PROCEEDING

INTERNATIONAL SEMINAR ON SCIENCE AND TECHNOLOGY 2014

October 23, 2014

Tegalboto Campus, University of Jember
Jember, Indonesia

UNIVERSITAS
JEMBER
1964-2014

PROCEEDING

INTERNATIONAL SEMINAR ON SCIENCE AND TECHNOLOGY 2014

October 23, 2014

Tegalboto Campus, University of Jember
Jember, Indonesia

Proceeding of The International Seminar on Science & Technology 2014 (**ISOSTECH '14**)

© University of Jember, Indonesia 2014
Jember University Press

The editing of this proceeding has been carried out by **Siswoyo & B. Kuswandi** with assisted by the Scientific Committee of ISOSTECH'14.

ISBN: 978-602-9030-59-4

Published by Jember University Press, University of Jember, Indonesia

Anggota IKAPI No. 127/JTI/2011

Foreword by Organising Committee

Assalamu'alaikum Wr. Wb.

Distinguished guests and delegates

On behalf of the organizing committee, I am deeply grateful to your present in the International Seminar on Science & Technology 2014 (**ISOSTECH '14**) that already held in Universitas Jember, Jember Indonesia on thirsday, 23 October 2014.

The **ISOSTECH '14** is jointly seminar between University of Jember (UNEJ), Indonesia and Universiti Sains Islam Malalaysia (USIM), it was arranged with substantive elements such as seminar pertaining to current advance on science and technology together with posters.

The seminar was provide an excellent platform for knowledge exchange between the academicians, researchers, scientists and engineers working in areas of mathematic and basic sciences, agricultural and food Technology, health sciences and enggineering as well as information technology. In addition, it provides an opportunity for the participants from Indonesia, Malaysia and Philiphine to share research findings, to establish networking and to encourage academic and student exchange and other participation in this exciting seminar.

We also would like to express our deep appreciation to the all organising committee members and steering committee, especially Dr. Zulfikar, on behalf of Rector, as Vice Rector of UNEJ who officially opens this seminar. Last but not least our appreciation to all participants especially delegate from USIM, IIU Malaysia and San Carlos University, Philiphines. We convey our great gratitude for your scientific speech and contribution. We do hope that all these research results are useful for further research progress and development in these fields.

Enjoy the conference proceeding and hope it will give inpiration on your research projects.

Wassalamu'alaikum Wr. Wb.

Mrs. L. Wulandary
Chairperson
University of Jember

Preface

The first International Seminar On Science & Technology 2014 (**ISOSTECH '14**), took place in University of Jember, Jember East Java Indonesia on 23 October 2014. This first seminar series is focused on all aspects related to recent advance in science and technology.

This proceeding contains papers that have been presented at **ISOSTECH '14** as plenary lectures, invited, oral and poster presentations. About 100 participants attended the conference, with 4 plenary lectures, 35 oral and 24 poster presentations. The proceeding of **ISOSTECH '14** has been published in electronic form as *.pdf file for simple and easy publication and to avoid heavy book of proceeding. We hope that this publication can be easily read, handled and transferred to other form. Furthermore, this paperless proceeding can be fruitful for all participants of the conference.

My sincerely thanks go to all the members of Scientific Committee for their valuable help in the review of the submitted papers, and also to the authors for their collaborative attitude. A special mention must go to **Mrs. L Wulandary**, our Conference Chairperson, who has put in a terrific amount of effort not only in general conference matter but also in the assembly of the papers for this proceeding. Finally, I congratulate the authors of all papers for producing the new and novel idea for research on mathematic and basic sciences, agricultural and food Technology, health sciences and enggineering as well as information technology.

Jember, October 2014

Siswoyo & B. Kuswandi
Editors

SCIENTIFIC COMMITTEE

Bambang Kuswandi
Lestyo Wulandari
Martinus H. Pandutama
Siswoyo
Adhitya Wardhono
Hadi Paramu

Table of Contents

Enzymatic Hydrolysis of *Jatropha curcas* Seed Cake and Utilisation of Its Hydrolysates for Single Cell Protein Production

Kahar Muzakhar

Biology Dept., Faculty of Mathematics and Natural Sciences, The University of Jember
Kalimantan Street 37, Jember Indonesia 68121, email; kaharmzk@unej.ac.id

Abstract - Production of jatropha seed cake hydrolyzates (JSCH) and its utilisation for a medium in single cell protein (SCP) production was examined. To produce JSCH, 5% of jatropha seed cake (JSC) powder was enzymatically hydrolysed using concentrated crude enzyme from *Trichoderma viride*. Hydrolysis released 27.3 mg/ml reducing sugar when done at 30^{o}C for 36 hours. JSCH consisted of 16.9 g/ml or 62% sugar as monosaccharide from the total reducing sugar produced, as analysed using Gas Chromatograph. Based on these results, the sugar-rich JSCH was then used for medium SCP *Saccharomyces cerevisiae* production. Aerobically fermentation at 30^{o}C and 120 rpm shaking for 48 hours produced 12.5 mg/ml SCP with efficiency 74.2%, respectively.

Keywords: Jatropha, Hydrolysate, SCP, fermentation

1. Introduction

In *J. curcas* oil processing, a significant quantity organic stuffs remain as polysaccharide and protein rich-JSC [1] is disposed as waste. JSC has been reported to possess phorbol esters [2], a potenial toxic compound to animals so that cannot be used as feed because of its toxic properties [3], [4]. These toxicities of JSC has been studied extensively in animals. When animals (e.g; fish, sheep and goat) were fed on phorbol ester containing feed, it could make decreasing of glucose level, dehydration diarrhea, and other anti-tick feeding effects [3], [5]. However, JSC is a source which can be used as organic fertilizer due to its high nitrogen content [6] [7], [8]. Therefore, it should be reasonably source for other useful products.

Due to the environmental concern, effective biological methods to manage and solve some of the environmental problems and other forms of pollution should be considered. Through bioconversion, the waste material can be converted to other useful product [9], which may also reduce the processing cost. Another important advantage of bioconversion that used some organisms such as fungi, bacteria or yeast is a long history of safety aspect of usage for the manufacture of food products destined for human consumption and is regarded to be nontoxic and nonpathogenic.

In the present study, utilizing JSC for producing ″sugar rich JSCH″ by hydrolysis using crude enzyme from *T. viride* and converting to SCP was reported.

2. Materials and Methods

2.1 Enzyme Production

Jatropha seed cake medium and *T. viride* were used for producing crude enzyme. In this step, optimisation of cultivation and harvesting of crude enzyme were done in series of days to obtain the crude enzyme with optimum activity. The activity was measured by reducing sugar produced during JSC hydrolysis. Detail of this step will be explained in Results and Discussion.

2.2 Degree of Hydrolysis and Total Sugar Analysis

The degree of hydrolysis was examined by incubating the reaction mixture of concentrated crude enzyme and JSC substrate at 30^{o}C. The JSCH as reducing sugars measured by the method of Nelson [10] as modified by Somogyi [11] using glucose as a standard sugar. The degree of hydrolysis of JSC was calculated as follows.

$$DH(\%) = \frac{TRS\,(w/v)}{TS\,(w/v)} * 100\% \qquad (1)$$

DH = Degree of Hydrolysis
TRS = Total Reducing Sugar
TS = Total Substrate

The total sugar content of JSCH was also measured according the phenol-sulphuric acid method [12].

2.3 Analysis of Monosaccharides

Analysis of sugar as monosaccharides was performed by using Gas Chromatograph as alditol acetates [13], [14] with a few modifications [15].

2.4 JSCH Production Medium for SCP Production

Five percent of JSC was hydrolyzed with concentrated crude enzyme at 30^{o}C under unbuffered condition for 48 hours and reducing sugar production was monitored every 6 hours. The total sugar also quantified using GC as described above.

2.5 Maintaining SCP

Saccharomycess cerevisiae was maintained at 30°C on 1.5% agar medium pH 6, with yeast-extract (0.3%), malt extract (0.3%), pepton (0.5%) and glucose (1%).

2.6 Analysis of SCP Production

Saccharomycess cerevisiae was cultured on JSCH medium aerobically in 500-ml shake flask using shaker set at 120 rpm and 30°C. Growth or biomass (mg/l) of *S. cerevisiae* was observed by measuring absorbance at 660 nm for every 12 hours. To relate the measured absorbance to biomass, the method of Kim *et al*. 1998 was adopted [16]. The sugar assimilation ability of *S. cerevisiae* SCP was determined by measuring the remaining of reducing sugar and GC analysis.

The assimilated sugar and assimilation efficiency were calculated as follows.

$$AS(g) = IS(g) - RS(g) \qquad (2)$$

$$AE(\%) = \left(\frac{AS(g)}{TS(g)} \right) * 100\% \qquad (3)$$

And the maximum yield of biomass was calculated as formula below.

$$Max.\ yield\ of\ biomass(\%) = \frac{Max.\ biomass(g)}{AS(g)} * 100\% \quad (4)$$

AS : Assimilated Sugar
IS : Initial Sugar
AE : Assimilation Efficiency
TS : Total Sugar

3. Results and Discussion

Enzymatically hydrolysis of JSC has been evaluated on this research. *T. viride* was seleted on this research because the genus *Trichoderma* easily grow and exploit the carbon as well as nitrogen from various biomass [17], [18]. Also, this species well known produced board spectrum of extracellular enzyme such as cellulase [18], [19], [20], [21], [22], [23], [24], xylanase [25], [26], exoglucanases and endoglucanases [27] and some hemicelluloses.

In this research, optimum crude enzyme production and activity of *T. viride* based on the measurement reducing sugar released during hydrolysis against JSC substrate. To optimise crude enzyme production, the solid state fementation was done on 10g JSC on flask and inoculated with *T. viride,* then incubated at 30°C. The enzyme was harvested and the activity daily monitored.

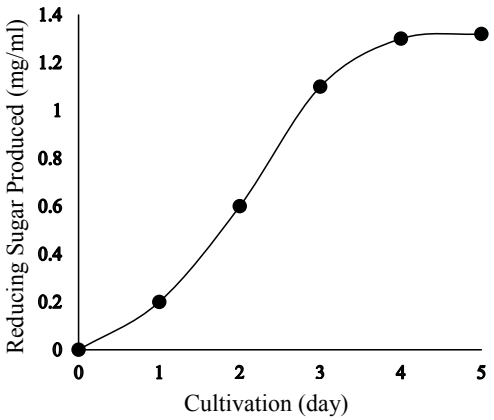

Figure 1. Optimisation of Enzyme Production of *T. viride*

It was observed and shown at Figure 1 that optimum extracellular production was found in fourth day cultivation. The enzyme able to hydrolyse JSC and produced reducing sugar at 1.3 mg/ml when hydrolysis of 1% JSC substrate was done in acetate buffer 50mM pH 5, incubated 37°C for 30 minutes.

According to optimisation result, large scale of extracellular enzyme production was done on 500 g JSC inoculated by *T. viride*, incubated at the same condition on 30°C for 4 days. The extracellular crude enzyme was harvested by 1% NaCl 500 ml and 0.1% toluene (v/v), followed by shaking at room temperature for 12 hours. The suspension was fitered and centrifuged 4000 rpm for 10 minutes to recover the supernatant as a source crude enzyme. Then the crude enzyme was concentrated to about one-tenth of the initial volume by ammonium sulphate precipitation at 70% saturation. The precipitate was dissolved and dialyzed against distilled water for 3 days. This solution was stored at 4°C till used for JSC hydrolysis.

Hydrolysis of 5% JSC was done without adjustment of pH resulting in 27.3 mg/ml reducing sugar in JSCH when done at 30°C for 36 hours. This evidence proved that concentrated enzyme hydrolysed JSC readily with the degree of hydrolysis 78%. Further analysis showed that 62% or 16.9 g/ml of JSCH is monosaccharide as analysed using GC. It was also elucidated by thin layer chromatography (TLC) that the spot was detected as glucose in JSCH (data not shown).

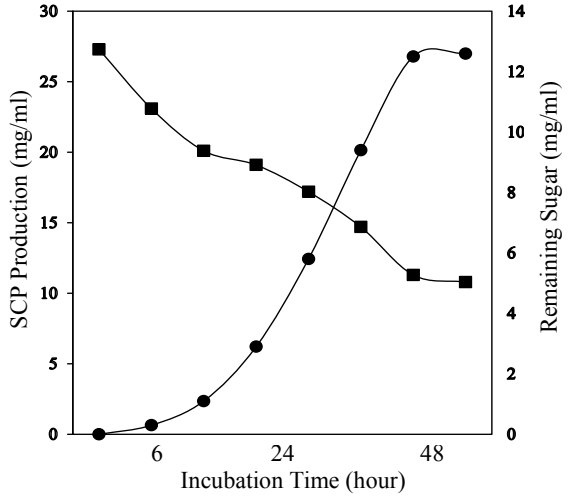

Figure 2. Growth Pattern of *S. cerevisiae* (●) and Remaining Sugar (■)

Tabel 1. SCP Production and Remaining Sugars

Incubation (Hour)	*SCP (mg/ml)*	*Sugars (mg/ml)*	
		Reducing Sugar	*Monosaccharides*
0	0	27.3	16.9
6	0.3	23.1	13.1
12	1.1	20.1	10.4
18	2.9	19.1	7.2
24	5.8	17.2	3.2
36	9.4	14.7	2.1
48	12.5	11.3	1.9
60	12.6	10.8	1.2

For application purposes, the SCP production was done with sugar-rich JSCH medium without any nutrient added. Growth of SCP *S. cerevisiae* biomass in medium was monitored every 6 hours. And remaining sugar was also quantified to ensure assimilation by *S. cerevisiae* happened which indicated decreasing amount of sugar in JSCH. This fermentation was done at 30^{o}C and 120 rpm shaking aerobically. Observation showed that SCP was produced maximum at 48 hours. As estimated at OD 660nm, the growth or biomass maximum of *S. cerevisiae* was 12.5 mg/ml. It were reported that some *yeast* [28], [29], [30]. Figure 2 showed that the remaining sugar also decrease gradually as reflected sugar utilisation occurred for SCP growth. As shown in Table 1, monosaccharides also decrease accordingly and 1.2 mg/ml remained after 60 hours. This indication revealed that *S. cerevisiae* assimilated monosaccharides in JSCH definitely. It was calculated that SCP production gave efficiency 74.2%, respectively.

4. Conclusion

Microbial utilisation of agricultural wastes JSC biomass to protein-rich food has been shown in this paper. The result of 62% sugar as monosaccharide production through enzymatic hydrolysis of 5% JSC and conversion of its hydrolysate to SCP production resulting in efficiency 74.2% which praiseworthy enough. However, scaling up to be industrially as well as optimizing and including the process with the point of view to increase in economic value must be evaluated.

5. Acknowledgments

Apart of this work was supported by competitive research funding Strategis Nasional 2014 scheme, Directorate General of Higher Education (DIKTI) Ministry of National Education and Culture - Indonesia

6. References

[1] S. Nithiyanantham, P. Siddhuraju, and G. Francis, "Potential of *Jatropha curcas* as a Biofuel, Animal Feed and Health Products," *J. Am. Oil Chem. Soc.*, vol. 89, no. 6, pp. 961–972, Jan. 2012.

[2] H. Makkar, J. Maes, W. De Greyt, and K. Becker, "Removal and Degradation of Phorbol Esters during Pre-treatment," *J. Am. Oil Chem. Soc.*, vol. 86, pp. 173–181, 2009.

[3] M. Das, H. S. Uppal, R. Singh, S. Beri, K. S. Mohan, V. C. Gupta, and A. Adholeya, "Bioresource Technology Co-composting of physic nut (*Jatropha curcas*) deoiled cake with rice straw and different animal dung," *Bioresour. Technol.*, vol. 102, no. 11, pp. 6541–6546, 2011.

[4] G. Kumar, B. Sen, and C. Lin, "Bioresource Technology Pretreatment and hydrolysis methods for recovery of fermentable sugars from de-oiled Jatropha waste," *Bioresour. Technol.*, vol. 145, pp. 275–279, 2013.

[5] S. Abdel-Shafy, S. M. Nasr, H. H. Abdel-Rahman, and S. M. Habeeb, "Effect of various levels of dietary *Jatropha curcas* seed meal on rabbits infested by the adult ticks of *Hyalomma marginatum* I. Animal performance, anti-tick feeding and haemogram.," *Trop. Anim. Health Prod.*, vol. 43, no. 2, pp. 347–57, Feb. 2011.

[6] B. R. Dave and A. P. Sudhir, "Utilization of Jatropha deoiled seed cake for production of cellulases under solid-state fermentation," pp. 1343–1353, 2012.

[7] H. P. S. A. Khalil, N. A. S. Aprilia, A. H. Bhat, M. Jawaid, M. T. Paridah, and D. Rudi, "A Jatropha biomass as renewable materials for biocomposites and its applications," *Renew. Sustain. Energy Rev.*, vol. 22, pp. 667–685, 2013.

[8] N. Contran, L. Chessa, M. Lubino, D. Bellavite, P. Paolo, and G. Enne, "State-of-the-art of the *Jatropha curcas* productive chain : From sowing

to biodiesel and by-products," *Ind. Crop. Prod.*, vol. 42, no. 2013, pp. 202–215, 2015.

[9] L. Jiang, Z. Fang, F. Guo, and L. Yang, "Bioresource Technology Production of 2 , 3-butanediol from acid hydrolysates of Jatropha hulls with *Klebsiella oxytoca*," *Bioresour. Technol.*, vol. 107, pp. 405–410, 2012.

[10] N. Nelson, "Article : A Photometric Adaptation of The Somogyi Method for The Determination of Glucose," *J. Biol. Chem.*, vol. 153, pp. 375–380, 1944.

[11] M. Somogyi, "Notes on Sugar Determination," *J. Biol. Chem.*, vol. 70, pp. 599–612, 1926.

[12] M. DuBois, K. a. Gilles, J. K. Hamilton, P. a. Rebers, and F. Smith, "Colorimetric Method for Determination of Sugars and Related Substances," *Anal. Chem.*, vol. 28, no. 3, pp. 350–356, Mar. 1956.

[13] R. G. Spiro, "Methods in Enzymology Study of the carbohydrates of glycoproteins Analysis of Monosaccharides," *Acad. Press. New York London Vol.XXVIII. Ginsbg. I.V., (ed.), Part B.*, vol. 28, 1972.

[14] S. Murao. M. Arai, "Characterization of Oligosaccharides from an Enzymatic Hydrolyzate of Red Yeast Cell Walls by Lytic Enzymes," *Agric. Biol. Chem.*, vol. 42, no. 9, pp. 1651–1659, 1978.

[15] K. Muzakhar, Sutoyo, and Siswoyo, "Sugar Production by Digesting of Oil Palm Empty Fruit Bunch Using Extracellular Enzymes From *Aspergillus niger* and *Trichoderma reesei* For Ethanol Production," in *ICBB 5th 2015*, 2014, pp. 1–7.

[16] J. K. Kim, K. Tak, and J. Moon, "A Continuous Fermentation of *Kluyveromyces Fragilis* for The Production Of A Highly Nutritious Protein Diet," *Aquac. Eng.*, vol. 18, no. 1, 1998.

[17] a G. Babu, J. Shim, K.-S. Bang, P. J. Shea, and B.-T. Oh, "*Trichoderma virens* PDR-28: a heavy metal-tolerant and plant growth-promoting fungus for remediation and bioenergy crop production on mine tailing soil.," *J. Environ. Manage.*, vol. 132, pp. 129–34, Jan. 2014.

[18] M. K. Bothwell, L. P. Walker, M. Prices, B. Engineering, R. Hall, and C. Biology, "Synergism Between Pure *Thermomonospora Fusca* and *Trichoderma*," vol. 4, no. 4, pp. 293–299, 1993.

[19] M. Irfan, M. Nadeem, and Q. Syed, "One-factor-at-a-time (OFAT) optimization of xylanase production from *Trichoderma viride*-IR05 in solid-state fermentation," *J. Radiat. Res. Appl. Sci.*, vol. 7, no. 3, pp. 317–326, Jul. 2014.

[20] M. Z. Alam, S. a Muyibi, and R. Wahid, "Statistical optimization of process conditions for cellulase production by liquid state bioconversion of domestic wastewater sludge.," *Bioresour. Technol.*, vol. 99, no. 11, pp. 4709–16, Jul. 2008.

[21] S. Bisaria, "Adsorption Characteristics of Cellulases from a Constitutive of *Trichoderma reesei*," vol. 86, no. 2, pp. 233–235, 1998.

[22] M. D. Busto and N. Ortega, "Location , Kinetics and Stability ff Cellulases Induced In *Trichoderma reesei* Cultures," vol. 57, pp. 187–192, 1996.

[23] H. R. Flodman and H. Noureddini, "Effects of intermittent mechanical mixing on solid-state fermentation of wet corn distillers grain with *Trichoderma reesei*," *Biochem. Eng. J.*, vol. 81, pp. 24–28, Dec. 2013.

[24] X. Jiang, A. Geng, N. He, and Q. Li, "New isolate of *Trichoderma viride* strain for enhanced cellulolytic enzyme complex production.," *J. Biosci. Bioeng.*, vol. 111, no. 2, pp. 121–7, Feb. 2011.

[25] L. D. S. Castro, A. C. C. Antoniêto, W. R. Pedersoli, R. Silva-Rocha, G. F. Persinoti, and R. N. Silva, "Expression pattern of cellulolytic and xylanolytic genes regulated by transcriptional factors XYR1 and CRE1 are affected by carbon source in *Trichoderma reesei*.," *Gene Expr. Patterns*, vol. 14, no. 2, pp. 88–95, Mar. 2014.

[26] G. S. Dhillon, H. S. Oberoi, S. Kaur, S. Bansal, and S. K. Brar, "Value-addition of agricultural wastes for augmented cellulase and xylanase production through solid-state tray fermentation employing mixed-culture of fungi," *Ind. Crops Prod.*, vol. 34, no. 1, pp. 1160–1167, Jul. 2011.

[27] H. Fang, C. Zhao, and X.-Y. Song, "Optimization of enzymatic hydrolysis of steam-exploded corn stover by two approaches: response surface methodology or using cellulase from mixed cultures of *Trichoderma reesei* RUT-C30 and *Aspergillus niger* NL02.," *Bioresour. Technol.*, vol. 101, no. 11, pp. 4111–9, Jun. 2010.

[28] T. Zhang, Z. Chi, and J. Shen, "A Highly Thermosensitive and Permeable Mutant of the Marine *Cryptococcus aureus* G7a Potentially Useful for Single-Cell Production and its Nutritive Components," *Mar. Biotechnol*, vol. 11, pp. 280–286, 2009.

[29] O. O. Ezekiel, O. C. Aworh, J. C. Preez, and L. Steyn, "Cultivation of *Candida utilis* on Cassava Peel Hydrolysates for Single-cell Protein Production Olufunke," vol. 2, pp. 452–461, 2012.

[30] L. Gao, Z. Chi, J. Sheng, X. Ni, and L. Wang, "Single-cell protein production from Jerusalem artichoke extract by a recently isolated marine yeast *Cryptococcus aureus* G7a and its nutritive analysis.," *Appl. Microbiol. Biotechnol.*, vol. 77, no. 4, pp. 825–32, Dec. 2007.