UNIVERSITATEA DE ȘTIINȚE AGRICOLE ȘI
MEDICINĂ VETERINARĂ "ION IONESCU DE LA BRAD" IAȘI

LUCRĂRI ȘTIINȚIFICE

VOL. 54

MEDICINĂ VETERINARĂ

NR. 2

EDITURA "ION IONESCU DE LA BRAD" IAȘI 2011

Volumul a fost editat cu sprijinul financiar al Ministerului Educației, Cercetării, Tineretului și Sportului

ISSN: 1454-7406

CUPRINS

NR.1

FERMENTATIONS OF VARIOUS WHEY TYPES WITH USING *Kluyveromyces lactis* IN THE PRODUCTION OF BIOETHANOL AND ORGANIC LIQUID FERTILIZER

Gemilang Lara Utama[1], Tita Roostita Balia[1], Kurnani Tubagus Benito Achmad[1,2], Sunardi[2], Iwan Setiawan[1]

[1]Faculty of Animal Husbandry University of Padjadjaran
Jl.Raya Bandung Sumedang KM.21 Tanjungsari Sumedang, Jawa Barat Indonesia
[2]Institute of Ecology University of Padjadjaran
e-mail : thmfsc@yahoo.co.id

ABSTRACT

The research aimed to determine Kluyveromyces lactis concentrations and whey type that appropriate in producing the best bioethanol production with distillery waste as organic liquid fertilizer. This experimental research was done based on Completely Randomized Design with 3 x 3 factorial patterns. Treatments consisted of two factors, namely the addition of different Kluyveromyces lactis concentrations (5%, 10% and 15% v/v) and combinations of whey types (neufchatel whey, feta whey and mixed neufchatel and feta whey 1:1), with three replications. Whey was fermented by Kluyveromyces lactis with the concentration of 5%, 7.5%, and 10% (v/v) at the temperature of 33ºC for 24 hours and then distillated twice at the temperature range 78 ° -100 °C. The first distillation gives bioethanol purity until 86% and the second one gives purity until 95%. The result showed that 5% concentration of Kluyveromyces lactis on neufchatel whey (K1W2) produce best bioethanol content of 1.94% and liquid organic fertilizer with N content of 0.1%; P of 0.067%; K of 0.135%; and pH of 5.7.

Keywords : **Whey, *Kluyveromyces lactis*, Bioethanol, Liquid Organic Fertilizer**

Introduction

Whey is byproduct of cheese-making industry which potential to cause pollution. One of the pollution potential came from high BOD-COD and low pH. Whey also donates Nitrogen (N) and Phosphate (P) in enough concentrations to cause eutrophication if continuously discarded in large numbers to the waters. Beside the potential of pollution, the nutrients of whey allow to be processed into a commodity.

One of the important nutrients of whey is lactose. The content of lactose in whey reaches 4-5% (Ghaly, *et al.*, 2000). The ability to utilize lactose as a carbon source is owned by the yeasts such as *Kluyveromyces lactis*. As mentioned by Ghaly, *et al,*, (1993) and Maullu *et al.*, (1999), lactose from whey is widely used as a carbon source in bioprocess media for *Kluyveromyces lactis* growth.

Kluyveromyces lactis were used in many industrial activities because of lactase producing ability (Rech *et al.*, 1999; Moeini *et al.*, 2004). With the lactase producing ability, lactose can be hydrolyzed directly into glucose and galactose by *Kluyveromyces lactis*, then through the glycolysis and metabolic pathways it's hydrolyzed into pyruvic acid. Pyruvic acid

became the most important branching point in the process of *Kluyveromyces lactis* metabolism, in a state of limited oxygen the oxidation of NADH cannot be perfect, so ethanol fermentation occur through pyruvat decarboxylation into acetaldehyde which subsequently hydrolyzed into bioethanol (Breunig and Steensma, 2003).

Bioethanol with the expected purity can be obtained by distillation process. Besides bioethanol, the distillation processes were also generating distillery waste which can be used as an alternative of liquid organic fertilizer. Many nutrients left that can be used as a source of NPK for the soil and plants.

To produce best bioethanol production and liquid organic fertilizer necessary to observe several things such as the concentration of starter and raw materials used. The starter concentrations in the production of bioethanol are varies greatly depending on the type of raw materials and fermentation conditions. Therefore, research aimed to determine *Kluyveromyces lactis* concentrations and whey type that appropriate in producing the best bioethanol production with distillery waste as organic liquid fertilizer.

Materials and Methods

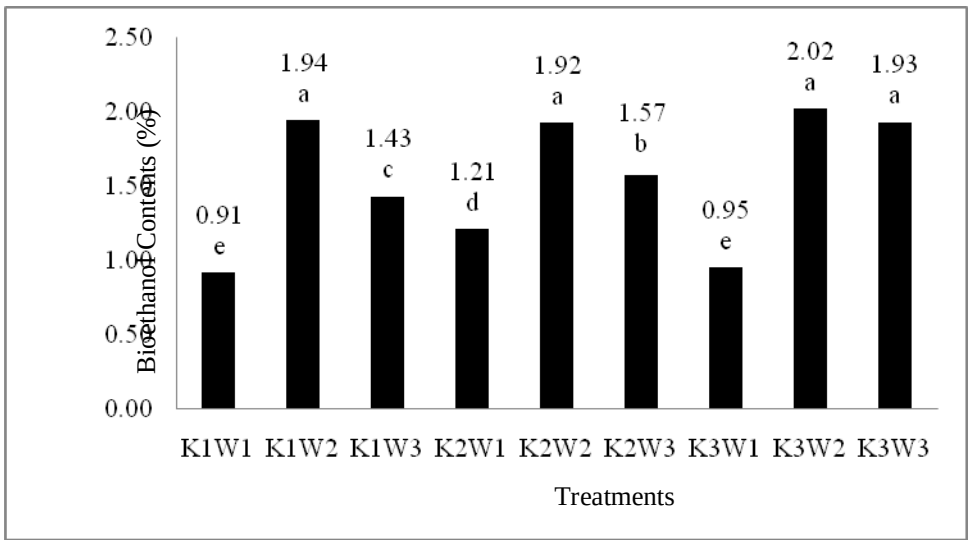
Neufchatel and feta whey was taken from PT. Yummy Food Utama East Jakarta and then analyzed at the Laboratory of Ruminant Nutrition and Feed Chemistry, Faculty of Animal Husbandry, University of Padjadjaran (2010) with results as follows: feta whey has a Lactose of 4.64%, Fat of 2.19%, Protein of 0.79%, Ash of 0.073% , Crude Fiber of 0.011%, Water of 92.29% and whey neufchatel have Lactose of 4.09%, Fat of 2.39%, Protein of 0.75%, Ash of 0.089%, Crude Fiber of 0.015%, Water of 92.67%. *Kluyveromyces lactis* was taken from The Laboratory of School of Life Science and Technology ITB.

All types of whey (neufchatel whey, feta whey and mixed neufchatel and feta whey 1:1) fermented by *Kluyveromyces lactis* with the concentration of 5%, 7.5%, and 10% (v/v) at the temperature of 33ºC (Steensma *et al.*, 1988) for 24 hours, it's because *Kluyveromyces lactis* through acceleration phase at 8-16 h at 35ºC (Barbosa *et al.*, 1985). After that, fermented whey was distillated twice at the temperature range 78 ° -100 °C (Erliza Hambali, *et al.*, 2009). The first distillation gives bioethanol purity until 86% and the second one gives purity until 95%. Bioethanol content were tested with using Gas Chromatography, N content with Kjedahl methods, P as P_2O_5 and K with *Atomic Absorbtion Spectrophotometer* (AAS), moreover pH with digital pH meter (Zenway).

Results and Discussions

Figure 1 showed that the highest average of bioethanol produced was 2.02%, which given by treatment combination of neufchatel whey with a 15% *K. lactis* concentration. While the lowest average of bioethanol produced was 0.91% that given by the treatment combination of feta whey with 5% concentration of *K. lactis*.

The production of bioethanol is not only due to the nutritional composition of the substrate but also influenced by pH, time, and also the amount of glucose and galactose which available on the substrate (Ramakrishnan and Hartley, 1993; Kargi, *et al.*, 2006; Dagbagli and Goksungur, 2008). All of the factors mentioned above was inter-related and affected the bioethanol contents. Kargi, *et al.*, (2006) states that pH 5-6 is the optimum pH in producing bioethanol with whey as raw material.



K_1 = 5 % *K.lactis* concentration, K_2 = 10% *K.lactis* concentration, K_3 = 15% *K.lactis* concentration
W_1 = *Feta* whey, W_2 = *Neufchatel* whey, W_3 = Mixed *feta* dan *neufchatel* whey 1 : 1

Figure 1. The average bioethanol contents that influenced by the treatment combination of *Kluyveromyces lactis* concentration in different types of whey

Similarly with Neri, *et al.* (2008) which showed at pH of 5.0 to 5.5, lactase enzyme produced by *K.lactis* through an adaptation phase and has a low activity and then lactase enzyme activity were increase and through the exponential phase along with the increasing pH of 5.5 to 6.0. Lactase enzyme activity by *K.lactis* reached peak at pH of 6.5, therefore the amount of glucose and galactose which can be hydrolyzed from feta and mix whey at a pH below 5.5 was not optimal, so the first 24 hours will be a shortage of glucose and production of bioethanol to be low. As mentioned Ramakrishnan and Hartley (1993) the first 24 hours *K.lactis* only utilize glucose and then break down galactose for bioethanol formation. Different with feta whey, neufchatel whey can produced higher bioethanol contents. Neufchatel whey has a pH of 5.7 which is the pH of *K.lactis* lactase activity experiencing exponential phase (Neri, *et al.*, 2008). Therefore, the amount of glucose and galactose which can be hydrolyzed would be better than feta whey and thus bioethanol produced becomes higher.

Beside the differences of whey types, the combinations of *K.lactis* concentration levels in different types of whey give significant effects on bioethanol contents. The high concentrations of *K.lactis* added resulted in higher competition in getting the available glucose so that glucose will be depleted and used only for respiration, it's resulting in a lack of glucose and results in lower amount of bioethanol produced (Mahmoud and Kosikowski, 1982).

Table 1. Average levels of NPK contents and pH of distillery waste affected by different types of whey and *K.lactis* concentration which compared with National Standard of Indonesia

Treatments	Results			
	N	P	K	pH
	%			
K1W1	0.12	0.060	0.127	5.4
K1W2	0.10	0.067	0.135	5.7
K1W3	0.12	0.058	0.112	5.4
K2W1	0.12	0.057	0.111	5.3
K2W2	0.11	0.062	0.130	5.6
K2W3	0.13	0.057	0.112	5.5
K3W1	0.13	0.049	0.116	5.4
K3W2	0.10	0.060	0.139	5.7
K3W3	0.11	0.055	0.171	5.5

K_1 = 5 % *K.lactis* concentration, K_2 = 10% *K.lactis* concentration, K_3 = 15% *K.lactis* concentration

W_1 = *Feta* whey, W_2 = *Neufchatel* whey, W_3 = Mixed *feta* dan *neufchatel* whey 1 : 1

Based on Table 1., note that the distillery waste at K2W3 and K3W1 treatment had a highest levels of N of 0.13%. *Kluyveromyces lactis* is one type of yeast that can utilize N for growth which derived from amino acids (Messenguy, *et al.*, 2006). The extracellular protease activities produced by *Kluyveromyces lactis* hydrolyze amino acids with peptides into N for metabolisms use (Walker, 1998). Nitrogen used by *Kluyveromyces lactis* and stored in their cells. Total Nitrogen in the yeast cells reached about 10% of the dry weight of yeast cells (Walker, 1998).

The results on Table 1 were also shown that K1W2 treatment gives highest P content of 0.067% on the distillery waste. Along with the increasing of *K.lactis* concentration the P content on the distillery waste was decreasing. It is caused by phosphorus in the form of nucleic acids and phospholipids contained in whey were an essential nutrient needed by *K.lactis* to grow (Parrondo, *et al*., 2009; Walker, 1998). Theobald, *et al.* (1996) states that *K.lactis* using phosphorus then it put in his cell in orthophosphate (H2PO4) form.

Distillery waste with highest K content was given by K3W3 treatments with the value of 0.171%. Walker (1998) mentions that K is macro-elements required as cofactor of various enzymes that involved in oxidative phosphorylation, biosynthesis of protein and carbohydrate metabolism. In the abnormal conditions, the role of Potassium in the metabolism of yeasts sometimes replaced by magnesium or sodium, but it will cause the slow rate of fermentation (Spencer, *et al.*, 1997).

Highest pH showed by K1W2 and K3W2 with pH of 5.7 and the lowest shown by K2W1 with the pH of 5.3. During the distillation, heating will trigger autolysis on yeast cells as mentioned by Stemwedel (2009), that heating at temperatures between 55-83ºC will cause autolysis of yeast cells which will lead the breakdown of cell wall and release the polysaccharides. Polysaccharides released can act as a source of carbon (C) and the hydroxyl group can trigger an increase of pH.

Conclusions

The results indicated that the 5% concentration level of *K.lactis* in whey neufchatel (K1W2) can be summed up as the best treatment to produce bioethanol. Even though the NPK content was not the highest; this treatment was taken as the best treatment on producing bioethanol because the treatment was not significant from other treatments that use a higher concentration of *K.lactis*. The addition of 5% concentration of *Kluyveromyces lactis* on neufchatel whey (K1W2) gives best bioethanol content of 1.94% and liquid organic fertilizer with N content of 0.1%; P of 0.067%; K of 0.135%; and pH of 5.7.

References

Barbosa., M.D.F.S., Silva, D.O., Pinheiro, A.J.R.,Guimaraes, W.V. and Borges, A.C. 1985. *Production of Beta-D-Galactosidase from Kluyveromyces fragilis Grown in Cheese Whey*. J. Dairy Sci., 68:1618-1623.

Breunig, K.D. and Yde Steensma, H. 2003. *Kluyveromyces lactis : Genetics, Physiology and Application*. Dalam J.H. de Winde (Ed.). *Functional Genetics of Industrial Yeasts*. Springer-Verlag Berlin Heidelberg.

Dagbagli, S. and Goksungur, Y. 2008. *Optimization of B-Galactosidase Production Using Kluyveromyces Lactis NRRL Y-8279 by Response Surface Methodology*. Electronic Journal of Biotechnology 11 (4).

Erliza Hambali, Siti Mujdalifah, Armansyah Halomoan. Tambunan, Abdul Waries Pattiwiri, dan Roy Hendroko. 2009. Teknologi Bioenergi. Agromedia Pustaka.

Ghaly, A.E. and E.A. Echiegu. 1993. *Kinetic of A Continuous Flow No-Mix Anaerobic Reactor*. Energy Sources 15(3) : 1-17.

Ghaly, A.E., D.R. Ramkumar, S.S. Sadaka and J.D. Rochon. 2000. *Effect of Reseeding and pH Control on the Performance of a Two-Stage Mesophilic Anaerobic Digester Operating on Acid Cheese Whey*. Journal of Canadian Agricultural Engineering 42(4) : 173-183.

Kargi., F and Omzichi, S. 2006. *Utilization of Cheese Whey Powder (CWP) for Ethanol Fermentations: Effects of Operating Parameters*. Enzyme and Microbial Technology 38 : 711–718.

Mahmoud, M. M. and Kosikowski, F.V. 1982. *Alcohol and Single Cell Protein Production by Kluyveromyces in Concentrated Whey Permeates with Reduced Ash*. J. Dairy Sci., 65:2082-2087.

Maullu, C., Lampis, G., Basile, T., Ingianni, A., Rossolini, G.M., Pompei, R. 1999. *Production of lysozymeenriched biomass from cheese industry by-products*. Journal of Applied Microbiology 86:182-186.

Messenguy, F., Andre, B. and Dubois, E. 2006. Diversity of Nitrogen Metabolism Among Yeast Species : Regulatory and Evolutionary Aspects. Dalam Rosa, C. and Gabor, P. (Ed.). Biodiversity and Ecophysiology of Yeasts. Springer-Verlag Berlin Heidelberg.

Moeini, H., Nahvi, I., Tavassoli, M. 2004. *Improvement of SCP production and BOD removal of whey with mixed yeast culture*. Elec. J. of Biot. 3 : 251 – 255.

Neri, D.F.M., Balcão, V.M., Carneiro-da-Cunh, M.G., Carvalho Jr., L.B., Teixeira, J.A. 2008. *Immobilization of ß-galactosidase from Kluyveromyces lactis onto α polysiloxane–polyvinyl Alcohol Magnetic (mPOS–PVA) Composite for Lactose Hydrolysis*. Catalysis Communications 9 : 2334–2339.

Parrondo, J. L., A. García, and M. Díaz. 2009. *Nutrient Balance and Metabolic Analysis in a Kluyveromyces Marxianus Fermentation With Lactose-Added Whey*. Brazillian J. of Chem. Engineering. 26(03): 445 – 456.

Ramakhrishnan, S. and Hartley, B.S. 1993. Fermentation of Lactose by Yeast Cells Secreting Recombinant Fungal Lactase. Journal of Applied and Environmental Microbiology. 59 (12) : 4230 – 4235.

Rech, R., Cassini, C.F., Secchi, A. and Ayub, M.A.Z. *Utilization of protein-hydrolyzed cheese whey for production of b-galactosidase by Kluyveromyces marxianus*. Journal of Industrial Microbiology and Biotechnology. 23(2) : 91-93.

Spencer, J.F.T., Spencer, D.M. and Figueroa, L.I.C. Yeasts as Living Objects: Yeast Nutrition. Dalam Spencer, J.F.T. and Spencer, D.M. (Ed.). *Yeasts : In Natural and Artificial Habitats*. Springer-Verlag Berlin Heidelberg.

Steensma., H.Y., de Jongh, F.C.M., Linnekamp., M. 1988. *The Use of Electrophoretic Karyotypes in the Classification of Yeast : Kluyveromyces marxianus and K. lactis.* Curr Genet 14:311-317.

Stemwedel, T.A., 2009. *Soluble Fertilizer for Organic Agriculture From Distiller's Yeast*. United States Patent Application. Through <www.freepatentsonline.com/y2009/0173122.html> [06/01/2010]

Theobald, U., Mohns, J. And Rizzi, M. 1996. *Determination of In Vivo Cytoplasmic Orthophosphate Concentration in Yeast.* Biotechnology Techniques. 10(2) : 297-302.

Walker, G.M. 1998. *Yeast Physiology and Biotechnology*. John Wiley and Sons Ltd.