International Journal of Technology (2017) 5: 898-908
ISSN 2086-9614

BIOETHANOL PRODUCTION FROM TOFU WASTE BY SIMULTANEOUS SACCHARIFICATION AND FERMENTATION (SSF) USING MICROBIAL CONSORTIUM

Fitria Febrianti[1*], Khaswar Syamsu[2,] Mulyorini Rahayuningsih[2]

[1]*Major Program of Biotechnology, Graduate School, Bogor Agricultural University, Darmaga Campus, Bogor 16680, Indonesia*
[2]*Department of Agroindustrial Technology, Faculty of Agricultural Technology, Bogor Agricultural University, Darmaga Campus, Bogor, 16680, Indonesia*

(Received: July 2016 / Revised: June 2017 / Accepted: October 2017)

ABSTRACT

Tofu waste can be used as a raw material for bioethanol production due to its high carbohydrate content in the form of starch. A microbial consortium, consisting of *Aspergillus niger* and *Saccharomyces cerevisiae.* The study's first objective wasto capture the amount of sugar produced from starch hydrolysis using single cultures of *Aspergillus niger*.The study's second objective wasto determine the amount of ethanol produced by the SSF technique. *Aspergillus niger* was used to produce an amylase enzyme that hydrolyzes starch into simple sugar.Then, *Saccharomyces cerevisiae* was used to produce bioethanol from the sugar produced earlier.The synthesis of bioethanol consists of two main stages, hydrolysis and fermentation. In previous studies, the hydrolysis and fermentation processes were performed separatelyusing a separated hydrolysis and fermentation (SHF)technique. This studyprocesses via a simultaneous saccharification and fermentation (SSF) technique which produced higher substrate efficiency, cell yield, and product yield compared to the SHF process.The characterization process showed that tofu waste flour was mainly composed of carbohydrates, which comprised 54.04±0.03% (dw) and had a starch content of 39.23±0.20 (dw). Sugar from the starch of the tofu waste was produced by batch system cultivation for 84 hours using *Aspergillus niger*. The highest sugar production (14.48 g/L) was achieved during the 48th hour. Then, *Saccharomyces cerevisiae* was used to convert the produced sugar into bioethanol. The production of bioethanol by SSF using a microbial consortium for 72 hours was 7.69 g/L of bioethanol, with a yield of bioethanol per substrate use (Yp/s) of 0.23 g ethanol/g substrate and a substrate conversion efficiency of 88%.

Keywords: *Aspergillus niger*; Bioethanol; *Saccharomyces cerevisiae*; Simultaneous saccharification and fermentation; Tofu waste

1. INTRODUCTION

Indonesia has one of the highest rates of energy consumption in the world. Based on data from the Directorate General of New Renewable Energy and Energy Conservation in the Ministry of Energy and Mineral Resources (2014), Indonesia's fuel consumption increased from 297.8 million barrels of fuel in 2005 to 394.052 million barrels of fuel in 2014. In contrast to this increased consumption, Indonesia's fuel production has significantly declined in recent years. While Indonesia produced 268.5 million barrels of fuel in 2005, fuel production decreased by more than 50% to 122.9 million barrels in 2014.

[*]Corresponding author's email: fitriafebri@gmail.com, Tel: +62-51-8624067, Fax: +62-51-8624067
Permalink/DOI: https://doi.org/10.14716/ijtech.v8i5.872

Because ofthis shortage, fuel must be imported from overseas.Bioethanol is an alternative renewable energy resource.It can be produced from biomass that is categorized into sugar, starch, and lignocellulose-based material (Kelly et al., 2009). Tofu waste is a potential bioethanol feedstock since 59.95% of its contents are carbohydrates (Yustina& Abadi, 2012) and starch (Sudaryati et al., 2013). Tofu waste is abundantly available in Indonesia and has low economic value. Currently, tofu waste's utility is limited to feed material.

Starch-based material requires a hydrolyzing agent to convert the starch into simple glucose. Based on Zambare (2010), *Aspergillus niger* mold has significant potential to produce amylase and glucoamylase enzymes that can hydrolyze starch into sugar.The amylase family has two major classes: (1) dextrin, fructose, glucose, lactose, and maltose; and (2) starch enzymesconsisting of amylase (EC 3.2.1.1) and glucoamylase (EC 3.2.1.3). Amylase can hydrolyze starch into maltose and glucose,while glucoamylase (GA) can produce single glucose units.

Saccharomyces cerevisiae can potentially be used as a fermentation agent to convert glucose into ethanol.Based on Hossain et al. (2017), utilization of agricultural waste using *Saccharomyces cerevisiae*with SSF technique can increase bioethanol production.

SSF can provide the following advantages: (1) increase the speed of the hydrolysis process by sugar conversion; (2) reduce the enzyme requirement; (3) increase product yield; and (4) reduce the need for sterilization (Zhang et al., 2011).SSF technique using both *Aspergillus niger*and *Saccharomyces cerevisiae* can produce ethanol simultaneously from the accumulation of sugar that was produced from the starch-based material.

The synthesis of bioethanol from starch consists of two main stages, hydrolysis and fermentation. In previous studies, the hydrolysis and fermentation processes were performed separatelyusing a separated hydrolysis and fermentation (SHF)technique. According to Ali et al. (2011), an SSF technique using a microbial consortium is more effective than SHF (Separated Hydrolysis and Fermentation) technique. SSF can produce a higher ethanol yield which ismore productive than SHF (Dahnum et al., 2015).

Based on the study by Arnata and Dewi (2013), using a microbial consortium such as *Trichoderma* spp., *Aspergillus* spp., or *Saccharomycescerevisiae* in a medium of cassava starch at the beginning of the cultivation process can increase the ethanol content by 11% (w/v) and increase efficiency by 40% (w/v) compared to a monoculture of *Saccharomyces cerevisiae*.

Currently, producing bioethanol as biofuel is not competitive compared to conventional fuel due to its higher production cost. There are at least two strategies to reduce the cost of production: (1) usingcheap and abundant substrates; and (2) improvingprocess technology to increase yield and productivity.A microbial consortium, consisting of *Aspergillus niger*and *Saccharomyces cerevisiae,*was used instead of commercial enzymes to minimize production costs. In this study, the synthesis of enzyme derived from microorganisms without the addition of synthetic enzyme.

This study were to find (1) the pattern of *Aspergillus niger* growth; (2) determine the highest sugar production time during cultivation; and (3) analyze tofu waste as a potential medium of bioethanol production using an SSF technique and a microbial consortium.

2. EXPERIMENTAL

2.1. Preparation of Tofu Waste Flour as Media

One kilogram of wet tofu waste was squeezed to obtain a starch suspension and dried at 80ºCfor 9 hours in an oven. Then the tofu waste was milled using a food processor, and a flour of waste tofu was then tested in dry weight form. The tofu waste flour was characterizedthroughproximate

analysis (AOAC, 1995) ofthe starch, amylose, and amylopectin using the Anthrone method (Sattler and Zerban, 1948).

2.2. Preparation Cultures of the Microbial Consortium

The isolated cultures utilizedin this study (*Saccharomyces cerevisiae*(IPBCC.Y. 05.544)and*Aspergillus niger*(IPB.93.265.CCBS420.64)) were obtained from the Institut Pertanian Bogor Culture Collection (IPBCC) at Bogor Agriculture University. Isolates of *Aspergillus niger* were refreshed on a Potato Dextrose Agar (PDA) medium (Bratachem, Indonesia). The culture was incubated at 25^{o}C, for 5–7 days before inoculation.Isolates of *Saccharomyces cerevisiae* were refreshedon a PDA medium and incubated for 3 days. Isolates were grown on 50 ml of Yeast Malt Broth (YMB)propagation medium (Bratachem, Indonesia) consisting of 5 g/l yeast extract, 5 g/l malt, 10 g/l glucose, and 5 g/l peptone in a 200 ml Erlenmeyer flask. Incubation was performed in a 125 rpm shaker at room temperature (±30^{o}C) for 24 hours before inoculation.

2.3. Cultivation of *Aspergillus niger*

Cultivation of *Aspergillus niger* was carried out to determine the microbial growth curve. Biomass yield, residual starch, total sugar, and total plate count (TPC) from the cultivation process were used to identify the pattern of growth and the required time for highest sugar production. Tofu waste flour witha concentration of 10% (w/v) was dissolved in 1.2 liters of distilled water and then 10% (v/v) inoculum was added to the substrate. The cultivation process was carried out at room temperature by using 150 rpm agitation and 1 vvm aeration. A sample was taken every 12 hours for 84 hours.

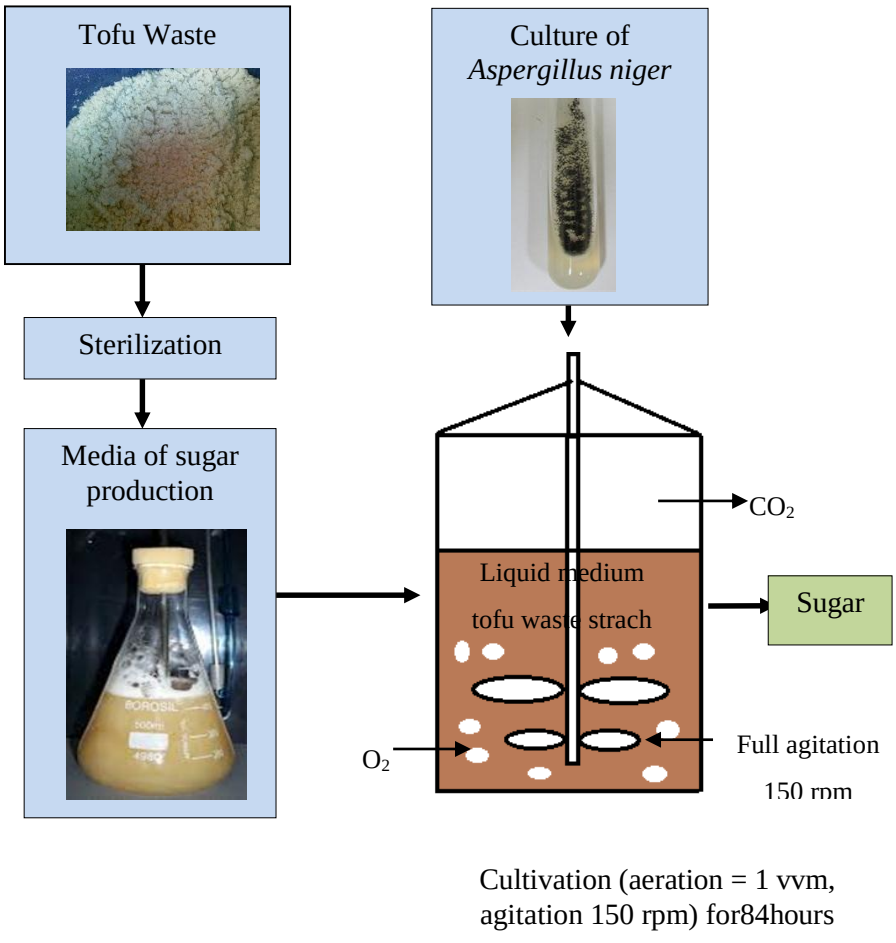

Figure 1 Schematic of *Aspergillus niger* cultivation

2.4. Simultaneous Saccharification and Fermentation (SSF)

Tofu waste flourwith a concentration of 10% (w/v) was dissolved in 1.2 liters of distilled water. Then, 10% (v/v) inoculum was added to the substrate. *Aspergillus niger* and *Saccharomyces cerevisiae*were inoculated at the beginning of cultivation. The bioreactor was in aerobic conditions;aeration and agitation were provided throughout the cultivation period. The cultivation process was carried out at room temperature by providing 150 rpm agitation and 1 vvm aeration. Biomass yield, residual starch, total sugar, TPC, and bioethanol content were measured every 12 hours for 72 hours. During cultivation by *Aspergillus niger*, sugar production was decline after 84th hour. Hence, SSF was carried out for 72 hours for bioethanol production to reduce processing time. *Chromatography* GC-17A Shimadzu LT-04-044 was used to identify the bioethanol content of the samples.

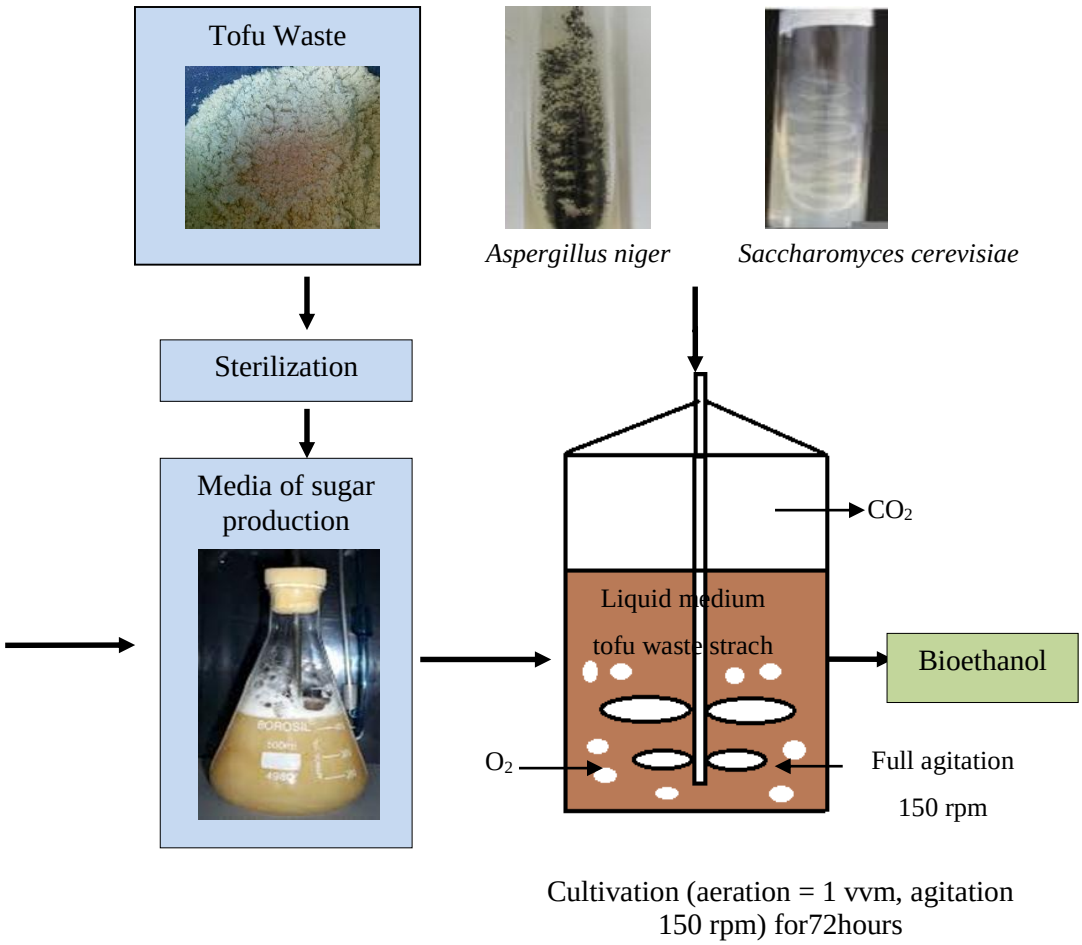

Figure 2 Schematic of Simultaneous Saccharification and Fermentation (SSF)

2.5. Cultivation Kinetic Parameters Measurement and Calculation

Samples from bioreactor were taken every 12 hours for 72 hours. Biomass yield, residual starch, total sugar, TPC, and bioethanol content were measured in the sample. The following kinetic parameters were measured and calculated as performance indicators of the cultivation process: totalof biomass produced (X), level of bioethanol produced (P), residual of starchsubstrate in the media (S), and maximum specific growth rate (μmax).The slope of the curve was obtained by plotting lnX against cultivation time (h) (Mangunwidjaja& Suryani, 1994; Farida et al., 2015).

The yield of biomass, bioethanol, product formation, and substrate efficiency were calculated per the following formulas.

Yield of biomass and bioethanol per substrate used:

$$Y_{x/s} = \frac{X - X0}{S_0 - S} \qquad Y_{p/s} = \frac{P - P0}{S_0 - S} \tag{1}$$

Yield of product formation of biomass:

$$Y_{p/x} = \frac{Pt - P0}{X - Xo} \tag{2}$$

Substrate efficiency:

$$(\Delta S/S0) = \frac{S0 - S}{S0} \times 100\% \tag{3}$$

3. RESULTS AND DISCUSSION

3.1. Characterization of Tofu Waste Flour

Drying was carried out to reduce the wet tofu waste's high water content. Reducing thesize of the tofu waste increases the surface area of the material, facilitating enzyme hydrolysis performance. In this study,1 kg of wet tofu waste produced 200–300 g of tofu waste flour. Tofu waste flour containsnutrients needed by microbes such as protein, fat, and glucose. The characteristics of tofu waste flour in proximate analysisare shown in Table 1.

Table 1 Proximate analysis of tofu waste flour

Component	Composition (%)	
	Literature	Result[d]
Water	5.74[a]	9.50 ±0.10
Ash (dw)	9.02[a]	8.16±0.20
Fat (dw)	14.49[a]	11.30 ±0.05
Protein (dw)	10.04[a]	17.02±0.02
Carbohydrate (dw)	59.95[a]	54.04±0.03
Starch	11.49[b]	39.23±0.20
Fiber	19.47[c]	12.38±0.10
Amylose	No literature	28.09±0.07
Amylopectin	No literature	73.00±0.10

These data have been processed. The mean ± standard deviation (n = 2); dw: dry weight
[a]Yustina& Abadi (2012), [b]Sudaryati et al. (2013),[c]Jenie et al. (2004), [d]Analysis Result.

Tofu waste is a perishable product due to its high water content. Environmental pollution will occur if the tofu waste is disposed of carelessly or without being processed. Tofu waste should be dried to reduce the water content, crushed, and sifted into aflour so it has more surface area and therebyinteract better with water and facilitate enzyme hydrolysis.

Tofu waste contains 39.23% of starch, a much higher proportion than the 11.49% of starch in soybean waste reported by (Sudaryati et al., 2013). This difference may be attributable to the duration of soybean harvesting. Starch content decreasesasharvest time increases. The plant enzymes able to hydrolyze starch into simple sugar also change over time (Winarno, 1997).

The amylose and amylopectin levels in the tofu waste flour in this study were 28.09±0.07% and 73.00±0.1%, respectively. The proportion of amylose and amylopectin from different sources vary depending on variety and growth location (Winarno, 1997). Protein was detected in about 17.02±0.02% (dw) of the tofu waste starch. Protein, one of the elements composing the cell membrane, is expected to be the main source of nitrogen for microorganism growth(Moore 1982).

Nitrogen is an essential macronutrient for the growth and formationof enzymes (Reed & Rehm, 1983). Protease enzymesare produced by *Aspergillus niger* (Parathaman et al., 2009).

3.2. Cultivation of *Aspergillus niger*

Figure 3 shows the growth of *Aspergillus niger* based on dry weightbiomass.The early phase of *Aspergillus niger*started at the 0 hourwhen the new culture was inoculated into the cultivation media.*Aspergillus niger* entered into the adaptation phase soon after inoculation. The microbial growth was low due to the new media environment during the adaptation phase. The lag phase followed the adaptation phase. The exponential phase occurredbetweenthe 24th and 48th hours. During this exponential phase, the microbes grew at their maximum growth rate.

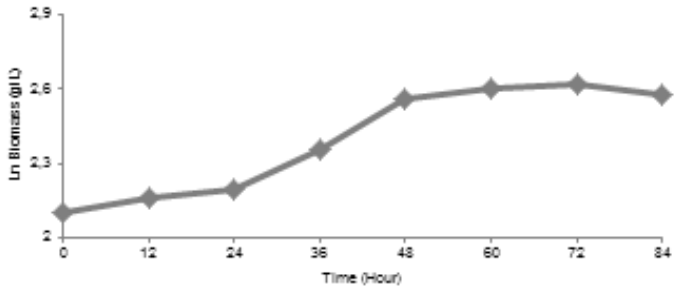

Figure 3 Growth pattern of *Aspergillus niger* during cultivation

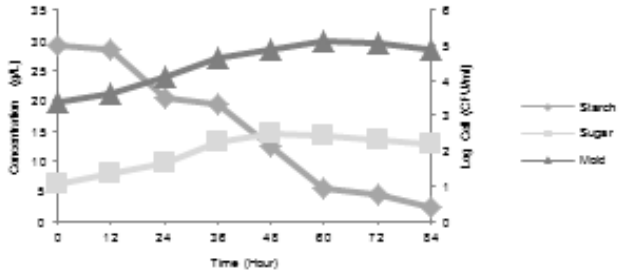

Figure 4 Cultivation results by the batch system of tofu waste using *Aspergillus niger*

During hours 0–12 (the adaptation phase), sugar production in*Aspergillus niger*increased slowly. During this adaptation phase, the α-amylase enzyme formed as a catalyst for starch hydrolysis (Ezugwu et al., 2015). Sugar production increasedrapidly along with the growth of *Aspergillus niger* from the 12th hour until the 48th hour.

The amylase and glucoamylase enzymes wereproduced by *Aspergillus niger*and cut off the polymer chains of starch into simplemonomer units. Starch concentration decreased along with mold growth as sugar production increased.*Aspergillus niger*growth reached the end of the exponential phase at the 48th hour. The maximum concentration of sugar achieved was 14.42 g/L.*Aspergillus niger*also consumes fats (Falony et al., 2006) and protein (Srinubabu et al., 2007).If polysaccharide content in the cultivation media is low, residual starch is reduced drastically.

During the cultivation process, the level of residual starch decreasesdue to the *Aspergillus niger*'s metabolic activity,which produces hydrolysis enzymes such as amylase (Bedan et al., 2014),glucoamylase (Parbat &Barkha, 2011), and invertase (Veanna et al., 2011). Starchof tofu waste, therefore, can be used as a carbon source for the growth of *Aspergillus niger*to producesugar. In addition,*Aspergillus niger*can also produce cellulase enzymes (Jayant et al., 2011). Hence, the fiber in tofu waste can also be used by *Aspergillus niger* to produce sugar.

3.3. Simultaneous Saccharification and Fermentation (SSF)

Bioethanol was produced from tofu waste starch via an SSF technique that used a microbial consortium consisting of *Aspergillus niger* and *Saccharomyces cerevisiae*. The *Aspergillus niger*functioned as a saccharification agent to convert starch into sugar; this sugar is subsequently converted through fermentation into bioethanol by the *Saccharomyces cerevisiae.* According to Nadir et al. (2009), using starch and an SSF technique with a mixture of bacteria is more effective at producing bioethanol than replacing the microbes or adding enzyme at each stage of the process. Bioethanol production from abundant raw materials can be obtained throughout the cultivation time, with lower costsand shorter processing times that increase productivity.

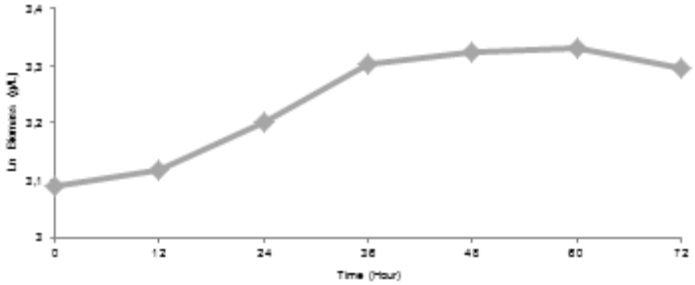

Figure 5 Growth of microbial consortium biomass during SSF

The cultivation process was carried out with full aeration of 185e vvm (Jagani et al., 2010). Aeration is needed for a group of molds and yeasts to produce cells. Oxygen in aerobic cultivation is the main factor affecting a microorganism's survival. The aeration process cannot be separated from the agitation process. The air flow from the compressor entered the medium to support aeration and agitation.

The exponential phase of microbial consortium occurredbetween the 24th and 48th hours. This mold phase is important because cell activity increases significantly.Enzymes can be harvested at the beginning of the exponential phase (Bedan et al., 2014). The results prove that enzyme accumulation occurred in this phase.

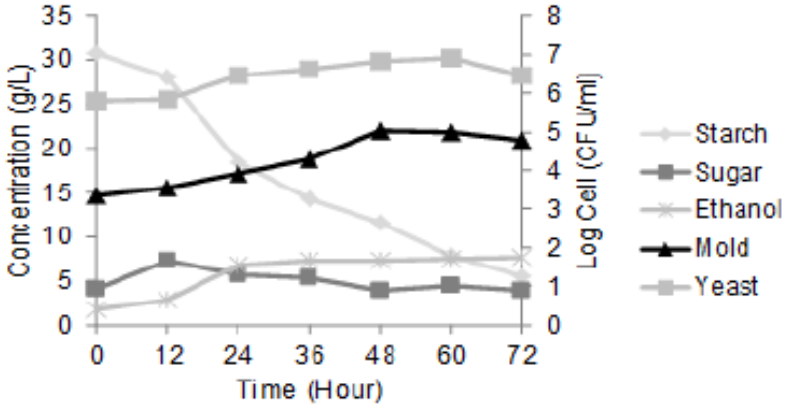

Figure 6 Cultivation results by SSF using a microbial consortium

The maximum bioethanol concentration, which was obtained during the 72nd hour, was 7.69 g/L. *Saccharomyces cerevisiae*cell numbers increased significantly until the 24th hour. *Saccharomyces cerevisiae*converted sugar into bioethanol by fermentation in addition to respiration. Sugar concentration from starch hydrolysis increased until the 12th hour, after which it began to decline, while yeast growth began to increase. These changes indicate that the sugar substrate had been

consumed by the mold and yeast for cell production and by the yeast for bioethanol formation. The ethanol concentration increased from 2.81 g/L at the 12th hour to 6.82 g/L at the 24th hour.

Starch hydrolysis activity was high because mold also needs sugar as a carbon and energy source for growth. Energy needs decreased since mold experiences sporulationat the end of fermentation. However, microbes are able to survive by consuming the remains of substrated and dead microbes because mold cell walls contain cellulose and chitin (Sharp, 2013), while yeast contains protein and manan (Huang, 2008). According to Moore (1982), the hyphae of mold can absorb simple molecules such as sugars, while the more complex forms of polymers such as cellulose, starch, and protein will be processed outside the cells using their extracellular enzymes.

The TPC results show the growth of molds and yeasts in the microbial consortium, which proves that both microbes can grow when tofu waste starch is used as a cultivation media. The cell doubling time of the mold was every 2–6 hours, while the yeast doubled every 20–120 minutes(Laskin, 1977). The maximum growth of molds was 5.4 log cell, which occurred at the 48th hour, while the maximum growth of the yeast was 6.90 log cell, which occurredat the 60th hour.

A microbial consortium consisting of*Aspergillus niger* and*Saccharomyces cerevisiae*was used for bioethanol production.*Saccharomyces cerevisiae*cannot produce hydrolase enzymes that canbreak down starch into glucose (Zhang et al., 2006). Hence, *Aspergillus niger* is required to produce amylolytic enzymes (Itelima et al., 2013). The amylase and glucoamylase enzymesin *Aspergillus niger*can be used to hydrolyze starch. *Saccharomyces cerevisiae*is required to convert the resulting sugar into ethanol.

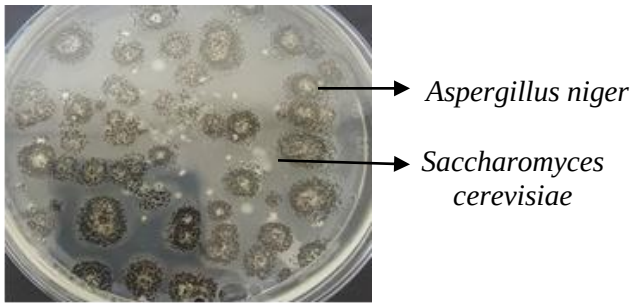

Figure 7 *Aspergillus niger* and *Saccharomyces cerevisiae* during SSF at the 72ndhour

3.4. Kinetics of Cultivation

Kinetics of cultivation can be used to describe cells' ability to respond to the environment. The yield of bioethanol per substrate uses (Yp/s), defined as the amount of nutrients used by microorganisms to form the product, was 0.24 g ethanol/g substrate. The yield of biomass per substrate (Yx/s), defined as the amount of substrate used by microorganisms to form cells, was 0.23 g biomass/g substrate. This result was higher than the study results of Pramashintaand Abdulah (2014), who produced 0.016 g biomass/g substrate from pineapple skin waste over 60 hours using *Saccharomyces cerevisiae*.

The yield of product formation per biomass (Yp/x) was 0.93 g ethanol/g biomass. The efficiency of the substrate (ΔS/S0) used in this study was 88%, higher than that achieved by Arnata and Dewi (2013), who obtained 82.92% from cassava. This result indicates that synthesizing bioethanol from tofu waste starch using an SSF technique and a microbial consortium to form biomass cells and/or products produces a high-efficiency substrate. The above parameters are important to describe the efficiency of product formation during fermentation,which is related to the optimalamount ofsubstrate converted into cells and product. The efficiency of product formation is generally expected to achieve maximum results from the existing substrate.

The synthesis of bioethanol from starch consists of two main stages, hydrolysis and fermentation. In previous studies, the hydrolysis and fermentation processes were performed separatelyusing a separated hydrolysis and fermentation (SHF)technique. This study, in contrast, carried out these processes via a simultaneous saccharification and fermentation (SSF) technique which produced higher substrate efficiency, cell yield, and product yield compared to the SHF process. According to Zhang et al. (2011) who worked with a potato substrate, ethanol production for the SSF technique was 110 g/L, while production with the SHF technique was 55 g/L. Separating the saccharification and fermentation stages as in the SHF technique results in amylase not reaching its maximum performance due to enzyme inhibition because of sugar accumulation. If amylase is inhibited, the saccharification process will stop even if the available starch has not yet been fully converted into sugar. The SSF technique has additional advantages because polysaccharides which have been converted into monosaccharides are not returned to polysaccharides, but can be directly fermented into ethanol.

4. CONCLUSION

This study's results clearly show thattofu waste is a viablemedium for bioethanol production.Tofu waste has a high carbohydrate content of 54.04±0.03% (dw), with 39.23±0.2% (dw) in the form of starch.*Aspergillus niger*achieved a peak sugar productionsof 14.42 g/L during the 48th hour of cultivation.

The processes of bioethanol 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. Beside that, the SSF technique can reduce the number of enzymes neededand reduce the need for sterilization. The SSF technique has proven to be superior in productivity and process time reduction compared to the SHF technique. The implementation of SSF technique using*Aspergillusniger* as the saccharification agent and *Saccharomyces cerevisiae* as the fermentation agent simultaneously produced 7.69 g/Lethanol.

5. REFERENCES

Ali, N., Mazharuddin, K., Majid, M., 2011. Ethanol Fuel Production through Microbial Extracellular Enzymatic Hydrolysis and Fermentation from Renewable Agrobased Cellulosic Waste.*International Journal of Pharma and Bio Sciences*, Volume 2(2), pp. 321–331

Arnata, I.W., Dewi, A., 2013. Bioprocess Engineering of Bioethanol Production from Cassava with Yeastand *Saccharomyces cerevi siae* Culture Technique.*Agrointek*, Volume 7(1), pp. 21–28

Association of Official Analytical Chemists (AOAC), 1995.*Official Methods of Analysis of the Association of Official of Analytic Chemist*, Washington, USA

Bedan, D.S., Aziz, G,M., Ali, A., 2014. Optimum Conditions for α-amylase Production by *Aspergillus niger* Mutant Isolate using Solid State Fermentation.*Current Research in Microbiology Biotechnology*, Volume 2(4), pp. 450–456

Dahnum, D., Sri,O.T.,Eka,T.,Muhammad, N., Haznan, A., 2015.Comparison of SHF and SSF Processes using Enzyme and Dry Yeast for Optimization of Bioethanol Production from Empty Fruit Bunch.*Energy Procedia Elsevier*, Volume 68, pp. 107–115

Directorate General of New Renewable Energy and Energy Conservation Ministry of Energy and Mineral Resources, 2014.*Indonesia's Fuel Production and Consumption*

Ezugwu, A.L., Eze, S.O.O, Chilaka, F.C., 2015. A Study of the Optimal Conditions for Glucoamylases Obtained from *Aspergillus niger* using Amylopectin from Cassava Starch as Carbon Source.*African Journal of Biotechnology*,Volume 14(3), pp. 2693–2702

Falony, G., Lose L., Martinez, H.,2006.Production of *A. Niger*Lipase.*Food Technology and Biotechnology*,Volume 44(2),pp. 235–240

Farida, I., Khaswar, S., Mulyorini, R., 2015. Direct Bioethanol Production from Breadfruit Starch (*Artocaspuscommunis Forst*) by Engineered Simultaneous Saccharification and Fermentation (ESSF) using Microbes Consortium.*International Journal of Renewable Energy Development*, Volume 4(1), pp. 25–31

Huang, G.L., 2008. Extraction of Two Active Polysaccharidesfrom the Yeast Cell Wall*Z.Naturforsch*,Volume63c, pp.919–921

Hossain, N., Zaini, J.H., Mahlia,T.M.I, 2017. A Review of Bioethanol Production from Plant-based Waste Biomass by Yeast Fermentation.*International Journal of Technology*, Volume 8(1), pp. 5–18

Itelima, J., Ogbonna, A., Pandukur, S., 2013.Simultaneous Saccharification and Fermentation of Corn Cobs to Bio-ethanol byCo-culture of *Aspergillus Niger* and *Saccharomyces Cerevisiae*.*International Journal of Environmental Science and Development*, Volume 4(2), pp. 239–242

Jagani, H., Karteek, H., Sagar, S., Gang, P., Vasanth, R., Raghu, C.H., Venkata, J.R., 2010. An Overview of Fermenter and the Design Considerations to Enhance its Productivity.*Pharmacologyonline*, Volume 1, pp 261–301

Jayant, M., Rashmi, J., Shailendra, M., Deepesh, Y.,2011.Production of Cellulase by Different Co-culture of *Aspergillus niger* and *Penicillium chrysogenum*from Waste Paper, Cotton Waste and Baggase.*Journal of Yeast and Fungal Study*, Volume 2(2), pp. 24–27

Jenie, B.S.L., Ridawati., Winiati P.R.,2004. Angkak Production by Monascus Purpureus in Tapioca Liquid Waste Medium, Tapioca Pulp and Tofu Waste.*Buletin Teknologi dan Industri Pangan*, Volume 5(3),pp. 60–64

Kelly, R., Lubbert, D., Hans, L., 2009. Starch andGlucan Acting Enzymes, Modulating their Properties by Directed Evolution.*Elsevier Journal of Biotechnology*, Volume 140(3-4), pp. 184–193

Laskin, A., 1977. *Biosystem Poised for Growth*. Paper presented at a meeting of the Commercial Development Association, held at Homestead, Virginia, USA

Mangunwidjaja, D., Suryani, A., 1994. *Bioprocess Engineering*. Penebar Swadaya, Jakarta, Indonesia

Moore, L.E.,1982.*Fundamentals of the Fungi*, Englewood Cliff, New Jersey, Prentice Hall

Nadir, N., Mel, M., Mia, K., 2009.Comparison of Sweet Sorghum and Cassava for Ethanol Production by using *Saccharomyces cerevisiae*.*Journal of Applied Science*, Volume 9, pp. 3068–3073

Parathaman, R., Alagusundaram, K., Indhumathi, J.,2009.Production of Protease from Rice Mill Wastes by *Aspergillus niger*in Solid State Fermentation.*World Journal of Agricultural Sciences*,Volume 5(3), pp. 308–312

Parbat, R., Barkha, S.,2011.Production of Glucoamylase by *Aspergillus oryzae*under Solid StateFermentation using Agro IndustrialProducts.*International Journal of Microbiological Study*, Volume 2(3), pp. 204–207

Pramashinta, A., Abdullah,2014.Kinetics of Pineapple Skin Waste Fermentation and Ethanol Productivity.*Methane*, Volume 10(31), pp. 12–17

Reed,G., Rhem, H.J.,1983.*Biotechnology Volume III.Industrial Microbiology*, Westport, Connecticut, AVI Publishing Company

Sattler, L. Zerban, F.W., 1948. The Dreywood Anthrone Reaction as Affected by Carbohydrate Structure. *Science*,pp. 108–207

Sharp, R.,2013.A Review of the Applications of Chitin and its Derivatives in Agriculture to Modify Plant-microbial Interactions and Improve Crop Yields.*Agronomy Journal*, Volume 3, pp. 757–793

Srinubabu, G., Lokeswari, N., Jayaraju, K.,2007.Screening of Nutritional Parameters for the Production of Protease from *Aspergillus oryzae.E-Journal of Chemistry*, Volume 4(2),pp. 208–215

Sudaryati., Mulyani, T., Setiawan, E.B.,2013.Study of the Substitution Tofu Waste and the Use of Sodium Bicarbonate in Tortilla Making.*Food Technology Program*, FTI UPN Veteran, Indonesia

Veanna, F., Aguilar, C.N., Rodríguez,R., 2011. Kinetic Studies of Invertase Production by Xerophilic *Aspergillus* and *Penicillium*Strains under Submerged Culture.*Micología Aplicada International*, Volume 23(2), pp. 37–45

Winarno, FG., 1997.*Food and Nutrition Chemicals*. PT. Jakarta, Indonesia, Gramedia Pustaka Utama

Yustina, I., Abadi, F.R.,2012.*The Potential of Flour from the Pulp of Soybean Processing Industry as Foodstuff*.National Symposiumof Food and Energy. Agriculture Faculty of Trunojoyo Madura, Indonesia

Zambare, V.,2010.Solid State Fermentation of *Aspergillus oryzae* for Glucoamylase Production on Agro Residues.*International Journal of Life Science*, Volume 4, pp. 16–25

Zhang, Y.P.H., Schell, D., McMillan, J.D.,2006. Methodological Analysis for Determination ofEnzymaticDigestibilityofCellulosicMaterials.*Biotechnology and Bioengineering*, Volume 6(1), pp. 188–194

Zhang. L., Hai, Z., Mingzhe, G., Yanlin, J., Xiaofeng, G., Qian, C., Jiafa, G., Zhongyan, W., 2011. Application of Simultaneous Saccharification and Fermentation (SSF) from Viscosity Reducing of Raw Sweet Potato for Bioethanol Production at Laboratory, Pilot and Industrial Scales.*Bioresource Technology*, Volume 102(6), pp. 4573–4579