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Direct Bioethanol Production from Breadfruit Starch (*Artocarpus communis Forst*) by Engineered Simultaneous Saccharification and Fermentation (ESSF) using Microbes Consortium

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ABSTRACT: Breadfruit (Artocarpus communis Forst.) is one of sources for bioethanol production, which has high starch content (89%). Bioethanol production from breadfruit starch was conducted by Simultaneous Saccharification and Fermentation (SSF) technology using microbes consortium. The aim of the research was to examine a method to produce bioethanol by SSF technology using microbes consortium at high yield and efficiency. The main research consisted of two treatments, namely Conventional SSF and Enginereed SSF (ESSF). The results showed that ConventionalSSF using aeration and agitation during cultivation could produce ethanol at 11.15 ± 0.18 g/L, with the yield of product (Yp/s) 0.34 g bioethanol/g substrate; and yield of biomass (Yx/s) 0.29 g cell/g substrate, respectively. A better result was obtained using Engineered SSF (ESSF) in which aeration was stopped after biomass condition has reached the end of the exponential phase. The bioethanol produced was 12.75 ± 0.04 g/L, with an conversion efficiency of 75% to products, yields of product (Yp/s) 0.41 g bioethanol/g substrate, and the yield of cell (Yx/s) 0.09 g cell/g substrate.

Keywords*:* Starch, breadfruit, Engineered Simultaneous Saccharification and Fermentation (ESSF), Microbes Consortium, bioethanol

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1. Introduction

Indonesia is one of countries with high energy consumption in the world. Based on the data from the Directorate General of New Renewable Energy and Energy Conservation Ministry of Energy and Mineral Resources (2013), in recent years Indonesia's energy consumption growth has reached 7% per year. This amount is above the world's energy consumption growth which is 2.6% per year. The high rate of consumption causes the depletion of petroleum resources faster than finding the new energy resources.

Bioethanol is an alternative fuel, which is more efficient, environmentally friendly, and is obtained from renewable natural resources (Szymanowska and Grajek 2011; Azmi et al. 2009). Bioethanol can be produced from renewable biomass such as agricultural crops containing starch or sugarcane. Moreover, the addition of small amounts of bioethanol at 10% into gasoline can reduce CO and NO emissions, therefore it can reduce the greenhouse effect (Balat et al. 2008; Imam and Capareda 2011).

Bioethanol can be produced from sugar or starchy materials such as molasses, sorghum, cassava, sweet potato, and sago (Syamsu 2008; Azmi at al. 2009; Saifuddin and Hussain 2011; Imam and Capareda 2011). The use of these plants has several advantages, namely: 1) easy to be grown and do not require difficult treatment for growing them hence cheaper production

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cost; 2) requires little fertilization and irrigation, 3) short growth period, and 4) the adaptation of plants to the environment and climates high (Azmi et al. 2009; Imam and Caperada 2011). Breadfruit is one of potential plants which can be used for production of bioethanol. The carbohydrate content of breadfruit is relatively high, i.e 79.46% (Adepeju et al. 2011).

The breadfruit production in Indonesia per hectare on average 4 – 20 tons on a single fruiting season (Adinugraha and Kartikawati 2004). However, the limitation of the use of breadfruit in Indonesia is due to the lack of information about the potential of this plant. This commodity is actually potential to be used as a material for producing alternative fuels.

The use of bioethanol as an alternative fuel is still not economically competitive to the petroleum based fuel. At least, there are two stategies to increase the competitiveness of bioethanol as an alternative fuel, namely: (a) to find a substrate which is cheap and abundantly available; (b) and to find a method or technology which is more efficient and productive to produce bioethanol.

Bioethanol production from breadfruit starch is conducted by Engineered Simultaneous saccharification and fermentation (ESSF). ESSF is Simultaneous Saccharification and Fermentation (SSF) technology which is combined with bioprocess engineering by switching the condition from fully aerobic into anaerobic. This switching condition will shift metabolism of yeast from respiration which is produce more cell (less bioethanol) into fermentation. The objective of this research is to investigate the use of breadfruit starch as a substrate by engineered simultaneous saccharification and fermentation (ESSF) using microbes consortium to produce bio-ethanol. Engineered SSF, at a certain time, will shift the metabolisms from respirative metabolism which produce more cells and less ethanol into fermentative metabolism which produces more ethanol and less cell. It is expected that engineered SSF will produce more ethanol than conventional SSF.

2. Materials and methods

2.1. Preparation of breadfruit starch for the media.

The breadfruit used was Lumut varieties obtained from the Mojokerto, East Java. One kg of breadfruit was extracted with 2 liters of tap water. The extract was then characterized, namely proximate analysis (AOAC 1995), carbohydrate was measured using by difference, and starch analysis (Antrhone method) (Sattler and Zerban 1948).

2.2 Inoculums of microbes' consortium.

Inoculums of microbes consortium was Ragi Tapai which was obtained from the local market. The brand of Ragi tapai was NKL (Na Kok Liong). An amount of 120 ml liquid medium extract of breadfruit starch was put into a the 250 ml Erlenmeyer, sterilized at 1210C for 15 minutes, and cooled. An amount of 0.5% (w/v) Ragi Tapai was inoculated into liquid medium. No other nutrient was added. The culture was incubated at room temperature (± 290C), at 200 rpm, for 24 hours before inoculation.

2.3. Simultaneous saccharification and fermentation.

An amount of 1.2 liters liquid breadfruit starch medium was sterilized at 1210C, for 15 minutes. An amount of 10% (v/v) inoculums of microbe consortium was inoculated into bioreactor containing sterile liquid medium of breadfruit extract. Aeration was set at 1 vvm, while agitation at a speed of 150 rpm at room temperature. Cultivation was kept at aerobic conditions.

2.4. Engineered Simultaneous saccharification and fermentation (ESSF)

The growth curve of the previous treatment was used as a reference for implementing the engineered SSF (ESSF). After the growth of microbe consortium reached late exponential phase, the aeration was stopped to allow anaerobic condition. Switching condition from aerobic to anaerobic condition after the biomass reached maximum amount is expected to switch the metabolism from respirative into fermentative which would produce more bioethanol. In the meantime, mould would be died and reserved as additional nutrient for yeast which can grow under anaerobic condition. Therefore, by this metabolism shifting, it is expected that the rest of substrate will be fully converted into bioethanol.

2.5. Measurement parameters (Kinetics of cultivation)

Samples were taken every 12 hours for 72 hours. Parameters measured and calculated as a performance indicator of cultivation process are as follows:

- Total biomass produced per 12 hours (X)
- Level of bioethanol produced (P)
- Residual of starch subtrate still present in the media (S) every 12 hours
- Maximum specific growth rate (μmax): slope of curve was obtained by plotting lnX againt time (hr)
- Mathematical model:

$$\frac{dx}{dt} = \mu x$$

If this equation is integrated between x0 to x_t in the time interval of t0 to t1, and resulted:

$$\mu = \frac{\ln x_t - \ln x_0}{\Delta t}$$

Where $\Delta t = t1-t0$

- Yield of biomass and bioethanol per substrate used

$$Y_{x/s} = \frac{X - Xo}{So - S} \text{ and } Y_{p/s} = \frac{P - Po}{So - S}$$

- Yield of product formation of cells (Yp/x)

$$Y_{p/x} = \frac{P - Po}{X - Xo}$$

- Efficiency of substrate used

$$= \frac{So - S}{So} x100\%$$

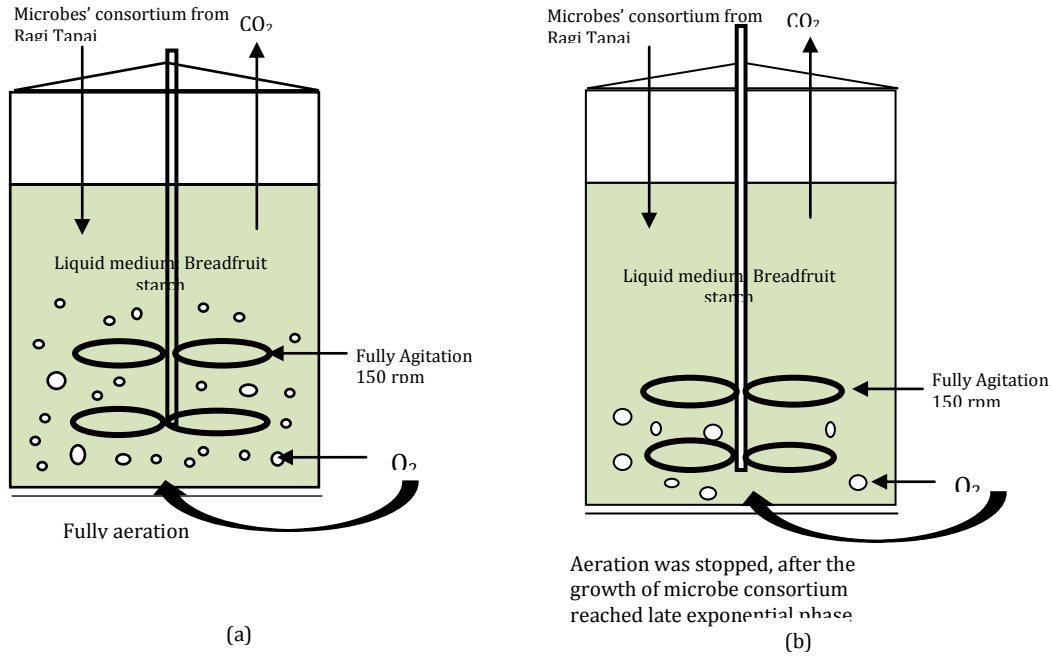

Figure 1. The experimental set-up : Configuration (a) Conventional SSF, (b) ESSF

3. Results and Discussion

3.1. Characterization of breadfruit starch.

Table 1 shows the results of the proximate analysis of breadfruit starch

Table 1
Chemical composition of breadfruit starch

Component	Composition (%)	
	Literature[a]	Research[b]
Moisture [wet basis]	5.45	75.96 ± 0.1
Ash [dry basis]	1.35	1.83 ± 0.02
Fat [dry basis]	0.37	2.16 ± 0.03
Protein [dry basis]	0.69	3.83 ± 0.00
Crude fiber [dry basis]	1.25	-
Carbohidrate [dry basis]	96.34	91.93 ± 0.09

Data: a and b has been processed; mean ± standard deviation (n=2)
[a]Akanbi *et al.* (2011), [b]Proximate analysis (2014)

The main components of the proximate analysis was carbohidrate (91.93%). The carbohydrate content of the breadfruit's starch was lower than literature. The protein, fat and ash content were higher than the breadfruit starch as reported by Akanbi *et al.* (2011).

The African breadfruit kernel has higher protein of 17.1% as studied by Akubor *et al.* (2000). Furthermore, the ash and the fat contents of breadfruit were higher than those of sago and sweet potato (Pangloli 1992; Syamsu 2008), but the carbohydrates were lower than that of sago (Supatmawati 2010).

Breadfruit starch content was analyzed quantitatively using Anthrone (Sattler and Zerban 1948). The result showed that the breadfruit contained 89% of starch in dry basis. This result was higher compared to the findings of earlier studies, which were 69%; 67.9%; and 58% (Graham and de Bravo 1981; Loos *et al.* 1981; Steve *et al.* 1995). The presence of starch implied that the material could be converted into bio-ethanol.

Protein and fat were still detected in the breadfruit starch. It was expected that the protein could be used as a nitrogen source for the growth of microbes' consortia. The primary nutrient for organisms other than carbon and oxygen is nitrogen. Nitrogen is an essential macronutrient for the growth and maintenance of the cell's ability to formatting enzyme (Rehm and Reed 1981). Microorganisms can grow at its maximum rate, although the nitrogen concentration was relatively low

(Griffin 1981). In the study reported by Azmi *et al*. (2009), the production of bioethanol from cassava starch as a substrate only adds 0.1% (w/v) peptone and distilled water without the addition of other nutrients.

3.2. *Simultaneous saccharification and fermentation performance*

In general, bio-ethanol production consists of two processes, they are hydrolysis and fermentation. Starch hydrolysis process consists of three steps, namely gelatinization, liquefaction and saccharification process. However, in this SSF technique, hydrolysis and fermentation process were carried out simultaneously; this could shorten time required. The use of the microbes' consortium in a simultaneous process was considered more efficient, because of without adding or replacing the enzymes and microbes in each process (Nadir *et al*. 2009). *Ragi tapai* was chosen based on its ability to produce high glucose and bioethanol yield directly from starch (Azmi *et al*. 2008).

The microbes' consortium was obtained from *Ragi Tapai* containing a mix of several microorganisms; it consists of fungi, yeasts, and bacteria (Merican and Quee-Lan 2004). Azmi *et al*. (2009) reported that at the end of fermentation, only yeast and mold that are observed in the medium. Dwidjoseputro and Wolf (1970), identified microorganisms from a Tape containing two yeast species, which are *Candida lactose* and *Pichia Malanga*. Djien (1972), found that in *Ragi Tapai* contained *Chlamydomucor oryzae,* five species of the genus *Mucor* and on species of *Rhizopus*, yeast *Pichia burtonii* and *Endomycopsis fibuliger*. Other researchers have identified that, there are other species in *Ragi Tapai*, such as *Candida utilis* and *Saccharomyces cerevisiae*, and bacteria *Pediococcus* sp. and *Bacillus* sp. (Gandjar 2003).

Based on the results of the starch analysis, breadfruit had 89% of starch. To avoid gelatinization process during autoclave heating, breadfruit starch content for the medium used was maximum only 6%. According to Winarno (1997), a mixture of starch and water is divided into two fractions, namely soluble fraction called amylose and insoluble fractions called amylopectin. Significant decreases of breadfruit starch levels affects of autoclave heating at 121^{0}C for 15 minutes to 3%. This could occur because autoclave heating had reduced the starch content for the effect of gelatinization process. Starch gelatinization causes damage in starch substance increased. In addition, the autoclave heating results in an increase of starch degradation (Jenie *et al*. 2012). Jenie *et al*. (2012) figured out that the beginning levels of starch in banana flour by 65.98% - 70.29%, decrease significantly as a result of autoclave heating by 62.12% - 66.81%.

3.3. Simultaneous Saccharification and Fermentation

For SSF performance in this study, there were two treatments performed. The first treatment was made in fully aerobic conditions, while the second treatment with bioprocess engineering. Consortium of microbes' biomass growth during cultivation of SSF can be seen in Figure 1. The growth curve was used as a reference for determining the time to stop the aeration on the second treatment (ESSF).

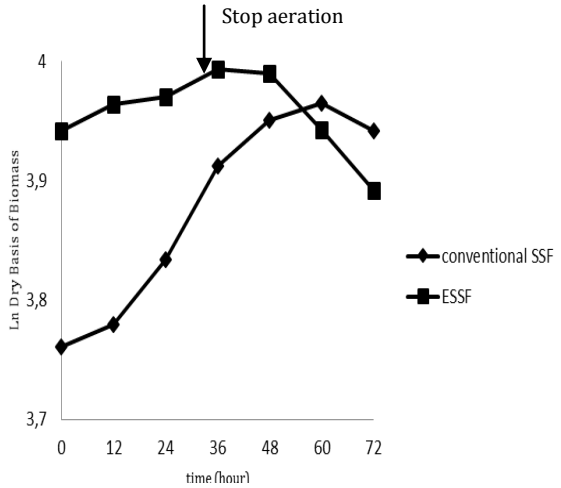

Figure 2 Biomass growth of microbes' consortium

In a batch system, the dry basis of biomass growth curve increases with time and decreases when the biomass reaches a maximum condition (Retledge and Kristiansen 2001). Adaptation occurred in the early of inoculation, then exponential phase at 24 – 36 h, further microbial growth slowed until the reaching of death phase at the end of the cultivation period. Based on the finding, the timing of the switching of aeration for the second treatment was at 36 h.

In ESSF, after switching condition into anaerobic, further microbial growth stationer at 36 – 48, until the reaching of death phase at the end of the cultivation period. Biomass in conventionalSSF and ESSF reached highest level after 60 h and 36 h of cultivation i.e. 52.73 g/L; 54.3 g/L, respectively. Supatmawati (2010) argued that at the same time with the substrate is approaching depletion, there is an accumulation of the metabolite products that decrease growth rate. Figure 3 shows several factors affecting the production of bioethanol. They were starch substrate, produced glucose, and the presence of microbes that catalyzed reactions during the production process. The maximum bioethanol obtained from SSF was 11.15 g/L. This was higher than the yield obtained by Supatmawati (2010) and Loebis (2008). They employed hydrolysis and fermentation techniques separately. Breadfruit starch through SSF techniques, could be used as a carbon source for growth of microbial consortia, in this case the growth of molds and yeasts.

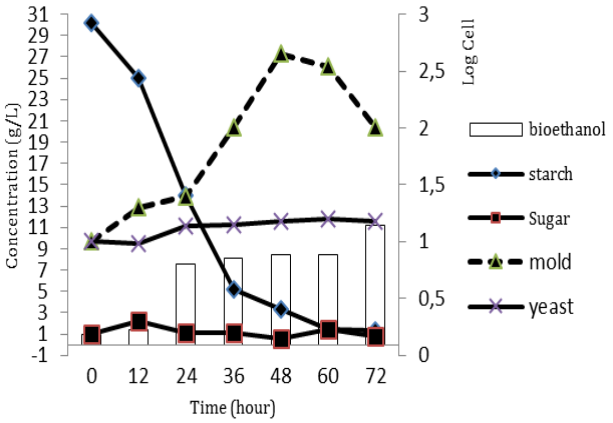

Figure 3 Bioethanol production during conventional SSF

In the process of starch saccharification, mold producing amylase, cellulase, or xylanase enzymes could change the components of the polysaccharide starch into glucose and other simple sugars. Figure 2 explains that the starch substrate concentration (30 g/L) decreased along with the growth of mold and the increase of sugar production. Nonetheless, in simultaneous technique, glucose and other simple sugars would be directly utilized by yeast to form a product and the cell.

Figure 3 shows that, in early inoculation yeast occurred at adaptation phase. At the same time, the production of sugar and bioethanol increased (1 g/L to 2.18 g/L) and (1 g/L to 1.6 g/L), respectively. After 12 h, yeast enterred into exponential phase, this resulted in decreased of sugar content while bioethanol increased (1.36 g/L to 7.56 g/L).

TPC results in Figure 4 shows the growth of molds and yeasts (microbes' consortium). It proved in *Ragi Tapai* there was more than one type of microbe. Azmi *et al*. (2009) reported that even though ragi tapai was mixed with several microorganisms, at the end of fermentation only the presence of yeast and mold were observed in the medium.

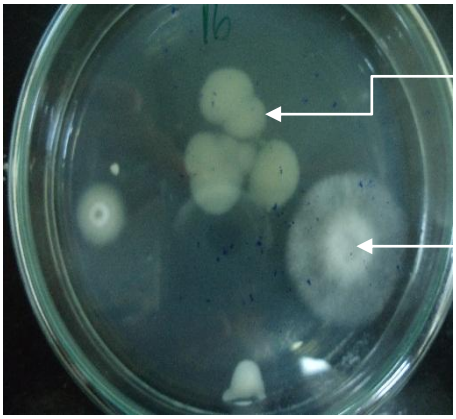

Figure 4 *Total plate count* result of microbes' consortium in the conventional SSF at 48 h

3.4. *Engineered SSF.*

In engineered SSF, bioethanol production was higher than without engineered (Conventional SSF). It can be seen in Figure 5, in which high bioethanol production, amounted to 12.75 g/L. The bioethanol production increased from 6.5 g/L at 36 h to 12.75 g/L at 72 h after switching condition from aerobic to anaerobic. After the switching condition, mold growth experienced stationary until death phase (at 36 – 72 h), while the growth of yeasts remained constant after 24 h. When oxigen was unavailable in the medium, mold that was absolute aerobic died. While for yeast, at the beginning of cultivation it required the oxygen for growing, so it lasted aerobic cultivation for doing respiration. After that, the formation of CO^2 would transform into an anaerobic reaction (Prescot and Dunn 1959).

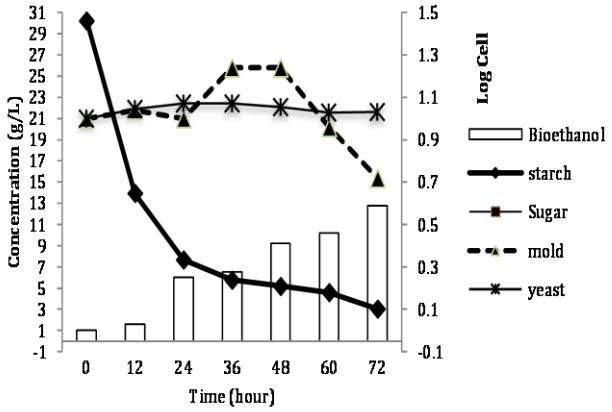

Figure 5 Bioethanol production during engineered SSF

Theoretically, in bioethanol fermentation which is anaerobic, one gram of glucose produces 0.51 grams of bioethanol (Kunkee and Mardon 1970). However, this result could not be achieved because there was a by-product produced during cultivation. In fact, only about 90-95% of the theoretical value that can be achieved (Underkofler and Hickey 1954). If the initial concentration of starch substrate of 30 g/L, theoretically it would produce glucose at 33.3 g/L. Of 33.3 g/L of glucose, it would stoichiometrically produce at maximum16.98 g/L of bioethanol. However, the highest bioethanol content could be produced by engineered SSF is 12.75 g/L, or only 75% of the possible maximum value.

Stationary phase of yeast occurred during 24 to 72 h. The growth of yeasts remained constant, because the yeast still can grow under anaerobic condition (anaerobic facultative). Yeast grows well forming cells under aerobic conditions. When the condition was switched into anaerobic, yeast produced bioethanol. This was evidenced by the growth of microbes with total plate count method at Figure 6.

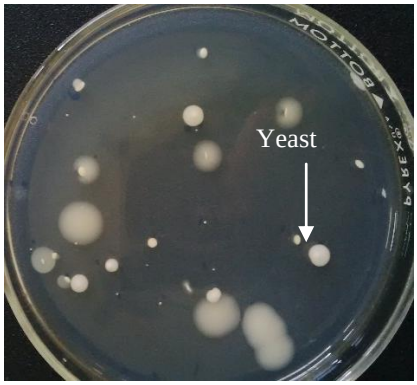

Figure 6 *Total plate count* result of microbes' consortium in the engineered SSF at 60 h

Total Plate Count in the engineered SSF (ESSF) at 60 h is shown in Figure 5. It shows the yeast grow dominantly rather than molds in anaerobic condition. TPC of mould decreased to the value of less than 10^3 CFU/ml.

3.5. The kinetics of cultivation in SSF

The data in Table 2 show cultivation kinetics calculations for conventional and engineered SSF. Specific growth rate (μ) was determined by calculating the slope of curve of ln X against time (hr). The maximum specific growth rates (μmax) in conventional SSF and ESSF are relatively equal, i.e 0.01/hour. Maximum specific growth rate was relatively low, probably because of culturing microbes in a low concentration of medium (Mangunwidjaja and Suryani 1994).

The yield of substrate used into biomass (Yx/s) for conventional SSF was 0.29 g biomass/g substrate, while the efficiency of substrate by 96%. Surya (2010) reported that the Yx/s in SSF utilizes waste of corn crop for 0.012 g biomass/g substrate, while its substrate utilization efficiency was 93%. It proves the potential of breadfruit starch used as a medium for bioethanol production by SSF technique using the microbes' consortium since the efficiency is quite high.

The value of Yx/s in engineered SSF (ESSF) which was lower than conventional SSF(0.09 g cell/g substrate) indicated that the microbes in engineered SSF utilized less substrate for biomass than that in conventional SSF. On the contrary, in ESSF more substrates in the medium was utilized to produce the products (bioethanol) as indicated by the value of Yp/s which was higher in ESSF (0.41 g bioethanol/g substrate) than that in conventional SSF (0.34 g bioethanol/g substrate). It proved that engineered SSF which switched the condition from aerobic into anaerobic condition has shifted the metabolism from respiration which produces more cell (less bioethanol) into fermentation which produce more bioethanol (less cell).

Value of Yp/s in this study was higher than studies by Subekti (2006), which implemented SHF technique (separated hydrolysis and fermentation), which was 0.154 g/g. Supatmawati (2010) reported that bioethanol produced from glucose syrup of sago was lower (10.12 g/L), but the Yp/s was higher (0.448 g/g). In engineered SHF, bioethanol was produced lower (10.69 g/L), but the Yp/s was higher (0.468 g/g) (Supatmawati 2010). SSF technique has more advantages; one of them is that the time required for the cultivation is lower than SHF. In addition, Engineered SSF technique produces bioethanol with the efficiency and yield are not different from SHF technique, or even higher.

Table 2
Results of the kinetics of fermentation in conventional and engineered SSF

SSF	μ max (/hour)	Yx/s	Yp/s	Yp/x	The substrates efficiency
Conventional	0.01	0.29	0.34	1.16	96%
Engineered	0.00	0.09	0.41	4.4	90%

4. Conclusions

The results of this study show that breadfruit starch is a good substrate for bioconversion into bio-bioethanol fuel, because it contains high level of starch (89%). The processes of bio-ethanol production from starch was previously carried out in three steps: hydrolysis, saccharification, and fermentation. But in SSF technique, the steps were only one, so the time required for cultivation is shorter, hence increase productivity. The use of the microbes' consortium in a simultaneous process was considered more efficient, because without adding or replacing the enzymes and microbes in each process. Engineered SSF technique offers process which has higher product yield (Yp/s) than conventional SSF.

The implementation of SSF technique using microbes' consortium produce the yield of substrate converted into product for 0.34 g/g, resulting in high substrate effieciency (96%). The combination of bioprocess engineering with SSF technique (engineered SSF)iscapable to produce bioethanol with Yp/s. The breadfruit starch has the potential as substrate for bio-ethanol, which in turn can improve the economy in rural areas in Indonesia.

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References

Adepeju AB, Gbadamosi SO, Adeniran AH, Omobuwajo TO. (2011). Functional and pasting characteristics of breadfruit (*Artocarpus artilis*) flours. *African Journal of Food Science*, Vol. 5(9), pp. 529-535, 15 September 2011.

Adinugraha HA, NK Kartikawati. (2004). Pertumbuhan Bibit Tanaman Sukun (*Arthocarpus altilis*) Hasil Perbanyakan secara Klonal di Persemaian. Yogyakarta [ID]: Prosiding Ekspose Hasil Litbang Bioteknologi dan Pemuliaan Tanaman Hutan.

Akanbi TO, Nazamid S, Adebowale AA, Farooq A, Olaoye AO. [2011]. Breadfruit starch-wheat flour noodles: preparation, proximate compositions, and culinary properties. International Food Research Journal 18: 1283-12897.

Akubor PI, Isolokwu PC, Ugbane O, Onimawo IA. [2000]. Proximate composition and functional properties of African breadfruit kernel and flour blends. *Food Res. Int.,* 33: 707-712.

[AOAC] Association of Official Analytical Chemists. [1995]. Official methods of analysis of the association of official of analytical chemist. Washington.

Azmi AS, Hasan M, Mei M. [2008]. Screening of microbes for producing ethanol from cassava starch. 15th *Regional Symposium of Malaysian Chemical Engineers (SOMChE)*, Vol I, pp. 177-181.

Azmi A, Hasan M, Mel M, Ngoh C. [2009]. Single-step bioconversion of starch to bioethanol by the coculture of ragi tapai and *Saccharomyces cerevisiae*. *Chemical Engineering Transactions* Vol 18. pp 557-562.

Balat M, H Balat, C. Oz. [2008]. Progress in bioethanol processing. *Progress Energy Combustion Sci.* 34: 551-573. DOI: 10.1016/J.PECS.2007.11.001.

Directorate General of New Renewable Energy and Energy Conservation Ministry of Energy and Mineral Resources. [2013]. Standar dan Mutu (Spesifikasi) Bahan Bakar Nabati (*Biofuel*) Jenis Bioetanol sebagai Bahan Bakar Lain yang Dipasarkan di dalam Negeri. Nomor 722 k/ 10/ DJE/2013.Availablehttp://www.ebtke.esdm.go.id/id/download/ doc_download/ 538-kepdirjen-standar-dan-mutu-bbn-jenis-bioetanol.html

Djien KS. 1972. Tape fermentation. *Applied Microbiology*, 23(5): 976 – 978.

Dwidjoseputro D, FT Wolf. [1970]. Microbiological studies of Indonesian fermented foodstuffs. *Mycopathol. mycol. Appl.* 41:211-222.

Gandjar I. [2003]. Tapai from cassava and cereals. *The first international symposium and workshop on insight into the world of indigenous fermented foods for technology development and food safety,* Kasetsart University.

Griffin HD. [1981]. *Fungal physiology*. New York: John Wiley and Sons, Inc.

Graham HD, de Bravo EN. [1981]. Composition of the Breadfruit. *J Food Sci* 46: 535-539.

Imam T, Capareda S. [2011]. Fermentation kinetics and ethanol production from different sweet sorghum varieties. *Int J Agric & Biol Eng* Vol. 4 Number 3.

Jenie BS, Putra R, Feri K. [2012]. Fermentasi kultur campuran bakteri asam laktat dan pemanasan autoklaf dalam meningkatkan kadar pati resisten dan sifat fungsional tepung pisang tanduk (*Musa paradisiacal formatypica*). *Jurnal pascapanen* 9 (1) 2012: 18-26. Bogor [ID]: Bogor Agricultural University.

Kunkee KD, CJ Mardon. [1970]. *Yeast in wine making*. London [UK]: Academic Press, London.

Loebis EH. [2008]. Optimasi proses hidrolisis kimiawi dan enzimatis tandan kosong kelapa sawit menjadi glukosa untuk produksi etanol. [Thesis]. Bogor [ID]: Bogor Agricultural University.

Loos PJ, Hood LF, Graham HD. [1981]. Isolation and Characterization of Starch from Breadfruit. *Cereal Chem*. 58(4): 282 – 286. Department and Institute of Science, Cornell University, Ithaca, NY 14853.

Mangunwidjaja D, Suryani A. 1994. *Teknologi bioproses*. Jakarta [ID]: Penebar Swadaya.

Merican Z, Quee–Lan Y. [2004]. *Tapai processing in Malaysia: A technology in transition*. Industrialization of Indigenous fermented foods, pp. 247-270, Marcel Dekker Inc., New York.

Nadir NM, Karim, RM Yunus, (2009). Comparison of sweet sorghum and cassava for ethanol production by using *Saccharomyces cerevisiae*. *J. Applied Sci*., 9: 3068-3073. DOI: 10.3923/jas.2009.3068.3073.

Pangloli. (1992). Potensi dan Pemanfaatan Sagu. Yogyakarta [ID]: Kanisius Press. 140p.

Prescott SC, Dunn. [1981]. *Industrial Microbiology*. Mc Grew Hill Book Co. Ltd, New York.

Rutledge C, Kristansen Bjorn. [2001]. *Basic Biotechnology*. Second Edition. Published by the press syndicate of the University of Cambridge. United Kingdom (US).

Rehm HJ, Reed G [1983]. Biotechnology Vol III. Industrial Microbiology. *AVI Publishing Company Inc*. Westport, Connecticut.

Saifuddin N, Hussain R. [2011]. Microwave Assisted Bioethanol Production from Sago Starch by Co-Culturing of Ragi Tapai and *Saccharomyces cerevisiae*. *Journal of Mathematics and Statistics* 7 (3): 198-206.

Sattler L, FW Zerban. [1948]. *The Dreywood anthrone reaction as affected by carbohydrate structure, Science*, 108:207.

Steve U, J Osuntogun, Bohanle A. [1995]. *Starch*. Volume 47, Issue 8, pages 289-294. Wiley-VCH Verlag GmbH& Co. KGaA, Weinheim.

Subekti H. [2006]. Produksi Etanol Dari Hidrolisat Fraksi Selulosa Tongkol Jagung Oleh Saccharomyces cereviseae. [Essay]. Bogor [ID]: Bogor Agricultural University.

Supatmawati. [2010]. Rekayasa bioproses produksi bioetanol dari hidrolisat pati sagu (Metroxylon sp.) menggunakan *Saccharomyces cerevisiae var. ellipsoids* pada kultivasi nir-sinambung dan semi sinambung. [Thesis]. Bogor [ID]: Bogor Agricultural University.

Surya L. [2010]. Produksi Bioetanol dari Limbah Tanaman Jagung melalui Sakarifikasi dan fermentasi simultan menggunakan biakan *Zymomonas mobilis* dan *Pichia stipitis*. [Essay]. Bogor [ID]: Bogor Agricultural University.

Syamsu K. [2008]. Rekayasa bioproses produksi bioetanol dari hidrolisat pati ubi jalar (*Ipomea batatas* L.) menggunakan *Saccharomyces cerevisiae*. *Laporan Badan Penelitian dan Pengembangan Departemen Penelitian*. Bogor [ID]: Bogor Agricultural University. pp 78.

Szymanowska D, Grajek W. [2011]. Energy-saving and by-products-free production of ethanol from granular corn starch. *BioTechnologia* vol. 92(1).pp 85-91.2011.

Underkofler LA, RJ Hickey. [1954]. *Industrial fermentation*. Chemical Publishing Co, New York.

USDA. [2004]. National Nutrient Database for Standard Reference, Release 16-1.

Widowati S, Richana N, Suarni, Raharto P, Sarasutha IGP. [2001]. Studi Potensi dan Peningkatan Dayaguna Sumber Pangan Lokal Untuk Penganekaragaman Pangan di Sulawesi Selatan. Bogor [ID]: Puslitbangtan Research.

Winarno FG. [1997]. *Kimia Pangan dan Gizi*. Jakarta [ID]: PT. Gramedia Pustaka Utama.