PAPER • OPEN ACCESS

Effect of Inoculum Dosage *Aspergillus niger* and *Rhizopus* oryzae mixture with Fermentation Time of Oil Seed Cake (*Jatropha curcas* L) to the content of Protein and Crude Fiber

To cite this article: T Kurniati *et al* 2017 *J. Phys.: Conf. Ser.* **824** 012064

IOP Conf. Series: Journal of Physics: Conf. Series **824** (2017) 012064 doi:10.1088/1742-6596/824/1/012064

Effect of Inoculum Dosage *Aspergillus niger* and *Rhizopus* oryzae mixture with Fermentation Time of Oil Seed Cake (*Jatropha curcas* L) to the content of Protein and Crude Fiber

T Kurniati, L Nurlaila and Iim

Department of Biology Education, Mathematics Education, Faculty of Tarbiyah UIN Sunan Gunung Jati Bandung. No. 105, Street of AH. Nasution Bandung Phone./Fax. 022-7803936.

*Corresponding author: tutikurniati1959@gmail.com

Abstract. *Jatropha curcas* L already widely cultivated for its seeds pressed oil used as an alternative fuel. This plant productivity per hectare obtained 2.5-5 tonnes of oil/ha / year and jatropha seed cake from 5.5 to 9.5 tonnes/ha/year, nutrient content of *Jatropha curcas* seed L potential to be used as feed material, However, the constraints faced was the low crude protein and high crude protein. The purpose of the research was to determine the dosage of inoculum and fermentation time of Jatropha seed cake by a mixture of *Aspergillus niger* and *Rhizopus oryzae* on crude protein and crude fibre. The study was conducted by an experimental method using a Completely Randomised Design (CRD) factorial design (3x3). The treatment consisted of a mixture of three dosage levels of *Aspergillus niger* and *Rhizopus oryzae* (= 0.2% d1, d2 and d3 = 0.3% = 0.4%) and three levels of fermentation time (w1 = 72 hours, 96 hours and w2 = w3 = 120 hours) each repeated three times. The parameters measured were crude protein and crude fibre. The results showed that dosages of 0.3% (*Aspergillus niger Rhizopus oryzae* 0.15% and 0.15%) and 72 hours (d2w1) is the dosage and the optimal time to generate the highest crude protein content of 21.11% and crude fibre amounted to 21.36%.

1. Introduction

One of the byproducts of agriculture which used as feed material is seed cake of Jatropha (*Jatropha curcas L).* Along with the processing and development of Jatropha curcas L oil, as an alternative energy producer of biodiesel. The oil extraction process results *from Jatropha* curcas L., potentially leading *Jatropha curcas L*. seed cake, amounting to 1 tonne/ha of production of 5 tonnes/ha of Jatropha curcas L. fruit, with oil production *Jatropha curcas L*., amounting to 2 tonnes/ha [1]. *Jatropha* seed cake is an agro-industrial waste from the pressing jatropha producing mainly oil used as an alternative fuel. The potential of *Jatropha* seed cake was excellent because it does not compete with human needs if it used as feed material.

Agro-industry waste used as feed material has a low quality, including Jatropha seed cake containing only 17% and crude fibre content is high at 24.66%. In order Jatropha seed cake can be used further as feed material, the need for treatment to be done before. One other alternative in the treatment of organic waste is through fermentation by using bioprocess technology of microorganisms that can improve the quality of feed materials [2], as well as fermentation by moulds. Through the process of fermentation, moulds will produce certain enzymes that are expected to break down complex organic materials to be digested to be a simple organic ingredient that easily absorbed. Mould used for fermentation includes *Aspergillus niger* and *Rhizopus oryzae*. *Aspergillus niger* produces the enzyme amylase,

IOP Conf. Series: Journal of Physics: Conf. Series **824** (2017) 012064 doi:10.1088/1742-6596/824/1/012064

protease and cellulose, while *Rhizopus oryzae* produces the enzyme amylase, protease, and lipase. Both types of these fungi have the same ability to break down protein and crude fibre.

The fermentation process is affected by the dosage and time. Dosage-related to the number of microbial populations and time related to the number of microbial growth on the substrate formed on the substrate. Fermentation using a mixture of both the fungus expected to improve the nutritional quality of Jatropha seed cake, especially crude protein and crude fibre, so it eventually used as feed material alternative sources of protein. But so far it has not been a lot of information about the use of Jatropha seed cake as feed and the use of a mixture of two types of mould as an inoculum in the fermentation process.
Based on the above, the author is interested in conducting research on "*Effect of Inoculum Dosage Aspergillus niger and Rhizopus oryzae mixture with Fermentation Time of Oil Seed Cake (Jatropha curcas L) to the content of Protein and Crude Fiber*".
".

Jatropha curcas L
Jatropha curcas L is a plant that is already familiar to the people of Indonesia. This plant is now widely cultivated for its seeds pressed oil used as a lubricating oil and alternative fuels. The productivity of these plants ranges between 3.5-4.5 kg of seeds/plant / year, with the rate of the population between 2500-3300 trees/ha. If the oil yield by 35%, then each hectare of land can be obtained 2.5-5 tonnes of oil/ha / year and Jatropha seed cake from 5.5 to 9.5 tonnes / ha / year.

Fermentation

Fermentation is an oxidation-reduction reaction in biological systems that produces energy in which the donor and acceptor of electrons in organic compounds, so that the resulting product is typical [3]. The moisture level of the solid substrate fermentation process varied between 30% -85%, however, the value of the optimum moisture is required for solid substrate fermentation depending on the organism and the substrates used. Application solid substrate fermentation has grown for the production of antibiotics, alcohol, hormones, biofuel, enzymes, organic acids, aroma compounds, also for the bioremediation of hazardous substances, waste detoxification Agroindustry, bio-pesticides, and maintenance of nutrition [4].

Aspergillus niger
Aspergillus niger has a characteristic that is a collection of single threads called hyphae or a collection of solid threads called mycelium white initially and then develops into yellow and heterotrophic life. The character of aerobic resulting in growing needs oxygen in sufficient quantities and multiple vegetative and generative through cell division and spores, the spores formed within an ascus (spore box). Meanwhile, another example is for a *α-amylase* activity of the highest in the koji fermented by Aspergillus oryzae requires a humidity of about 35% [5].

Rhizopus oryzae.
Rhizopus is a member of Zygomycetes. Rhizopus members often used in the fermentation process is Rhizopus oligosporus and Rhizopus oryzae. Rhizopus oryzae hyphae are not septate and colourless (clear), specialised into three forms, rhizoid, sporangiophore, and sporangium.

Whitish colonies and become gray-brown with age raised, and reached a height of approximately 10 mm, length 150-2000 µm and a diameter of 4-6 lm. Sporangia is round or semi-round, spiked walls, dark brown and a diameter of 50-200 lm. Columella is ovoid-shaped or spherical, 30-120 µm diameter and smooth walled or slightly rough. Sporangiophores round, ovoid, often shaped polygonal, striped on the surface and had a length of about 4-10 µm chlamydospore round shape, diameter 10-35µm. This species has an optimum growth temperature of 35 0C, minimum and maximum 50-70C 350-440 (-490)C. Ikasari and Mitchell (1994) states that as an example of the optimum moisture for protease production by *Rhizopus oligosporus* through solid substrate fermentation is 47%. According to Sarette et al. (1992), other studies show, during fermentation by *Rhizopus oligosporus* entire activity of the enzyme produced by this fungus is strongly affected water activity (a_w), such as enzymes polysaccharides.

Crude protein

Proteins are complex organic compounds that have a high molecular weight. As well as carbohydrates and lipids, proteins contain the elements carbon, hydrogen and oxygen, but also all proteins contain nitrogen.

Crude fibre

Crude fibre contains cellulose and lignin-hemicellulose. Hemicellulose cellulose and is a component of the cell walls of plants and can not be digested by monogastric animals (single bellied). Meanwhile, Medium ruminant animals have substances microorganisms, so the cattle have a greater ability to digest cellulose and hemicellulose, which is enzymatical. Lignin did not include in carbohydrates, but it is in the plant and part or unity in carbohydrates. These substances together form the cellulose component called lignocellulose, which has digestibility coefficient is tiny. In young plants, lignin content will be low but will increase with increasing age of the plant and reached the highest level when the plants are grown [6].

2. Methods

The first stage process, Jatropha curcas L. seed cake sterilised by autoclaving at a temperature of 121^0C at 1 atm pressure for 15 minutes. After sterile and then drained until it reaches a temperature of 30-350C, and then inoculated with the inoculum mix. Then incubated in a fermentation chamber at a temperature of 300C for a long 72 hours, 96 hours and 120 hours, and each treatment repeated three times.

The second stage is an experimental method, namely by applying experimental design Completely Randomised Design (CRD). The treatment consisted of a mixture of three dosage levels of *Aspergillus niger* and *Rhizopus oryzae* (= 0.2% d1, d2 and d3 = 0.3% = 0.4%) and three levels of fermentation time (w1 = 72 hours, 96 hours and w2 = w3 = 120 hours) each repeated three times. The parameters observed are crude protein and crude fibre. To see the effect of the treatment of the observed variables were statistically analysed by the method of variance followed by Duncan's multiple range tests.

3. Result

3.1 Effect of Treatment of Crude Protein Content

Protein is an organic compound of containing the elements carbon, hydrogen, oxygen and nitrogen as a constituent. The use of crude protein known in the analysis of animal food ingredients, all the nitrogen present in a feed material calculated as crude protein contained in the material. The higher the nitrogen content of material, the greater the amount of crude protein will be.

The results of the analysis of the crude protein content of Jatropha seed cake fermented in each treatment presented in Table 1.

Table 1. Crude protein content of Jatropha curcas Fermentation results.

Dosage	Time	Repeating 1	2	3	average
		………………………..…%	....		
d1	w1	19,42	19,68	19,46	19,52
d1	w2	19,31	19,63	19,56	19,50
d1	w3	20,08	19,72	19,96	19,92
d2	w1	20,92	21,22	21,19	21,11
d2	w2	19,11	19,23	19,17	19,17
d2	w3	17,98	18,19	17,92	18,03
d3	w1	18,66	18,78	18,75	18,73
d3	w2	18,84	18,75	18,69	18,76
d3	w3	19,97	20,05	19,89	19,97

Note : d1= inoculum dosage 0, 2%
d2= inoculum dosage 0, 3%
d3= inoculum dosage 0, 4%
w1= fermentation time 72 hours
w2= fermentation time 96 hours
w3= fermentation time 120 hours

Table 1 shows that the 0.3% dosage of inoculum fermentation time of 72 hours (d2w1) produces the average value the highest crude protein content of 21.11%, while 0.3% inoculum dosage fermentation time of 120 hours (d2w3) produces the protein content rude lowest at 18.03%. Based on the analysis of variance is known that the dosage of inoculum, fermentation time and interaction significant ($P < 0.05$) crude protein substrate. Furthermore, to determine the differences among the treatments, Duncan's multiple range tests are done as in Table 2.

Table 2. Duncan's Multiple Range Test Effect of Treatment on Crude Protein of Substrates Content

Treatment	mean	significance α 0,05
	………%...........	
d2w3	18,03	a
d3w1	18,73	b
d3w2	18,76	b
d2w2	19,17	c
d1w2	19,50	d
d1w1	19,52	d
d1w3	19,92	e
d3w3	19,97	e

d2w1	21,11	f

Note: a different letter on the significance column indicates significantly different (P <0.05).

Duncan's multiple range test results (Table 2) shows that the crude protein content of the substrate d2w1 significantly (P <0.05) higher than other treatments. The crude protein content of the substrate and d1w3 d3w3 treatment show no significant differences (P> 0.05), but both are significantly (P <0.05) higher than treatment d1w1, d1w2, d2w2, d3w2, d3w1 and d2w3. The crude protein content of the substrate and d1w2 d1w1 treatment show no significant differences (P> 0.05), but both are significantly (P <0.05) higher than treatment d2w2, d3w2, d3w1 and d2w3. Crude protein substrate at d2w2 treatment is significantly (P <0.05) greater than treatment d3w2, d3w1 and d2w3. The crude protein content of the substrate and d3w1 d3w2 treatment show no significant differences (P> 0.05), but both are significantly (P <0.05) higher than d2w3 treatment. Crude protein substrate at d2w3 treatment is significantly (P <0.05) lower than other treatments.

3.2 Effect of Treatment of Crude Fibre Content

Crude fibre is a structural carbohydrate composed of cellulose, hemicellulose and lignin which are difficult to be digested by the digestive system of poultry. Microbes producing cellulolytic enzymes can degrade crude fibre, so the crude fibre content of a substrate decreased.

The results of the analysis of crude fibre content of Jatropha seed cake fermented in each treatment shown in Table 3.

Table 3. Crude Fibre Content of *Jatropha curcas* of Fermentation.

Dosage	time	Repeating			average
		1	2	3	
		……………………..…%	....		
d1	w1	20,49	20,19	19,68	20,12
d1	w2	20,01	19,21	19,31	19,51
d1	w3	21,11	20,02	19,50	20,21
d2	w1	20,63	21,64	21,81	21,36
d2	w2	22,29	21,63	21,36	21,76
d2	w3	21,18	20,42	20,71	20,77
d3	w1	21,92	21,84	21,97	21,91
d3	w2	21,44	22,86	21,19	21,83
d3	w3	21,24	20,55	20,49	20,76

Table 3 shows that the 0.2% dosage of inoculum fermentation time 96 hours (d1w2) produces the average value of crude fibre content of the lowest of 19.51%, while 0.4% inoculum dosage fermentation time of 72 hours (d3w1) produces fibre highest rough that is equal to 21.91%. Based on the analysis of variance is known that inoculum dosage significantly (P <0.05) to the crude fibre content of the fermented substrate, but the fermentation time is not significant (P> 0.05) to the crude fibre content of the substrate and no interaction.

Furthermore, to determine the differences among the treatments, then Duncan's multiple range tests as shown in Table 4.

Table 4. Duncan's Multiple Range Test Effect of Treatment of Crude Fibre Content of Substrates

Treatment	Mean	significance α 0,05
	………%...........	
d1	19,95	A
d2	21,30	B
d3	21,50	b

Note: a different letter on the significance column indicates significantly different (P <0.05).

Duncan's multiple range test results (Table 4) shows that the content of crude fibre d1 dosage significantly (P <0.05) lower than the dosage d2 and d3, respectivel*y*. The crude fibre content of the substrate on d2 and d3 is not shown significant differences (P> 0.05).

3. Discussion

The optimal dosage of the fermentation of Jatropha seed cake that is a dosage of 0.3% (*Aspergillus niger* 0.15% and *Rhizopus oryzae* 0.15%) with a time of ferment for 72 hours (d2w1). If a dosage is lower or smaller, thus it is lower crude protein content. Similarly, if the fermentation time increased to 96 and 120 hours (d2w2 and d2w3) the crude protein content is decline. It is caused by a mixture of two microbes that Aspergillus niger and *Rhizopus oryzae* are on the dynamics of the different growth rate. Rhizopus oryzae mycelia were growing faster, followed by mycelial growth of *Aspergillus niger*. Aspergillus niger uses proteins which are formed by *Rhizopus oryzae* for growth. So the addition fermentation time affects the decline in crude protein content of the fermented substrate.

The increased crude protein which occurs during the process of fermentation is caused by the work of the mould and the addition of protein donated from cell growth of mould [7]. The increase in crude protein content of fermented also caused due to falling moulds use food ingredients such as carbohydrates and fat for growth and breeding. The fungus can synthesise a protein by taking carbon source of carbohydrate (glucose, sucrose and maltose), a nitrogen source of inorganic or organic material and minerals from its substrate [8]. Also, because during the incubation period, foods derived from the substrate used for growth of mould forming body protein derived from *Rhizopus oryzae*. The more the growth of mould, then the protein substrate will increase [9]. The protein content increases proportionally substrate caused by the reduced carbohydrate content [10] Mechanical fermentation done through the utilisation of biochemical properties of a particular microbe or a mixture of some bacteria [11]. The longer of the fermentation time caused an increasing chance of mould colonies to growth and fermentation so that the number of colonies has increased and will increase the protein content [12].

The increase in crude protein content in the substrate research results come from the ability of *Aspergillus niger* and *Rhizopus oryzae* in overhauling protein complex into simple amino acids and nitrogen. Microbial fermentation by mould and yeasts can improve the nutrition of agricultural waste fermentation products and agriculture industry [13].

Inoculum dosage of *Aspergillus niger* and *Rhizopus oryzae* fermentation of Jatropha seed cake significantly affect crude fibre content of the substrate fermented. The higher dosage of inoculum, the higher crude fibre content of the substrate will be. It is caused by *Rhizopus oryzae* mycelial growth which is faster than the mycelia *Aspergillus niger*. *Rhizopus oryzae* mycelia wall contains a lot of cellulose and chitin substances (compounds that have the same function with the plant cell wall), so that is an increase in crude fibre content of the substrate fermented. The production of cellulolytic enzymes produced by *Aspergillus niger* is not optimal because of the slow growth of the fungus, so the addition of fermentation time does not affect the crude fibre content of the substrate. Mycelia is a collection of hyphae, hyphae surrounded by a cell wall composed of polysaccharides, and the highest content of cell wall in most of the mold are cellulose [8]. The high content of crude fibre on the substrate fermented due to the crude fibre of mould mycelia. Along the slow growth of mould mycelia result can not be opened and a coarse substrate is not accompanied by the opening of other nutrients (fat and starch) led to increasing content of crude fibre fermentation results [14].

4. Conclusion

Based on the results of this study concluded that a dosage of 0.3% (Aspergillus niger 0.15% and *Rhizopus oryzae* 0,15%) and 72 hours (d2w1) is the dosage and the optimal time to produce the highest crude protein content of 21.11 % and amounted to 21.36% crude fibre.

References

[1] Becker, K., dan H.P.S Makkar, 2000. Makalah pada Daimler Chrysler/The world Bank Environment Forum Magdeburg 1999.
[2] Djunaidi, I.H. Natsir, M.H Hardini, D. Tastra, I.K Fatah, G.S.A. 2002. Jurnal Ilmu-Ilmu Hayati 14 (2)
[3] Winarno, F.G. 1980. Kimia Pangan dan Gizi. PT Gramedia, Jakarta.
[4] Perez-Guerra, N., Tarrado-Agrasar, A., Lopez-Macias, C., Pastrana, 2003. Electronic Journal of Environmental
[5] Jung-Yin, L. (2005). J.Fish
[6] Tillman, A. D. H. Hartadi., S. reksohadoprojo., S. Prawirokusumo dan S. Lebdosukojo. 1991. *Ilmu Makanan Ternak Dasar*. Cetakan ke enam. Gadjah Mada University Press, Yogyakarta.
[7] Tangendjaya, B. 1993. Jurnal Ilmu Peternakan Vol.6 No.2; 34-38
[8] Fardiaz, S. 1992. Mikrobiologi Pangan 1. PT Gramedia Pustaka Utama, Jakarta.
[9] Setiyatwan, H. 2007. Jurnal Ilmu Ternak. 7 (2): 113-116
[10] Kurniati, Tuti. 2011. ISSN 1970- 891 Volume V No. 1 -2
[11] Salmah. 2004. Analisa Pertumbuhan Mikroba pada Fermentasi. USU.
[12] Tampoebolon, B.I.M.2009. *Kajian Perbedaan Aras dan Lama Pemeranan Fermentasi Ampas Sagu dengan Aspergillus niger Terhadap Kandungan Protein Kasar dan Serat Kasar.* Seminar Nasional Kebangkitan Peternakan, Semarang Hal 235-243.
[13] Ringpfeili, M.negeli, B.Kreuteri, T.H. Moo Young, M.Rolz , C.1987. *Recommend Method for Caracterization of Agricultural Residues and Feeds Products Derived Through Biocenversation.* Pure and Appl. Chem.59 (5):723-730.

[14] Nurhayati, O.Sjofjan dan Koentjoko.2006. J. Indon. Trop. Anim. Agric. 31.