Scientific Bulletin. Series F. Biotechnologies, Vol. XXI, 2017
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

EFFICIENCY OF POWDER INOCULUM AND MICROORGANISM ENCAPSULATION ON HYDROLYZATE SUGAR FERMENTATION OF NEWSPAPER CELLULOSE FOR BIOETHANOL PRODUCTION

Ratu SAFITRI[1,2*], Mia MIRANTI[1], Ismi Dwi ASTUTI[1], Lien KARTIAWATI[1], Jetty NURHAYATI[1]

[1]University Padjadjaran, Faculty of Mathematics and Natural Sciences, Department of Biology, Jl. Raya Bandung Sumedang Km. 21, Jatinangor, Jawa Barat 45363, Indonesia
[2]Post Graduate School Universitas Padjadjaran, Program Study Of Biotechnology, Jl. Dipati Ukur no. 35, Bandung, Indonesia

*Corresponding author email: ratusafitrie@yahoo.com

Abstract

Immobilization of cells is a method for placing the microbial cells on a carrier material, wherein the catalytic activity of the cells is still high after storage for a long periode of time. The purpose of this study was to determine the optimum formula for the carrier material inoculum for bioethanol fermentation. The carrier material tested were based on different combination of wheat flour: rice flour, tapioca starch: corn starch and alginate. The study consisted of three phases: First phase is the preparation of the encapsulation of Zymomonas mobilis, Kluyveromyces marxianus, and Saccharomyces cerevisiae. The Second phase is the enzyme hydrolysis process and the third is fermentation of the hydrolysate into bioethanol by the consortium on the best carrier. The method used in this research is descriptive and experimental. Descriptive method carried out in the second phase. While the experimental method with completely randomized design is used in the research phase I and III. The consortium used were KI (Z.mobilis and S.cerevisiae), K2 (Z. mobilis and K marxianus), and K3 (Z. mobilis, S.cerevisiae and K. marxianus). The data were statistically analyzed by ANOVA, and followed by Duncan Test in 5% significance level. The results showed that S.cerevisiae and K.marxianus on formula with a combination of wheat flour: rice flour is the best carrier material with cell density about 3,719x10^{10} CFU / ml and 3,027x10^{10} CFU / ml. Meanwhile, alginate is the best carrier for Z.mobilis with cell density about 3,576x10^{10} CFU / ml. Best consortium in the process of bioethanol fermentation from cellulose waste is Z.mobilis and S.cerevisiae (K1), which have the highest ethanol concentration about 7.167%, the efficiency of fermentation about 61.2%, specific growth rate of 0,072 cells / hour, ethanol yield ($Y_{p/s}$) 0,23 g/g, microbial yield ($Y_{x/s}$) 0,33 g/g and a maximum ethanol productivity (q_p) of 0,58 g/g/h.

Key words: *Fermentation, Encapsulation, hydrolysis enzyme, carrier material, consortium.*

INTRODUCTION

Bioethanol is one form of renewable energy, derived from biological sources, it is environmentally friendly because has a high oxygen content (35%) burning more completely and high value-octane, producing lower CO emissions (19-25%), (Kusumaningati et al., 2013). Waste paper with high cellulose contain can be used as raw material for bioethanol as it as a monomer constituent is glucose that can be fermented into ethanol. Based on the test results on the chemical composition of newspaper used which conducted in industrial engineering of agriculture faculty laboratories in Padjadjaran University, old newspaper used containing cellulose 43.17%, 27.18% hemicellulose, lignin 16.11%, 11.27% starch content, and content of extractive 2,27%. In the manufacture of bioethanol, there are two processes that need to be considered, hydrolysis and fermentation. Hydrolysis is the process breaks down complex sugars into reducing sugars which classified as simple sugars. Fermentation is the process of breaking down sugars into alcohol and carbon dioxide which caused by the activity of microbial cells. Microorganisms that ferment bioethanol should be able to ferment the monosaccharide in the media. Microorganisms which most often used for bioethanol fermentation process is *Saccharomyces cerevisiae, Kluyveromyces marxianus and Zymomonas mobilis.* However, the microrganisms that have relatively short lifetimes will be an obstacle in large-scale ethanol production, so we need a way to get catalytic activity of the microrganisms remains high in the long term. Immobilization of cells is a

method for placing of microbial cells in a certain space with a long period of time, in which the catalytic activity of the cells is still high. One of the cell immobilization technologies is dried culture. There are several techniques for obtaining dried cultures: spray-dried, freeze-dried and the powder culture. Methods powder culture is the most efficient method because it does not require elaborate preparation (Pumphan at al., 2013). Drying using vacuum drying is one method that can be applied for preservation of microorganisms in powder form. Method of drying by vacuum drying to maintain the viability of microorganisms when using the appropriate heating medium and performed under conditions appropriate to the nature of the dried microorganisms, (Goderska, 2012). Aside from the powder method of culture, there is also a method of encapsulation with alginate. The advantage of the alginate encapsulation process that alginate can form a semipermeable membrane that will protect the microorganisms from the environment is less supportive. The carrier is one of the success factors of immobilized cells (Devi, 2014), this study used a carrier material consists of a combination of wheat flour and rice flour, tapioca flour and a combination of corn flour and encapsulation with alginate. Three formulas of carrier material were considered to meet the requirements of the good carrier material nontoxic to the inoculant, the moisture capacity is relatively good, pH neutral, cheap, easily processed and available (El-Fattah et al., 2013). High starch content in the starch and corn flour will protect the microorganisms from high temperature during vacuum drying. The combination of different types of flours as a carrier material more effectively protect the microorganisms compared to one type of flour, because the nutrients in combination flour can support the growth of microbial cells more. Starch is composed of 70-80% amylopectin which has a high ability to bind water product, so that microbial cells are trapped and protected during the heating process will be higher (Alonso, 2016). Alginate has a high fiber and essential minerals that can be used as a carrier matrix of active compound (Goderska, 2012). The principles of encapsulation are that the nutrients and metabolites can be diffuse through the membrane. The membrane serves as a barrier keep the contents of the cell and minimize contamination entering (Goderska, 2012). Until now, the uses of microorganism inoculum encapsulation is exclusive to the food products. It is expected from this study to be a significant step to help bioethanol entre-preneurs by providing ready-made microbial culture powder which can be stored longer and Its activities remains high.

MATERIALS AND METHODS

Materials

Materials used in this study are: alginate, CaCl2 2%; α-amylase; amyloglucosidase; hemicellulase; cellulase; Strains of *K. marxianus, S.cerevisiae,* and *Z. mobilis,* DNS reagent (3,5-dinitrosalicylic acid); potassium dichromate, Wheat flour, Rice flour, Tapioca flour, Cornstarch, YEPD (yeast extract peptone dextrose).

Methods

The method used in this research is descriptive and experimental methods carried out in a laboratory scale. This study includes three stages. Phase I is the stage of manufacture and encapsulation of microbial inoculant powder, III phase is the fermentation using immobilized microbial cells in the best carrier material. Research stages I and III were analyzed with experimentally. I phase is done with a completely randomized design (CRD), factorial design 3x3 with three replications. The first factor is microbial fermentation (M) and the second factor is the formulation of the carrier material (F). Phase III study conducted by completely randomized design (CRD) 3x7 factorial design with three replications. The first factor is a consortium of microbes in the carrier material (M) and the second factor is the sampling time (T) comprising of 11 degree. The results were then analyzed by analysis of variance (ANOVA). If they are different, we continue with Duncan Multiple Range Test at 5% significance level. This study includes three stages. The First Stage is manufacture and encapsulation of microbial inoculant powder, the second stage is the preparation of the substrate and hydrolysis process, and the third stage is a stage of fermentation using microbial

cells immobile in the best carrier material. Fermentation study conducted with a completely randomized design (CRD) 3x7 factorial design with three replications. The first factor is a consortium of microorganisms in the carrier material (M) and the second factor is the sampling time (T) comprising of 7 levels. The results were then analyzed by analysis of variance (ANOVA) and Duncan's Multiple Range Test at 5% significance level.

Preparation of inoculum Powders

Formula 1 is composed of rice flour and wheat flour and formula 2 consists of corn starch and tapioca flour with a ratio respectively of 50%: 50%. Carrier material is added sterile distilled water at a ratio of 1:10. Microbial suspension is added to the carrier material (1: 1). Cultures were then incubated for 6 days. Viability of microorganisms was performed every 2 days by counting the microbial population. Microbial cultures are then dried using vacuum drying at 45°C for 9 hours.

Encapsulation of Microbial cultures

The density of Microbial biomass suspension is 5 McFarland which has been calculated using total plate count (TPC) as the initial biomass. Furthermore, the microorganisms are suspended in 3 ml to 10 ml of sterile saline, then added 60 ml Alginate 3% (w / v). The mixture obtained is dripped into a solution of 0.1 M CaCl2, thus forming a bead. The beads will harden within 15 minutes. Beads were washed with 0.85% NaCl solution and to reduce excess Ca ions and washed again with distilled water. Bead was stored at a temperature of 4 ° C and is ready for use as an inoculum.

Pretreatment of Newspapers used as cellulosic waste and Enzymatic Hydrolysis

Desizing process of newpaper used is done by soaking the paper in water, then dried in the sun and ground into powder paper with ± 40 mesh sizes. Then delignification process was made by adding 4% NaOH solution. The mixture is allowed to stand for 24 hours, and then rinsed with distilled water. Flour paper dried at 105°C for 6 hours was refined by grinding.

Enzymatic hydrolysis consists of liquefaction by α-amylase enzyme hydrolysis of hemicellulose with hemicellulase, and saccharification with cellulase enzymes and amyloglucosidase combined. The paper flour was mixing with: α-amylase enzyme (0.52 mL/g), hemicellulase enzyme (0.001 g/g), cellulase enzyme (0.83 mL/g) amyloglucosidase doses (0, 56 mL/g) and a reducing sugar content set at 20% is used as a substrate for fermentation. Then into the substrate medium is added (NH4) 2SO4 4% (w/v) and peptone 1% (w/v). A total of 200 ml of substrate is put into containers and sterilized.

Fermentation of hydrolyzate by Microbial Consortium in Materials Carrier Fermentation was conducted by SSF (Simultaneous Saccharification Fermentation) using microbial consortia in the best carrier at phase 1, which were a consortium I (*Z.mobilis* and *S. cerevisiae), Consortium II (Z. mobilis* and *K marxianus)*, and consortium III (*Z. mobilis, S.cerevisiae* and *K. marxianus).* Inoculum in the form of powder is added as much as 10% (g/v) into the fermentation substrate. The mixture was fermented at a temperature of 28°C for 120 hours with agitation speed of 150 rpm. During the fermentation, samples were taken every 12 hours to measure parameters of a reducing sugar, ethanol and the number of microbial populations.

RESULTS AND DISCUSSIONS

Viability of the microorganism in the inoculum powder and encapsulated in alginate Formulation 1 was inoculum in a carrier material mixture of rice flour and wheat flour containing *S. cerevisiae, Z. mobilis,* and *K. marxianus.* Formula 2 was the inoculum in a carrier material mixture of cornstarch and tapioca starch containing *S. cerevisiae, Z. mobilis,* and *K. marxianus.*

Table 1. The viability of cells in culture inoculum powder in Formula 1 and Formula 2

Species of microorganism	Cell density (CFU/ml) of Microorganism in formula 1 (a mixture of rice flour and wheat flour)	Cell density (CFU / ml) of Microorganism in formula 2 (a mixture of corn flour and tapioca flour)
S.cerevisiae	3,36x10^{10}	3,42 x10^{10}
Z.mobilis,	3,37x10^{10}	3,17x10^{10}
K.marxianus	3,63x10^{10}	3,17x10^{10}

Furthermore, each inoculum in the carrier material is incubated for 6 days, the incubation process aims to adapt and grow microorganisms in each formula. The results showed that all microorganisms can adapt well to the formula 1 and 2. It showed by the increasing number of microbial populations during the incubation process. According to Alonso (2016), a carrier material should also serve as a medium for microbial growth. After cultivated for 6 days, the inoculum powder was then dried using vacuum drying. High cell densities in the inoculum powder showed that microorganisms can adapt well in formula 1 and formula 2. After drying, the density of microbial cells decreased, this is caused by heating during the drying process, the water content in formula reduced carrier material and microorganisms that are not bound to the matrix formula death. Microorganisms with most decreased cell density, is *Z.mobilis* in formula 1 and 2, with the percentage of the decline reached respectively 73.9%, 82.6%. In formula 1 and 2 cell density for *K.marxianus* was decreased reached at respectively 36.9% and 38%. Meanwhile, *S.cerevisiae* in formula 1 had the lowest decrease in the cell density of about 20.6%, but in the formula 2, *S.cerevisiae* has decreased by about 50%. These results indicate that *Z.mobilis* is more vulnerable to heating process compared *to K.marxianus* and *S.cerevisiae.* Chosen alginate as the carrier, based on the preservation of microorganism cells in alginate can protect the microbial population and can be stored in a long time without reducing the microbial population

(El-Fattah et al. 2013). In formula 3, initial Biomass (alginate) of S. cerevisiae, Z.mobilis and K.marxianus is 3.93 $x10^{10}$ CFU / ml; $3,903x10^{10}$ CFU / ml; and $3,406x10^{10}$ CFU / ml respectively. Once encapsulated, the cell density of *S.cerevisiae* was $1,81x10^{10}$ CFU / ml; *K.marxianus* $1,934x10^{10}$ CFU / ml; and *Z.mobilis* $3,674x10^{10}$ CFU / ml. These results show that alginate can highly maintain the viability of microorganisms, especially in *Z.mobilis* with a cell density of $3,67x10^{10}$ CFU / ml. This is the same conclusion with Yang et al. 2016. that the number *Z.mobilis* encapsulated in alginate able to maintain their viability. The results of cell viability in the carrier material are analyzed using Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (Table 2).

Table 2. Duncan's multiple range test results of cell viability in the carrier material in Formula 1, Formula 2 and alginate

Cell density of microorganisms			
Carrier Materials	*S. cerevisiae*	*Z. mobilis*	*K. marxianus*
Wheat flour and rice flour (F1)	3.719 B c	1.673 A a	3.027 B b
Tapioca starch and corn flour (F2)	2.273 A b	0.995 A b	2.98 B a
Alginate (F3)	1.81 A a	3.576 B b	1.934 A b

Description: Capital letters are the same, read vertically, showed no significant difference (p> 0.05) and the same small letters, read to the horizontal direction indicates not significantly different (p> 0.05).

Table 2 shows that *S.cerevisiae* in the formulation of rice flour and wheat flour (F1) produces the highest cell viability which equal to 3.72 $x10^{10}$ CFU / ml, it also shows that the alginate Z.mobilis has a high viability is 3.57 $x10^{10}$ CFU / ml. While *K.marxianus* have high viability in all the flours but *K. Marxianus* not produce high viability in the alginate. From these results it can be seen that the combination of wheat flour and rice flour is the best carrier for *S. cerevisiae* and *K. marxianus* because it can maintain the viability of microbial cells during the process of preservation. Wheat flour and rice flour contains amylopectin amounted to respectively 83% and 75%. High content of amylopectin will help maintain the viability of microorganisms during the drying process using vacuum drying. Protein content in wheat flour also helps in the process of microbial protection. The existence of gliadin and glutenin proteins in the media can reduce heat conductivity. Interaction between flour and water to form gluten dough shaped matrix that is compact and has a strong structure that can trap and protect the cells during drying (Jobbehdar et al. 2013).

Ethanol Fermentation by Microbial Consortia in the Inoculum powder of Sugar hydrolyzate result of hydrolysis cellulose newspaper will be used as the substrate in the fermentation process. Hydrolysis and fermentation processes will be efficient and effective if will be implemented in on an ongoing basis without a long pause; it is often known as Simultaneous Sacharificatian and Fermentation (SSF). In this

study the fermentation of sugar hydrolyzate is fermented by *S.cerevisiae* and *K.marxianus* preserved in flour and rice, as well as *Z.mobilis* preserved in alginate. Levels of ethanol during the fermentation process of used paper can be seen in Fig. 1. Data were statistically analyzed using Analysis of Variance (ANOVA).

Figure 1. Levels of ethanol produced during fermentation.

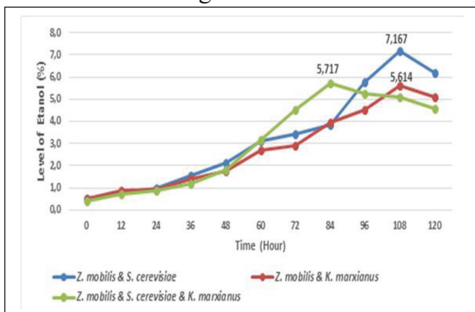

Table 3. Duncan's Multiple Range Test of the ethanol content (%) during the fermentation process of bioethanol from cellulose of a newspaper hydrolyzate.

Sampling Time	Consortium		
	Z. mobilis & S. cerevisiae	*Z. mobilis & K. marxianus*	*Z. mobilis & S. cerevisiae & K. marxianus*
0	0.517 A a	0.502 A a	0.415 A a
12	0.751 A a	0.854 A a	0.707 AB a
24	0.971 AB a	0.927 A a	0.883 AB a
36	1.572 BC b	1.411 AB b	1.176 AB a
48	2.143 C a	1.777 ABC a	1.791 B a
60	3.11 D a	2.685 BCD a	3.168 C a
72	3.447 DE a	2.89 CD a	4.501 D a
84	3.857 E a	3.959 DE ab	5.717 E b
96	5.746 F a	4.53 EF a	5.263 DE a
108	7.167 G b	5.614 F a	5.116 DE a
120	6.186 F a	5.102 EF a	4.589 DE a

Description: Capital letters are the same, read vertically, showed no significant difference ($p > 0.05$) and the same small letters, read to the horizontal direction indicates not significantly different ($p > 0.05$).

In the Table 3. The result showed that the longer the fermentation time, the concentration of ethanol produced is higher. This is because the number of cells and microbial activity is increasing as well, so the more the amount of sugar that is converted into ethanol. However, after 120 hours the ethanol content decreases, this is partly due to the sugar hydrolyzate as a nutrient is depleted. Ethanol concentration is highest at 108th hour, reaching 7.17%. Subsequently the ethanol concentration decreased after 120 hours which may result from ethanol began to be converted into organic acids and evaporation. According to Jayanti (2011) that ethanol fermentation results have a low level of concentration which is about 5-20%. If the ethanol concentration exceeds 15%, the ethanol will damage the cell wall and causing leakage of the plasma membrane, whereas according to Patterson and Ricke (2015) bacterial growth is inhibited by ethanol at a concentration of 10%. The use *Z.mobilis* and *Saccharomyces* sp. immobilized in agar-gelatin matrix will produce ethanol in the amount of 76 g / L in 48 hours of fermentation (Ryu, 1997). While according to Behera et al. (2012), the use of single-*Z.mobilis* cultured cells immobilized in alginate matrix will produce ethanol at 59 g / L. In addition, the fermentation using mixed cultures is considered more efficient than the fermentation using a single culture; this is due to the merger some enzyme activity to convert sugars to ethanol. Bioethanol fermentation by two cultures *Z.mobilis* and *Saccharomyces sp*. immobilized in agar-gelatin matrix, resulting in higher concentrations than ethanol fermentation by *Z.mobilis* and *Saccharomyces* sp. which is not immobilized. Reducing sugar content, cell density, and reducing the ability of microorganisms to ferment sugars during fermentation are the factors that determine the level of concentration of ethanol produced.

The reseach showed that fermentation of reducing sugars of old newpapers cellulose by the consortium of *Z.mobilis* and *S.cerevisiae* produce microbial population, high ethanol concentration and low residual sugar. The results of the calculation of the efficiency of fermentation, showing that the consortium *Z.mobilis* and *S.cerevisiae* value fermentation efficiency 61.2%, with the value of the specific

growth rate (µmaks) of 0072 cells / hour. This consortium also produces ethanol yield value results (Yp / s) and biomass (Yx / s) high of 0.23 g / g and 0.33 g / g. The maximum value of ethanol production (qp) was 0.58 g / g / j. This means that the fermentation of ethanol by a consortium *S.cerevisiae* and *Z.mobilis* is very efficient process. The results also showed that the microorganism population continues to rise, followed by a decrease in the concentration of reducing sugars proven that reducing sugar is used as a nutrient substrate for metabolic processes and cell formation. As the microbial population increases, then the ethanol content increased. *Z. mobilis,* produce enzymes including glucokinase and Fructokinase that convert sugar into ethanol via the Entner-Doudoroff (ED). ED pathway metabolizes glucose reduction via 2-keto-3deoksi-6-phosphogluconate to form pyruvate; then pyruvate by pyruvate decarboxylase is converted into acetaldehyde which is then converted into ethanol (Eram and Ma, 2013). *Saccharomyces cerevisiae* it is capable to convert monosaccharides C-5 and C-6, such as glucose, fructose and galactose into ethanol. *Saccharomyces cerevisiae* has the enzymes as invertase and zymase that work together to transform sugars into ethanol. If sugar is available in the form of a disaccharide sugar, then the enzyme invertase will work and hydrolyze the disaccharide into monosaccharides. Furthermore, zymase enzyme will transform into alcohol and CO2 monosaccharide (Azizah et al., 2012). Based on this research, it is known that the use of microorganisms encapsulated in a polysaccharide matrix and alginate capable of fermenting ethanol with quite high in the fermentation process for 5 days. Ethanol fermentation process using cell immobilization system gives better results comparing with the conventional because when using cell immobilization, product separation will be easier and cell stability can be maintained.

CONCLUSIONS

The combination of rice flour and wheat flour produces high viability in *S.cerevisiae* with cell density reaches $3{,}719x10^{10}$ CFU / ml and in *K.marxianus* its cell density reaches $3{,}027x10^{10}$ CFU / ml. While the 3% alginate is the carrier material for *Z.mobilis,* its produce high cell viability, reaching $3{,}624x10^{10}$ CFU / ml. Consortium *Z.mobilis* and *S.cerevisiae* in the fermentation of bioethanol from waste paper produce the highest ethanol content is 7.17% with the value of fermentation efficiency reached 61.2%.

ACKNOWLEDGEMENTS

We are very grateful to the Padjadjaran University, which has funded the research and publication through the Leadership Academy Grant Scheme.

REFERENCES

Alonso, S. 2016. Novel Preservation Techniques for Microbial Cultures. Food Engineering Series. Springer International Publishing Switzerland. DOI 10.1007/978-3-319-42457-6_2

Azizah, N., A.N.Al-Baarri, S. Mulyani. 2012. Influence of fermentation time on Alcohol levels, pH and Gas Production at Ethanol Fermentation of Whey with Pineapple peel Substitutes. *Jurnal Aplikasi Teknologi Pangan*. 1(2):72-77.

Behera, S., Mohanty, R. C., Ray, R. C. 2012. Ethanol Fermentation Of Sugarcane Molasses By *Zymomonas mobilis* Mtcc 92 Immobilized In *Luffa cylindrica* L. Sponge Discs And Ca-Alginate Matrices. *Brazilian Journal of Microbiology*. ISSN 1517-8382.

Devi, H.S. 2014. Viability of *Bacillus* sp. BK 17 on Various Materials Carrier. *Skripsi*. Universitas Sumatera Utara. Medan.

El-Fattah, D.A.A., Eweda, W.E., Zayed, M. S., Hassanein, M. K. 2013. Effect of Carrier Materials, Sterilization Method, and Storage Temperature on Survival and Biological Activities of *Azotobacter chroococcum* Inoculant. *Annals of Agricultural Science*. 58(2): 111-118.

Eram, M.S., and K. Ma. 2013. Decarboxylation of Pyruvate to Acetaldehyde for Ethanol Production by Hyperthermophiles. *Biomolecules* **2013**, *3*, 578-596; doi: 10.3390/ biom3030578.

Goderska, Kamila. 2012. Different Methods of Probiotics Stabilization. Licensee. InTech. Chapter 24. http://dx.doi.org/10.5772/50313

Jayanti, R.T. 2011. Effect of pH, Temperature of Hydrolysis enzyme α-amylase, concentrations of Yeast, For Ethanol Production Using Starch Bran. Skripsi,. Surakarta, Department of Biology, FMIPA, Universitas Sebelas Maret.

Jobbehdar, S.B., C. Soukoulis, L.Yonekura, and I. Fisk. 2013. Optimization of Spray-Drying Process Conditions for the Production of Maximally Viable Microencapsulated L. acidophilus NCIMB 701748. Drying Technology, 31: 1274–1283, 2013 Published

with license by Taylor & Francis. DOI: 10.1080/07373937.2013.788509

Kusumaningati, M.A., S.Nurhatika, dan A. Muhibuddin. 2013. Effect of inoculum concentration of bacteria Zymomonas mobilis and fermentation time on Ethanol Production from Waste Vegetable and Fruit, Wonokromo local Market, Surabaya *Jurnal Sains Dan Seni Pomits*. 2(2): 2337-3520.

Patterson, J.A and S. Ricke. 2015. Effect of ethanol and methanol on growth of ruminal bacteria Selenomonas ruminantium and Butyrivibrio fibrisolven. Journal of Environmental Science and Health Part B Pesticides Food Contaminants and Agricultural Wastes 50(1):62-7. doi: 10.1080/03601234.2015.965639

Pumphan, K., Sornplang, P., Uriyapongson, S. 2013. Cultivation of Lactobacillus sp. and Production of Probiotic Powder as Animal Feed Additive. *Pakistan Journal of Nutrition*. 12(6): 567-570.

Ryu, Sangryeol and K. Lee. 1997. Compariosn of Immobilization Matrix for Ethanol Fermentation by Zymomonas mobilis and saccharomyces cerevisae. *Journal of Microbiology and Biotechnology*. Vol. 7 No. 6 , 438-440.

Yang, S., Q. Fei, Y. Zhang, L. M. Contreras, S. M. Utturkar, S.D.Brown, M. E. Himmel and M. Zhang. 2016. Zymomonas mobilis as a model system for production of biofuels and biochemicals. Minireview. Microbial Biotechnology published by John Wiley & Sons Ltd and Society for Applied Microbiology.