colonies on 10^{-5}



188
189

Figure 4. Staining of bacilli in the genus *Bacillus* in rumen contents on NA media

190 **4. Discussion**

191 4.1. Enzyme activity test

192 Statistical analysis showed that no interaction effect ($P > 0.05$) existed between the
193 rumen source (cattle and buffalo) and microbe energy source on the activities of cellulase,

194 laccases, LiP and MnP. The data in Table 1 show that LiP enzyme activity ranged from 2.46
195 U/ml to 3.48 U/ml. LiP enzyme activity in this study was higher than that in the work of Ilmi
196 and Kuswytasari (2013), who found that LiP from *Gliomastix* sp. T3.7 had the enzyme activity
197 of 0.06–1.022 in corn hump waste at pH 5 and 25 °C–35 °C. The data showed the rumen
198 contents were the source of the bioactivator that was ingested and remained dominant in the
199 form of fiber from forage consumed by livestock. For all treatments, the main energy source
200 was molasses and soybean soaking water. Therefore, the main energy source in this work was
201 very representative and supplied the energy needed by the microbes in the rumen to grow and
202 exhibit high production. The average cellulase activity in this study was considerably higher
203 than that in a previous work (Murtiyaningsih and Hazmi, 2017) that obtained the highest
204 enzyme activity of 0.028279 U/m for cellulase from cellulolytic soil bacteria. This difference
205 was due to the large number of microbes in the rumen contents and the addition of molasses
206 and tofu soaking wastewater that further increased microbial growth. Another study (Astuti et
207 al., 2020) found 40×10^{12} total colonies in 1 ml of local bioactivator rumen contents mixed
208 with molasses, soybean soaking water and palm oil fronds.

209 In this work, the average laccase enzyme activity ranged from 11.0 U/ml to 20.11 U/ml,
210 LiP activity ranged from 1.74 U/ml to 4.93 U/ml and MnP activity ranged from 2.40 U/ml to
211 7.06 U/ml. Heterologous laccase production has been used for obtaining increased amount of
212 the enzyme (Debnath and Saha, 2020). The activity of laccase and LiP enzymes in this study
213 was much higher than the results of the study of Dimawarnita et al. (2019) who found LiP
214 activity of 0.269 U/ml and laccase activity of 0.35 U/ml in *Pleurotus ostreatus* in oil palm empty
215 fruit bunch media. Research of Fithri et al (2020), Laccase could degrade lignin with apparent
216 damage the lignocellulose substrate of corn cob and rice straw. Laccase, LiP and MnP activities
217 of 2.02, 1.677 and 0.33 U/ml, respectively, were observed in sugarcane fermentation by 10%
218 *Phanerochaete chrysosporium* because the microbes that thrived in the local activator were
219 supplied with palm fronds and leaves with high lignin contents. Dhakar et al. (2014) maximum
220 laccase production was observe as 28.2 U/l. Rumen microbes develop and produce enzymes
221 on the basis of given feed (Astuti et al., 2021).

222

223 4.2. Identification of microbial morphology

224 The morphology of the fungi and bacteria present in the local bioactivator rumen
225 contents was analysed. The microbes were identified on the basis of the best results of the
226 enzyme activity evaluation.

227 Figure 2 shows that the bacterial colonies were circular, small, spread out and thin and
228 had flat colony edges, a white colour and transparent structure. Morphological observation
229 revealed that the fungal colonies had a circular colony shape, convex elevation, uneven edges,
230 white colour and transparent structure (Figures 2 and 3). The shape of the colonies in this study
231 was the same as that of the colonies reported by Yogyaswari et al. (2016), who found that the
232 bacterial isolates from the rumen contents of Fries Holland cows formed white colonies. The
233 microscopy observations demonstrated that all of the bacterial isolates were Gram-positive
234 bacilli (Table 2 and Figure 4).

235 The Gram staining results for the isolates showed the bacteria were Gram-positive
236 bacteria that were negative for H₂S content and positive for catalase content. Furthermore,
237 microscopic and macroscopic analyses revealed that the bacterial isolates shared similar
238 bacillus shapes and belonged to two genera, namely, *Bacillus* sp. 1 and *Bacillus* sp. 2 (Table
239 2).

240 The bacterial identification results demonstrated that *Bacillus* sp. 1 was present in the local
241 bioactivator comprising rumen content added with palm fronds and that *Bacillus* sp. 2 was
242 present in the local bioactivator comprising rumen content added with fronds and palm leaves.
243 In addition, the microscopic and macroscopic observations showed that the bacterial isolates
244 in Figure 3 all had similar bacillus shapes. Kumar et al., (2021) state some bacterial strains
245 could lignin-degrading such as *Bacillus sp. CS-1*, *Bacillus pumilus*, *Bacillus atrophaeus*,
246 *Bacillus sp, ect*.

247

248 **5. Conclusion**

249 On the basis of the research results, the best bioactivator that can be used to improve
250 feed quality was concluded to be rumen content mixed with molasses, soybean soaking water
251 and oil palm fronds and leaves. It had the cellulase, laccase, LiP and MnP activities of 2.2,
252 15.91, 4.11 and 7.06 U/ml, respectively. Bacterial identification revealed that *Bacillus* sp. was
253 present under the best treatment.

254

255 **References**

256 Agbabiaka, L.A., Madubuike, F.N., Amadi, S.A., 2012. Studies on nutrients and anti-nutrients
257 of rumen digesta from three most domesticated ruminants in Nigeria. Pakistan Journal of
258 Nutrition, 11 (7), 580- 582. <https://doi.org/10.3923/pjn.2012.678.680>.
259 Astuti T., Akbar, S.A., Afrini, D., Rofiq, M.N., Humaira, I., 2020. The identification of fungi
260 colonies total on the rumen content of cow and buffalo with addition of leaves and oil

261 palm frond. World Journal of Advanced Research and Reviews 02(08), 314-317.
262 <https://doi.org/10.30574/wjarr.2020.8.2.0444>.

263 Astuti T., Rofiq, M.N., Nurhaita, Santoso, U., 2019. Analysis of fibre fraction of palm oil frond
264 fermented with different microbes and soluble carbohydrates addition as ruminant
265 feeding. IOP Conference Series: Earth and Environmental Science 347(1).
266 <https://doi.org/10.1088/1755-1315/347/1/012059>.

267 Astuti T, Jamarun, N., Arief, Yanti, G., 2021. Effect fermentation of sugarcane shoots with
268 phanerochaetechrysosporium on the activity of lacase enzymes, lignin peroxidase and
269 manganese peroxidase. IOP Conference Series: Earth and Environmental Science
270 709(1):3–7. <https://doi.org/10.1088/1755-1315/709/1/012065>.

271 Beilen, Jan B.van, Li, Z., 2002. Enzyme technology: an overview. Current Opinion in
272 Biotechnology 13 4, 338–44. [https://doi.org/10.1016/S0958-1669\(02\)00334-8](https://doi.org/10.1016/S0958-1669(02)00334-8).

273 Brown, Margaret E; Chang, Michelle CY. 2014. *Exploring bacterial lignin degradation*.
274 *Current Opinion in Chemical Biology*, 19(), 1–7. doi:10.1016/j.cbpa.2013.11.015

275 Debnath, R., Saha, T., 2020. An insight into the production strategies and applications of the
276 ligninolytic enzyme laccase from bacteria and fungi. Biocatalysis and Agriculture
277 Biotechnology 26, 101645. <https://doi.org/10.1016/j.bcab.2020.101645>.

278 Elfaki, M.O.A., Abdelatti, K.A., Malik, H.E.E., 2015. Effect of dietary dried rumen content on
279 broiler performance, plasma constituents and carcass characteristics. Global Journal of
280 Animal Scientific Research 3(1), 264-270.

281 Fithri. L., Puspaningsih, N.N.T., Asmarani, O., Ni'matuzahroh, Dewi, G.D.F., Arizandy,
282 R.Y., 2020. Characterization of fungal laccase isolated from oil palm empty fruit bunches
283 (OPEFB) and its degradation from the agriculture waste. Biocatalysis and Agricultural
284 Biotechnology 27, 101676. <https://doi.org/10.1016/j.bcab.2020.101676>.

285 Flint, Harry J.; Bayer, Edward A.; Rincon, Marco T.; Lamed, Raphael; White, Bryan A. (2008).
286 *Polysaccharide utilization by gut bacteria: potential for new insights from genomic*
287 *analysis*. , 6(2), 121–131. doi:10.1038/nrmicro1817

288 Glenn, J.K., Morgan, M.A., Mayfield, M.B., Kuwahara, M., Gold, M.H., 1983. An
289 extracellular H₂O₂-requiring enzyme preparation involved in lignin biodegradation by the
290 white rot basidiomycete Phanerochaete chrysosporium. Biochemical and Biophysical
291 Research Communications 114(3),1077–83. [https://doi.org/10.1016/0006-](https://doi.org/10.1016/0006-291x(83)90672-1)
292 [291x\(83\)90672-1](https://doi.org/10.1016/0006-291x(83)90672-1).

293 Guo, G., Yuan, X.J., Li, L.X., Wen, A.Y., Shao, T., 2014. Effects of fibrolytic enzymes,
294 molasses and lactic acid bacteria on fermentation quality of mixed silage of corn and

295 hulloless-barely straw in the Tibetan Plateau. *Grassl. Sci.* 60, 240–246.
 296 <https://doi.org/10.1111/grs.12060>.

297 Dimawarnita, Firda, Panji, T., Faramitha, Y., 2019. Peningkatan kemurnian selulosa dan
 298 karboksimetil selulosa (CMC) hasil konversi limbah TKKS melalui perlakuan NaOH
 299 12%. *E-Journal Menara Perkebunan* 87(2), 95–103.
 300 <https://doi.org/10.22302/iribb.jur.mp.v87i2.339>.

301 Dhakar K, Jain, R., Tamta, S., Pandey, A., 2014. Prolonged laccase production by a cold and
 302 pH tolerant strain of *penicillium pinophilum* (MCC1049)
 303 isolated from a low temperature environment. *Enzyme Research*, 120708
 304 <https://doi.org/10.1155/2014/120708>.

305 Hattaka, A., 1994. Lignin-modifying enzymes from selected white-rot fungi: production and
 306 role from in lignin degradation. *FEMS Microbiology Reviews* 13,125–35.
 307 <https://doi.org/10.1016/j.biortech.2013.02.042>.

308 Huey, H.S., 2008. Enzymatics enhanced production of gaharu oil: effect of enzyme loading
 309 and duration time. MSc. Thesis. University of Malaysia Pahang.

310 Ilmi, I.M., Kuswytasari, N.D., 2013. Aktifitas enzim lignin peroksidase oleh *gliomastix* sp.
 311 T3.7 pada limbah bonggol jagung dengan berbagai pH dan suhu. *Jurnal Sains dan Seni*
 312 2(1), 38-42.

313 Jovanović, M., Čuperlović, M.,, 1977. Nutritive value of rumen contents for monogastric
 314 animals. *Anim. Feed Sci. Technology* 2(4), 351-360. [https://doi.org/10.1016/0377-](https://doi.org/10.1016/0377-8401(77)90007-4)
 315 [8401\(77\)90007-4](https://doi.org/10.1016/0377-8401(77)90007-4).

316 Kameshwar, A.K.S., Qin, W., 2016. Lignin degrading fungal enzymes in: Production of
 317 Biofuels and Chemical from lignin. *Production of Biofuels and Chemicals from Lignin*,
 318 81–130.

319 Kumar M, Raj Morya, Asmita Gupta, Vivek Kumar, and I.S. Thakur. 2021. Bacterial Mediated
 320 Depolymerization And Degradation of Lignin. In book : *Environmental Microbiology and*
 321 *Biotechnology*. pp 83-103

322 Lambertz, Camilla; Ece, Selin; Fischer, Rainer; Commandeur, Ulrich. 2016. *Progress and*
 323 *obstacles in the production and application of recombinant lignin-degrading peroxidases.*
 324 *Bioengineered*, (), 145–154. doi:10.1080/21655979.2016.1191705

325 Leonowicz, A., Grzywnowicz, K., 1981. Quantitative estimation of laccase forms in some
 326 white-rot fungi using syringaldazine as a substrate. *Enzyme and Microbial Technology*
 327 3(1),55–58. [https://doi.org/10.1016/0141-0229\(81\)90036-3](https://doi.org/10.1016/0141-0229(81)90036-3).

328 Murtiyaningsih H, and Muhammad Hazmi, 2017. Isolation and cellulase enzyme activities

329 assays in cellulolytic bacteria origin from soil waste. *Agritop*. Vol 15 (2). 293-308

330 Pandey, A., Soccol, C.R., Nigam, P., Soccol, V.T., Vandenberg, L.P.S., Mohan, R., 2000.

331 Biotechnological potential of agro-industrial residues. II: cassava bagasse. *Bioresource*

332 *Technology* 74(1), 81–87. [https://doi.org/10.1016/S0960-8524\(99\)00143-1](https://doi.org/10.1016/S0960-8524(99)00143-1).

333 Pollegioni, L., Tonin, F., Rosini, E., 2015. Lignin-degrading enzymes. *FEBS J.* 282(7), 1190-

334 1213. <https://doi.org/org/10.1111/febs.13224>.

335 Ribeiro, G.O.; Gruninger, R.J.; Badhan, A.; McAllister, T.A. 2016. Mining the rumen for

336 fibrolytic feed enzymes. *Animal Frontiers*, 6(2), 20–26. doi:10.2527/af.2016-0019

337 Richana, N. 2002. Produksi dan prospek enzim xilanase dalam pengembangan bioindustri di

338 Indonesia. *Buletin AgroBio* 5(1), 29–36.

339 Sánchez C. 2009. Lignocellulosic residues: Biodegradation and bioconversion by fungi. ,

340 27(2), 185–194. doi:10.1016/j.biotechadv.2008.11.001

341 Tafsin, M., Hanafi, N.D., Yunilas, Mulianda, R., 2019. Nutrient quality of oil palm frond

342 fermented by local microorganism (MOL) with different dosage and incubation time. *IOP*

343 *Conf. Series: Earth and Environmental Science* 260 (2019) 012050 IOP Publishing.

344 <https://doi.org/10.1088/1755-1315/260/1/012050>

345 Tian J.-H., Pourcher, A.-M., Bouchez, Gelhaye, E., Peu, P., 2014, Occurrence of lignin

346 degradation genotypes and phenotypes among prokaryotes. *Appl Microbiol Biotechnol*

347 98, 9527-9544. <https://doi.org/10.1007/s00253-014-6142-4>.

348 Tien, M., Kirk, T.T., 1984. Lignin-degrading enzyme from *Phanerochaete chrysosporium*:

349 purification, characterization, and catalytic properties of a unique H₂O₂-requiring

350 oxygenase. *Proceedings of the National Academy of Sciences* 81(8), 2280–84.

351 <https://doi.org/10.1073/pnas.81.8.2280>.

352 Troger, C., Niranjana, K., 2010. Sustainable chitin extraction and chitosan modification for

353 application in the food industry. *International Conference on Food Innovation*.

354 Wariishi, H., Valli, K., Gold, M.H., 1992. Manganese(II) oxidation by manganese peroxidase

355 from the basidiomycete *Phanerochaete chrysosporium*. kinetic mechanism and role of

356 chelators. *Journal of Biological Chemistry* 267(33), 23688–95.

357 [https://doi.org/10.1016/s0021-9258\(18\)35893-9](https://doi.org/10.1016/s0021-9258(18)35893-9).

358 Wina, E., 2005. The Technology of Utilizing Microorganism in Feed To Improve Ruminant

359 Productivity in Indonesia: A Review. *Wartazoa* 15(4):173–86.

360 <http://repository.pertanian.go.id/handle/123456789/4636>.

361 Yogyaswari, Ayoe, S., Rukmi, M.G.I., Raharjo, B., 2016. Ekplorasi bakteri selulolitik dari

362 cairan rumen sapi peranakan fries holland (PFH) dan limousine peranakan ongole

363 (Limpopo). Jurnal Biologi 5(4), 70–80.
364 <https://ejournal3.undip.ac.id/index.php/biologi/article/view/19516>.
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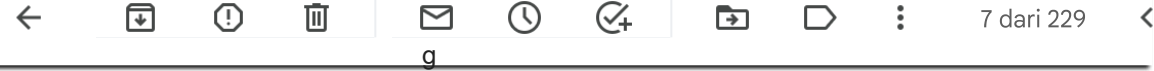


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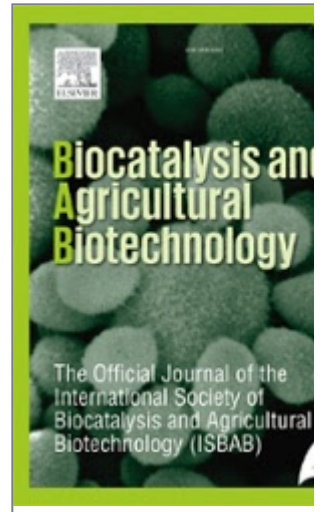
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Activity of cellulase and ligninase enzymes in a local bioactivator from cattle and buffalo rumen contents

Tri Astuti^{a,*}, Syahro Ali Akbar^a, Muhamad Nasir Rofiq^b, Novirman Jamarun^c, Nurul Huda^d, Ahmad Fudholi^{b,e}

^a Department of Animal Science, Faculty of Agriculture University of Mahaputra Muhammad Yamin, Indonesia

^b National Research and Innovation Agency (BRIN), Indonesia

^c Faculty of Animal Science, Andalas University, Padang, West Sumatera, Indonesia

^d Universiti Malaysia Sabah, Malaysia

^e Solar Energy Research Institute, Universiti Kebangsaan Malaysia, 43600, Bangi, Selangor, Malaysia

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ABSTRACT

Lignin is the main component of agricultural and plantation wastes, such as bagasse, straw and oil palm fronds. Lignocellulosic bonds in lignin, cellulose and hemicellulose can be broken down by enzymes. Numerous studies have utilised plantation waste as feed ingredients. Lignin is the limiting factor that affects the digestibility of this material. Therefore, the lignin content of plantation waste must be reduced before it is used as a feed ingredient. The use of local bioenzymes will be effective in breaking lignocellulose bonds. Thus, finding sources of enzymes that are easy to obtain, inexpensive to produce and effective as lignocellulose-degrading enzymes is necessary. This study aims to determine the activity of cellulase and ligninase enzymes in a bioactivator from rumen contents incubated for 7 days with different enzyme energy sources. The treatments included cattle and buffalo rumen contents added with molasses, palm frond, palm leaf extract and each enzyme. The parameters observed were the enzyme activities of cellulase, laccase, lignin and manganese peroxidase (MnP). Microbial identification was also performed. The results of statistical analysis showed insignificant differences ($P > 0.05$) amongst the parameters of the enzyme activities of cellulase (2.22–3.51 U/ml), laccase (10.62–20.11 U/ml), lignin peroxidase (1.74–4.93 U/ml) and MnP (2.40–7.06 U/ml). *Lactobacillus* sp. was identified through bacterial identification. Therefore, the live microbes discovered in the local microorganism solution originated from the study environment and not from the rumen contents.

1. Introduction

Animal feeding is a major factor determining the success of livestock businesses. Many studies have used agricultural and plantation by-products as substitutes for field grass in ruminant feed. These by-products mostly contain cellulose, hemicellulose and lignin that consist of lignocellulosic bonds. The digestibility of ruminant feed ingredients is influenced by the contents of lignin, cellulose and soluble substances. These rumen microorganisms could not for lignin degradation because they do not generate enzymes with ligninolytic activity (Pollegioni et al., 2015). Oil palm fronds as animal feed berries are low crude protein about 1.6%, high crude fiber content reaching 56.1%, and contain 79.27% ADF, 64.25% NDF, 12.91% cellulose, 15.34% hemicellulose and 15.34% lignins (Astuti et al., 2019; Tafsir et al., 2019). Lignin is a complex, heterogeneous phenylpropanoid polymer that accounts for 25%–30% of

* Corresponding author.

E-mail address: tri.astuti@ummy.ac.id (T. Astuti).

plant biomass. It is resistant to microbial degradation under natural conditions. Lignin and cellulose are the main plant components that are enzymatically degraded by bioactivators, such as the enzymes cellulase and ligninase. Ligninolytic microbial systems, which are primarily composed of lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase (Glenn et al., 1983), have been used to improve digestibility and nutritional value. LiP and MnP are extracellular peroxidase enzymes that use H_2O_2 to degrade lignin, while laccase is a copper-containing enzyme that uses molecular oxygen to degrade lignin (Hattaka, 1994). Laccase is a metalloenzyme that can degrade lignin during the bio-catalysis process (Kameshwar and Qin, 2016). The use of commercial enzymes for animal feeding will need additional livestock operational costs. Therefore, because of it is necessary to find sources of natural enzymes that are cheap and easy to produce.

Using microbes for enzyme production has several advantages, including low production costs, short production times, high growth speeds, and easy control. The bacteria responsible for lignin degradation can be found in environments such as soil, digestive system of herbivora, wood-eating insects, effluents from paper industry, sludge, etc. (Brown and Chang, 2014; Tian et al., 2014). The contents of the rumen cattle contain crude protein, about 18.52–19.56%, Amino acids include lysine, leucine, alanine, aspartate, arginine, valine, threonine and low methionine (Jovanović and Čuperović, 1977). Rumen contents It also contains high crude fiber, calcium, phosphorus and magnesium (Agbabiaka et al., 2012; Elfaki et al., 2015). Ruminant microbes will produce enzymes in accordance with the given substrate. For example, ruminant microbes will produce enzymes that degrade fiber when given straw and enzymes that break down tannins when given calliandra (Wina, 2005). Lignin and cellulose are the main plant components that are enzymatically degraded by cellulose and lignase. These enzymes are produced by bioactivators (Pandey et al., 2000). Molasses is the waste of sugar mills; that can use as a fermentation stimulant to increase the rate of silage acidification by providing fermentable sugars for the growth of Lactic Acid Bacteria (Guo et al., 2014).

Enzymes are biopolymer molecules that are composed of a series of amino acids in an ordered and fixed composition and chain arrangement. They are proteins that are produced and used by living cells to catalyse chemical reactions with a high level of specificity and increased reaction rates (Beilen et al., 2002; Richana, 2002). Thus, enzymes have various advantages over conventional processes using chemicals. However, the main obstacle to the industrial application of enzymes is the high price of enzymes, and cannot be used repeatedly (Huey, 2008; Troger and Niranjana, 2010). Lignin-degrading or ligninolytic enzymes include laccase (polyphenol oxidase), LiP and MnP; all three are extracellular multienzymes that participate in lignin depolymerisation (Sánchez, 2010).

Cellulases are complex enzymes that gradually cut cellulose chains into glucose. Fungi, bacteria and ruminants produce cellulase. The commercial production of enzymes usually applies fungi or bacteria. Although the production of cellulases from ruminants has been underappreciated, cellulases originating from ruminants are being produced given their high availability. The rumen microbes are considered the most efficient microbial system in degrading lignocellulosic biomass (Flint et al., 2008), because they can produce enzymes from their environment and can be used in the feed and food industry, cellulose biofuels, and other industrial processes (Ribeiro et al., 2016). In contrast to commercial enzymes, cellulases have the potential to be produced from beef rumen fluid. Therefore, this study aims to determine the activity of cellulase and ligninase as a local bioactivator produced by incubating rumen contents with several different ingredients as the microbe substrate. This research can produce complex enzymes (cellulase, MnP, LiP and laccase) from waste materials that are cheap and easy to obtain.

2. Materials and methods

The materials used in this study were cattle and buffalo rumen contents, molasses, soybean soaking wastewater, oil palm fronds, oil palm leaves and some chemicals for enzyme activity measurement.

2.1. Bioactivator process

The rumen contents of cattle and buffalo were collected from an abattoir and placed in tubes. All the rumen contents were added with molasses and tofu soaking water. The treatments were the addition of palm leaves, palm fronds and a mixture of these materials, which had high lignin contents. Palm fronds and leaves were added to induce the bioactivator to produce ligninase. The rumen contents, molasses and tofu soaking wastewater were mixed at the ratio of 1:1:8 with 10% oil palm fronds and leaves. The tubes were tightly closed. Then, a hole was made in the middle and connected with a hose to a small bottle filled with water to catch fermentation waste. The tubes were incubated for as long as 10 days as shown in Fig. 1.

2.2. LiP activity test

A total of 0.2 ml of enzyme filtrate (sample extract and phosphate buffer shaken for 1 h and then centrifuged for 10 min at 10000 rpm), 0.05 ml of 5 mM H_2O_2 , 0.1 ml of 8 mM veratrine alcohol, 0.2 ml of 0.05 M acetate buffer (pH 3) and 0.45 ml of distilled water were added to a cuvette and then shaken. The absorbance of the solution at 310 nm was recorded at 0 and 30 min intervals. One unit of LiP enzyme activity was defined as the amount of enzyme that caused the conversion of 1 μ mol (1 μ mol = 10^{-6} mol) veratril alcohol per minute (Tien and Kirk, 1984).

2.3. MnP activity test

A total of 0.1 ml of 50 mM Na-lactate buffer (pH 5) was added with 0.1 ml of 4 mM guaiacol, 0.2 ml of 1 mM $MnSO_4$, 0.1 ml of 1 mM H_2O_2 and 0.3 ml of distilled water, as well as 0.2 ml of enzyme filtrates. The solution was then checked and read at the wavelength of 465 nm at 0 and 30 min (Leonowicz and Grzywnowicz, 1981).



Fig. 1. Fermentation with the local bioactivator.

2.4. Laccase activity test

A total of 0.4 ml of enzyme filtrate was added with 0.5 ml of acetate buffer (pH 5) and 0.1 ml of 1 mM 2'-azinobis-3-ethylbenzothiazole-6-sulphonic acid. Measurements using spectrophotometer were taken at 420 nm at 0 and 30 min (Wariishi et al., 1992).

Enzyme activity tests were conducted at the biotechnology laboratory of the Faculty of Animal Husbandry, Andalas University, Padang. Microbial morphology identification was performed at the Baso Veterinary Centre Laboratory, Bukit Tinggi. The analysis was continued by identifying the morphology of the fungi and bacteria present in the local bioactivator rumen contents. Microbes were identified on the basis of the results of the best enzyme activity evaluation. Samples were inoculated onto sodium agar (NA) medium for the identification of bacteria, and potato dextrose agar (PDA) was used as the medium for fungal/mould growth. The samples were diluted to the concentration of 10^{-1} – 10^{-10} , and the selected isolates were further analysed on the basis of colony distribution.

2.5. Experimental design

A 2×4 factorial randomised block design with three replications for each treatment was used in this study. Factor A was the type of rumen content: A1 = rumen cattle and A2 = buffalo rumen. Factor B was the microbial energy substrate: B1 = molasses, B2 = molasses + palm frond extract, B3 = molasses + palm leaf extract and B4 = molasses + palm frond and leaf extract. Enzyme activity data were processed by using analysis of variance. The bioactivator was identified by using the described method. Significant differences ($P < 0.05$) were further tested by using Duncan's multiple range tests.

The variables were observed through the isolation and identification of the bioactivator (fungi and bacteria) based on morphology and cellulase and ligninase activity tests on the crude bioenzymes in rumen content. Morphological data were obtained through descriptive analysis.

3. Results

3.1. Enzyme activity test

The activities of cellulase, laccase, LiP and MnP were tested. These enzymes are essential for lignin degradation. The average results of the enzyme activity test for the local bioactivator in rumen content are shown in Table 1.

3.2. Identification of microbial morphology

The results of the chemical analysis are shown in Table 2. Microscopic and macroscopic analyses revealed that the bacterial colonies in Fig. 2, the fungal colonies in Fig. 3 and the bacterial isolates in Fig. 4 had similar bacillus shapes.

4. Discussion

4.1. Enzyme activity test

Statistical analysis showed that no interaction effect ($P > 0.05$) existed between the rumen source (cattle and buffalo) and microbe energy source on the activities of cellulase, laccases, LiP and MnP. The data in Table 1 show that LiP enzyme activity ranged from 2.46 U/ml to 3.48 U/ml. LiP enzyme activity in this study was higher than that in the work of Ilmi and Kuswytasari (2013), who found that LiP from *Gliomastix* sp. T3.7 had the enzyme activity of 0.06–1.022 in corn hump waste at pH 5 and 25 °C–35 °C. The data showed the rumen contents were the source of the bioactivator that was ingested and remained dominant in the form of fiber from forage consumed by livestock. For all treatments, the main energy source was molasses and soybean soaking water. Therefore, the main energy source in this work was very representative and supplied the energy needed by the microbes in the rumen to grow and exhibit high production. The average cellulase activity in this study was considerably higher than that in a previous work (Murtiyaningsih and Hazmi, 2017) that obtained the highest enzyme activity of 0.028279 U/m for cellulase from cellulolytic soil bac-

Table 1
Average activity (U/ml) of the local bioactivator in rumen contents.

Factor B	Factor A				Average
	B ₁	B ₂	B ₃	B ₄	
Cellulase enzymes					
A1	3.51	2.41	2.81	2.22	2.74
A2	3.44	2.52	3.31	3.64	3.23
	3.48	2.46	3.06	2.93	2.98
Lacasse Enzymes					
A1	20.11	16.40	11.70	15.91	16.03
A2	11.00	12.86	10.62	14.59	12.27
	15.55	14.63	11.16	15.25	14.15
Enzim Lignin Peroksidase					
A1	4.06	4.93	2.80	4.11	3.97
A2	2.61	1.74	4.16	3.80	3.08
	3.34	3.34	3.48	3.95	3.53
Manganese Peroxidase Enzymes					
A1	2.40	5.61	3.39	7.06	4.61
A2	6.86	4.17	3.18	5.18	4.85
	4.63	4.89	3.29	6.12	4.73

Note: A1 = cow rumen content, A2 = buffalo rumen content, B1 = rumen content only, B2 = rumen content and palm fronds, B3 = palm leaves and B4 = leaves and palm fronds.

Table 2
Chemical examination results for microbial isolates on NA media.

No	Treatment	NA 1	NA 2
1.	Colony (color, shape, traits)	White	White
2.	Grams (morphology, spora)	+ bacteri	+ bacteri
3.	Aerobic/Anaerobic	A	A spora
4.	TSIA	M/K	M/K
5.	Gas	-	+
6.	H ₂ S	-	-
7.	catalase	+	+
8.	Oxidase	-	-
9.	Motility	+	+
10.	Indole	-	-
11.	Urea	+	+
12.	Citrate	-	-
13.	Lactose	-	+
14.	Glucose	-	+
15.	Sucrose	-	-
16.	Mannitol	-	+
17.	MR	+	+
18.	VP	+	+
19.	OF	-	+
20.	Nitrat	-	+
21.	Gelatin	+	+
22.	Genus	<i>Basillus, sp 1</i>	<i>Basillus sp 2</i>

teria. This difference was due to the large number of microbes in the rumen contents and the addition of molasses and tofu soaking wastewater that further increased microbial growth. Another study (Astuti et al., 2020) found 40×10^{12} total colonies in 1 ml of local bioactivator rumen contents mixed with molasses, soybean soaking water and palm oil fronds.

In this work, the average laccase enzyme activity ranged from 11.0 U/ml to 20.11 U/ml, LiP activity ranged from 1.74 U/ml to 4.93 U/ml and MnP activity ranged from 2.40 U/ml to 7.06 U/ml. Heterologous laccase production has been used for obtaining increased amount of the enzyme (Debnath and Saha, 2020). The activity of laccase and LiP enzymes in this study was much higher than the results of the study of Dimawarnita et al. (2019) who found LiP activity of 0.269 U/ml and laccase activity of 0.35 U/ml in *Pleurotus ostreatus* in oil palm empty fruit bunch media. Research of Fithri et al. (2020), Laccase could degrade lignin with apparent damage the lignocellulose substrate of corn cob and rice straw. Laccase, LiP and MnP activities of 2.02, 1.677 and 0.33 U/ml, respectively, were observed in sugarcane fermentation by 10% *Phanerochaete chrysosporium* because the microbes that thrived in the local activator were supplied with palm fronds and leaves with high lignin contents. Dhakar et al. (2014) maximum laccase production was observed as 28.2 U/l. Rumen microbes develop and produce enzymes on the basis of given feed (Astuti et al., 2021).

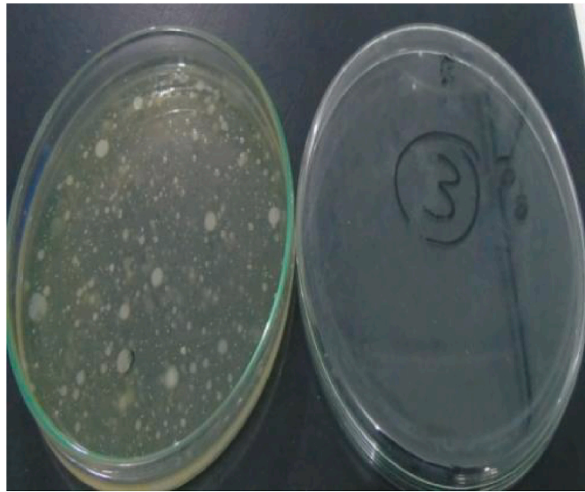


Fig. 2. Bacterial colonies on 10^{-8} .

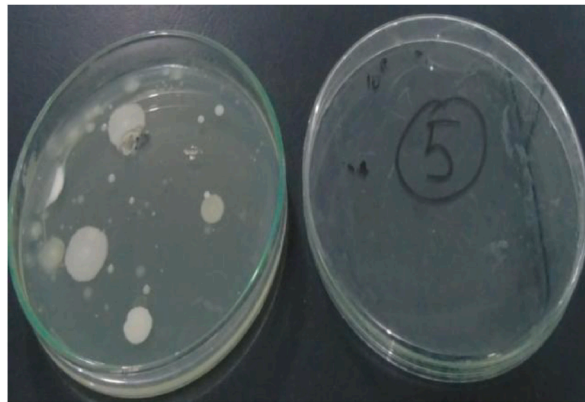


Fig. 3. Fungal colonies on 10^{-5} .

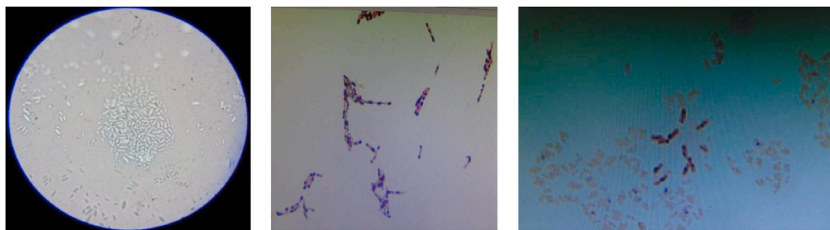


Fig. 4. Staining of bacilli in the genus *Bacillus* in rumen contents on NA media.

4.2. Identification of microbial morphology

The morphology of the fungi and bacteria present in the local bioactivator rumen contents was analysed. The microbes were identified on the basis of the best results of the enzyme activity evaluation.

Fig. 2 shows that the bacterial colonies were circular, small, spread out and thin and had flat colony edges, a white colour and transparent structure. Morphological observation revealed that the fungal colonies had a circular colony shape, convex elevation, uneven edges, white colour and transparent structure (Figs. 2 and 3). The shape of the colonies in this study was the same as that of the colonies reported by [Yogyaswari et al. \(2016\)](#), who found that the bacterial isolates from the rumen contents of Fries Holland cows formed white colonies. The microscopy observations demonstrated that all of the bacterial isolates were Gram-positive bacilli (Table 2 and Fig. 4).

The Gram staining results for the isolates showed the bacteria were Gram-positive bacteria that were negative for H_2S content and positive for catalase content. Furthermore, microscopic and macroscopic analyses revealed that the bacterial isolates shared similar bacillus shapes and belonged to two genera, namely, *Bacillus* sp. 1 and *Bacillus* sp. 2 (Table 2).

The bacterial identification results demonstrated that *Bacillus* sp. 1 was present in the local bioactivator comprising rumen content added with palm fronds and that *Bacillus* sp. 2 was present in the local bioactivator comprising rumen content added with fronds and palm leaves. In addition, the microscopic and macroscopic observations showed that the bacterial isolates in Fig. 3 all had similar bacillus shapes. Some bacterial strains could lignin-degrading such as *Bacillus* sp.CS-1, *Bacillus pumilus*, *Bacillus atrophaeus*, *Bacillus* sp, ect. Kumar et al. (2021) state some bacterial strains could lignin-degrading such as *Bacillus* sp.CS-1, *Bacillus pumilus*, *Bacillus atrophaeus*, *Bacillus* sp, ect.

5. Conclusion

On the basis of the research results, the best bioactivator that can be used to improve feed quality was concluded to be rumen content mixed with molasses, soybean soaking water and oil palm fronds and leaves. It had the cellulase, laccase, LiP and MnP activities of 2.2, 15.91, 4.11 and 7.06 U/ml, respectively. Bacterial identification revealed that *Bacillus* sp. was present under the best treatment.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

References

- Agbabiaka, L.A., Madubuike, F.N., Amadi, S.A., 2012. Studies on nutrients and anti-nutrients of rumen digesta from three most domesticated ruminants in Nigeria. *Pakistan J. Nutr.* 11 (7), 580–582. <https://doi.org/10.3923/pjn.2012.678.680>.
- Astuti, T., Rofiq, M.N., Nurhaita, Santoso, U., 2019. Analysis of fibre fraction of palm oil frond fermented with different microbes and soluble carbohydrates addition as ruminant feeding. *IOP Conf. Ser. Earth Environ. Sci.* 347 (1). <https://doi.org/10.1088/1755-1315/347/1/012059>.
- Astuti, T., Akbar, S.A., Afrini, D., Rofiq, M.N., Humaira, I., 2020. The identification of fungi colonies total on the rumen content of cow and buffalo with addition of leaves and oil palm frond. *World J. Adv. Res. Rev.* 2 (8), 314–317. <https://doi.org/10.30574/wjarr.2020.8.2.0444>.
- Astuti, T., Jamarun, N., Arief, Yanti, G., 2021. Effect fermentation of sugarcane shoots with phanerochaetechrysosporium on the activity of laccase enzymes, lignin peroxidase and manganese peroxidase. *IOP Conf. Ser. Earth Environ. Sci.* 709 (1), 3–7. <https://doi.org/10.1088/1755-1315/709/1/012065>.
- Beilen, Jan, B.van, Li, Z., 2002. Enzyme technology: an overview. *Curr. Opin. Biotechnol.* 13 (4), 338–344. [https://doi.org/10.1016/S0958-1669\(02\)00334-8](https://doi.org/10.1016/S0958-1669(02)00334-8).
- Brown, M.E., Chang, M.C.Y., 2014. Exploring bacterial lignin degradation. *Curr. Opin. Chem. Biol.* 19, 1–7. <https://doi.org/10.1016/j.cbpa.2013.11.015>.
- Debnath, R., Saha, T., 2020. An insight into the production strategies and applications of the ligninolytic enzyme laccase from bacteria and fungi. *Biocatal. Agricult. Biotechnol.* 26, 101645. <https://doi.org/10.1016/j.cbab.2020.101645>.
- Dhakar, K., Jain, R., Tamta, S., Pandey, A., 2014. Prolonged laccase production by a cold and pH tolerant strain of penicillium pinophilum (MCC1049) isolated from a low temperature environment. *Enzym. Res.* 120708. <https://doi.org/10.1155/2014/120708>.
- Dimawarnita, Firda, Panji, T., Faramitha, Y., 2019. Peningkatan kemurnian selulosa dan karboksimetil selulosa (CMC) hasil konversi limbah TKKS melalui perlakuan NaOH 12%. *E-Journal Menara Perkebunan* 87 (2), 95–103. <https://doi.org/10.22302/iribb.jur.mp.v87i2.339>.
- Elfaki, M.O.A., Abdelatti, K.A., Malik, H.E.E., 2015. Effect of dietary dried rumen content on broiler performance, plasma constituents and carcass characteristics. *Global J. Anim. Scientific Res.* 3 (1), 264–270.
- Fithri, L., Puspaningsih, N.N.T., Asmarani, O., Ni' matuzahroh, Dewi, G.D.F., Arizandy, R.Y., 2020. Characterization of fungal laccase isolated from oil palm empty fruit bunches (OPEFB) and its degradation from the agriculture waste. *Biocatal. Agric. Biotechnol.* 27, 101676. <https://doi.org/10.1016/j.cbab.2020.101676>.
- Flint, H.J., Bayer, E.A., Rincon, M.T., Lamed, R., White, B.A., 2008. Polysaccharide utilization by gut bacteria: potential for new insights from genomic analysis. *Nat. Rev. Microbiol.* 6 (2), 121–131. <https://doi.org/10.1038/nrmicro1817>.
- Glenn, J.K., Morgan, M.A., Mayfield, M.B., Kuwahara, M., Gold, M.H., 1983. An extracellular H₂O₂-requiring enzyme preparation involved in lignin biodegradation by the white rot basidiomycete *Phanerochaete chrysosporium*. *Biochem. Biophys. Res. Commun.* 114 (3), 1077–1083. [https://doi.org/10.1016/0006-291x\(83\)90672-1](https://doi.org/10.1016/0006-291x(83)90672-1).
- Guo, G., Yuan, X.J., Li, L.X., Wen, A.Y., Shao, T., 2014. Effects of fibrolytic enzymes, molasses and lactic acid bacteria on fermentation quality of mixed silage of corn and hullless-barely straw in the Tibetan Plateau. *Grassl. Sci.* 60, 240–246. <https://doi.org/10.1111/grs.12060>.
- Hattaka, A., 1994. Lignin-modifying enzymes from selected white-rot fungi: production and role from in lignin degradation. *FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Rev.* 13, 125–135. <https://doi.org/10.1016/j.biortech.2013.02.042>.
- Huey, H.S., 2008. *Enzymatics Enhanced Production of Gaharu Oil: Effect of Enzyme Loading and Duration Time*. MSc. Thesis. University of Malaysia Pahang.
- Ilmi, I.M., Kuswytasari, N.D., 2013. Aktifitas enzim lignin peroksidase oleh gliomastix sp. T3.7 pada limbah bonggol jagung dengan berbagai pH dan suhu. *Jurnal Sains dan Seni* 2 (1), 38–42.
- Jovanović, M., Čuperlović, M., 1977. Nutritive value of rumen contents for monogastric animals. *Anim. Feed Sci. Technol.* 2 (4), 351–360. [https://doi.org/10.1016/0377-8401\(77\)90007-4](https://doi.org/10.1016/0377-8401(77)90007-4).
- Kameshwar, A.K.S., Qin, W., 2016. Lignin degrading fungal enzymes in: production of Biofuels and Chemical from lignin. *Prod. Biofuels Chem. Lignin* 81–130.
- Kumar, M., Morya, R., Gupta, A., Kumar, V., Thakur, I.S., 2021. Bacterial mediated depolymerization and degradation of lignin. *Environ. Microbiol. Biotechnol.* 83–103. https://doi.org/10.1007/978-981-15-7493-1_4.
- Leonowicz, A., Grzywnowicz, K., 1981. Quantitative estimation of laccase forms in some white-rot fungi using syringaldazine as a substrate. *Enzym. Microb. Technol.* 3 (1), 55–58. [https://doi.org/10.1016/0141-0229\(81\)90036-3](https://doi.org/10.1016/0141-0229(81)90036-3).
- Murtiyaningsih, H., Hazmi, M., 2013. Isolasi dan uji aktivitas enzim selulase pada bakteri selulolitik asal tanah sampah. *Agritrop* 15 (2), 293–308. <https://core.ac.uk/download/pdf/229212333.pdf>.
- Pandey, A., Soccol, C.R., Nigam, P., Soccol, V.T., Vandenberghe, L.P.S., Mohan, R., 2000. Biotechnological potential of agro-industrial residues. II: cassava bagasse. *Bioresour. Technol.* 74 (1), 81–87. [https://doi.org/10.1016/S0960-8524\(99\)00143-1](https://doi.org/10.1016/S0960-8524(99)00143-1).
- Pollegioni, L., Tonin, F., Rosini, E., 2015. Lignin-degrading enzymes. *FEBS J.* 282 (7), 1190–1213. <https://doi.org/10.1111/febs.13224>.
- Ribeirio, G.O., Gruninger, R.J., Badhan, A., McAllister, T.A., 2016. Mining the rumen for fibrolytic feed enzymes. *Anim. Front.* 6 (2), 20–26. <https://doi.org/10.2527/af.2016-0019>.
- Richana, N., 2002. Produksi dan prospek enzim xilanase dalam pengembangan bioindustri di Indonesia. *Buletin AgroBio* 5 (1), 29–36.
- Sánchez, C., 2010. Cultivation of *Pleurotus ostreatus* and other edible mushrooms. *Appl. Microbiol. Biotechnol.* 85, 1321–1337. <https://doi.org/10.1007/s00253-009-2343-7>.
- Tafsin, M., Hanafi, N.D., Yunilas, Mulianda, R., 2019. Nutrient quality of oil palm frond fermented by local microorganism (MOL) with different dosage and incubation

- time. In: IOP Conf. Series: Earth and Environmental Science, 260. IOP Publishing, 012050. <https://doi.org/10.1088/1755-1315/260/1/012050>.
- Tian, J.-H., Pourcher, A.-M., Bouchez, E., Peu, P., 2014. Occurrence of lignin degradation genotypes and phenotypes among prokaryotes. *Appl. Microbiol. Biotechnol.* 98, 9527–9544. <https://doi.org/10.1007/s00253-014-6142-4>.
- Tien, M., Kirk, T.T., 1984. Lignin-degrading enzyme from *Phanerochaete chrysosporium*: purification, characterization, and catalytic properties of a unique H₂O₂-requiring oxygenase. *Proc. Natl. Acad. Sci. USA* 81 (8), 2280–2284. <https://doi.org/10.1073/pnas.81.8.2280>.
- Troger, C., Niranjan, K., 2010. Sustainable chitin extraction and chitosan modification for application in the food industry. In: *International Conference on Food Innovation*.
- Wariishi, H., Valli, K., Gold, M.H., 1992. Manganese(II) oxidation by anganese peroxidase from the basidiomycete phanerochaete chrysosporium. kinetic mechanism and role of chelators. *J. Biol. Chem.* 267 (33), 23688–23695. [https://doi.org/10.1016/s0021-9258\(18\)35893-9](https://doi.org/10.1016/s0021-9258(18)35893-9).
- Wina, E., 2005. The technology of utilizing microorganism in feed to improve ruminant productivity in Indonesia: a review. *Wartazoa* 15 (4), 173–186. <http://repository.pertanian.go.id/handle/123456789/4636>.
- Yogyaswari, Ayoe, S., Rukmi, M.G.I., Raharjo, B., 2016. Eklorasi bakteri selulolitik dari cairan rumen sapi peranakan fries holland (PFH) dan limousine peranakan ongole (Limpo). *J. Biol.* 5 (4), 70–80. <https://ejournal3.undip.ac.id/index.php/biologi/article/view/19516>.