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REDUCING CHEESE-MAKING BY-PRODUCT DISPOSAL THROUGH ETHANOL FERMENTATION AND THE UTILIZATION OF DISTILLERY WASTE FOR FERTILIZER

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ABSTRACT: Whey as cheese-making by-product has become a threat toward the sustainability of production process at small medium enterprises (SMEs) cheese producer. High organic contents lead high pollution load to the environment, because until now the producer still dispose the waste to the stream or land. Whey utilization through simple ethanol fermentation could reduce high organic content and highly implementable in SMEs level because its easiness. The research aimed to determine waste minimization through ethanol fermentation and the utilization of distillery wastes for fertilizer. Research was done experimentally with substrate variation (whey and napa cabbage) with and without 10% molases addition that fermented by indigenous yeasts consortium (*Candida lambica* and *Prototheca zopfii*) on various temperature (24-27°C and 17-21°C) for 96 hours. The ethanol contents measured by using dichromate oxidation methods. After fermentation finished substrates distilled two stages, the first stage distillery wastes were analyzed for the contents of N (Kjeldahl), P_2O_5 (Bray I) and Potassium (AAS). Results showed that the combination of whey and napa cabbage (1:1) with 10% molasses addition that fermented by *Candida lambica* and *Prototheca zopfii* on 17-21°C resulted in 11.06% of bioethanol contents in 72 hours fermentation. After two stages distillation, 11.2% substrates can converted into ethanol and 37.9% of water resulted from second stage distillation that can disposed to the environment. Meanwhile, 50.9% of first stage distillery wastes has 0.56% N, 0.83% P and 0.35 K which suitable with the Indonesian Agriculture Ministerial Decree No.28/2009 of minimum technical requirement for organic fertilizer. Ethanol fermentation from cheese whey with napa cabbage wastes and 10% molasses addition that fermented by *Candida lambica* and *Prototheca zopfii* consortium and the utilization of its distillery wastes for fertilizer could minimize wastes up to 62.1%.

Keywords: cheese whey, napa cabbage, ethanol, fertilizer, wastes minimization

1. INTRODUCTION

Cheese making by-products which was known as whey increasingly to the attention of cheese producers especially in Small Medium Enterprises (SMEs) scale. Cheese whey often discharged directly to the environment and supposed to be one of the causes of pollution. Cheese whey has low acidity (pH) so that can cause problems, especially if disposed into stream that have low water discharge. The organic matter left behind on cheese whey was potential to cause eutrophication if discharged directly into water bodies. Meanwhile, organic materials owned allow cheese whey to be processed into commodities.

Organic materials with the largest number owned by cheese whey is lactose. Lactose is a specific carbohydrate owned by dairy products and its content up to 5% [1]. As carbon source for microorganisms, lactose widely used in bioprocess medium for the growth of lactic microorganisms. Lactic microorganisms synthesized lactose into glucose and galactose, then metabolized through the glycolytic pathway to generate energy, organic acids and ethanol [2][3].

Several indigenous yeasts and yeast-like microorganisms have good ability in ethanol fermentation from wastes. *Candida lambica* isolated from mozzarella whey to ferment ethanol up to 0.15% within 48 hours at room temperature and have a sugar content and resistance to high ethanol [4]. *Prototheca zopfii* was yeast-like organism that capable in fermenting ethanol in extreme conditions, this type was found in some milk-based and cellulose-based wastes [5][6].

The addition of sugar complex based substrates such as cellulose is one way that can be done to increase the ethanol contents resulting from cheese whey fermentation. Napa cabbage waste was a potential cellulose-based substrate for ethanol fermentation [7]. The addition of other cellulose-based substrate such as molasses in cheese whey fermentation resulting in 2.06% ethanol with 92.71% sugar conversion [8].

Fermented substrates were purified by distillation. Distillation at temperature of 78º-

100°C resulted in ethanol evaporation, and through the condensing unit ethanol with desired purity will be released.

The distillation also resulted distillery waste that potentially polluting the environment because it's contains high organic materials. Undiluted organic load on distillery wastes could results toxic effect on aquatic organisms and the color could also block out sunlight thus hamper photosynthesis inside the waters [9]. Distillery wastes have low pH of 3.8-4.4, BOD of 45000-60000 ppm and COD of 70000-98000 ppm with suspended solids of 2000-14000 ppm [10].

The distillery wastes with the organic materials contained could applied as organic liquid fertilizer [11]. Common characteristic of distillery wastes showed the contents of 0.10-0.12% N, 0.05-0.15% P and 0.5-1.2% K [10]. Meanwhile, distillery wastes generated from the residue of neufchatel whey fermentation contains 0.1% N, 0.07% P and 0.14% K [12].

Cheese whey utilization through ethanol fermentation with the utilization of distillery wastes for fertilizer aimed to minimize wastes disposal into environment. Aside from being able to decrease organic material load through reduction of BOD and COD, the amount of wastes to be released can be reduced.

2. MATERIALS AND METHODS

2.1 Materials

Cheese whey taken from Koperasi Peternak Bandung Selatan (KPBS), napa cabbage (*Brassica rapa subsp. pekinensis*) wastes and molasses from Pangalengan traditional market, Bandung District.

2.2 Methods

2.2.1 Yeasts isolates prepartion

Candida lambica isolated from cheese whey and *Prototheca zopfii* isolated from broccoli leaves. The yeasts isolates grown in Nutrient Broth/NB with the addition of 3% yeast extract (Kraft Foods Inc.) and 10 ppm amoxycilline (PT. Kimia Farma), then incubated for 36 hours at room temperature (26-28°C) [4].

2.2.2 Ethanol production

Cheese whey and napa cabbage wastes (1:1) mixed then set into two replications treatments i.e. : with and without 10% molasses addition, incubation temperature of low temperature (17-20°C) and room temperature (24-27°C) for 96 hours fermentation. Ethanol contents measured with dichromate oxidation methods every 24 hours [13]. Data analyzed with multivariate regression analysis (SPSS 18).

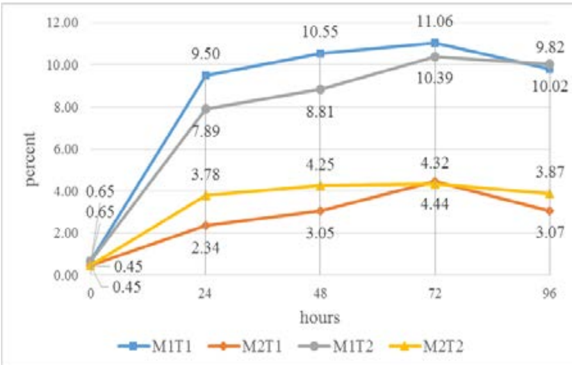
2.1.3 Distillery wastes characterization

Fermented substrates distilled two stages at 78-100°C. Residue of first stage distillation measured on the contents of Nitrogen/N (Kejldahl), Phospate/ (P_2O_5)/P (Bray I), and Potassium/K (*Atomic Absorbtion Spectrophotometer*/AAS) then compared with the ministerial decree No.8/2009 for minimum technical requirement for organic fertilizer. Volume of distillery wastes measured as basis for waste minimization calculation.

Use at most three levels of headings that correspond to chapters, sections and subsections. The first level headings for chapter titles should be in 10pt, bold, justified, and upper case font. Leave one-blank line before and after the first level headings, respectively.

3. RESULTS AND DISCUSSIONS

Results of ethanol fermentation (Fig.1) shown increased until 72 hours and decreased at 96 hours. Highest ethanol contents (11.06%) resulted by treatment of 10% molasses addition that incubated at low temperature for 72 hours. Analysis of multivariate regressions showed that the variables of molasses addition, temperature and incubation time significantly affected the ethanol contents with R^2=0.945 and the multivariate regression equation of Y = 15,12 – 6,11X1 – 0,06X2 + 0,33X3.



Note :
M1T1 : 10% Molasses addition, low temperature
M2T1 : 0% Molasses addition, low temperature
M1T2 : 10% Molasses addition, room temperature
M2T2 : 0% Molasses addition, room temperature

Fig. 1 Ethanol production

The addition of 10% molasses could produce 11.06% ethanol contents. Molasses contains 50% sugars that can be fermented and enhanced ethanol productivity [14][15]. The mixture of cheese whey and molasses as much as 10% was an effective and efficient treatment for ethanol fermentation [8].

Ethanol contents declined at 96 hours

incubation. At this stage, yeasts has been through stationary phase and lead to death phase. The optimum incubation time for ethanol fermentation from cheese whey with the addition of cellulose-based substrates was 72 hours. Incubation until 96 hours will reduce the ethanol levels because it's converted to other compounds such as esters [16].

Low temperature (17-21°C) produced the best ethanol contents on 72 hours of fermentation. At low temperatures, yeasts sensitivity to the toxicity of ethanol to be lower so that the fermentation continues, meanwhile at warmer temperatures (> 20°C) loss of viability of yeasts happens faster [17].

After the fermentation done, fermented substrates was distilled 2 stages and the distillery wastes were characterized then compared to the Indonesian Agriculture Ministerial Decree No.28/2009 of minimum technical requirement for organic fertilizer (Table 1). The results showed that the distillery wastes characteristics was suitable with the requirements.

Table 1 Distillery wastes characteristics

Items	%	Decree No.28/2009
Nitrogen (N)	0.56	< 2
P_2O_5 (P)	0.83	< 2
Potassium (K)	0.35	< 2

The potential of distillery wastes as organic fertilizer can be characterized from the raw materials. Cheeses whey still contain 0.06% N, 0.38% P, and 0.12% K, while the molasses had 0.20% N, 0.27% P, and 0.11% K [8]. In addition, there were 1.3% N and various minerals including P and K in napa cabbage [18].

Aside from cheese whey, napa cabbage and molasses, the number of N, P and K on the distillery wastes could derived from yeast cells that assimilate and utilize the elements. Yeasts were able to utilize methionine, histidine and lysine that available in cheese whey as the primary nitrogen source for growth and keep it inside the cell up to 10% of the dry weight of yeast cells [19][20]. Phosphorus contained in cheese whey were essential nutrients required for the growth of yeasts then stored inside the cell in the form of orthophosphate (H2PO4) [21][22]. Meanwhile potassium were macro minerals needed by yeasts as cofactor of various enzymes involved in oxidative phosphorylation, biosynthesis of protein and carbohydrate metabolism [19].

Volume of distillate and the residue of 2 stages distillation were measured and shown at Table 2.

Table 2 Distillation results volume

Items	ml	%	
Fermented substrates	2000	100	
Residue stage 1	1018	50.9	Fertilizer
Distillate 1	982	49.1	
Residue stage 2	224	11.2	Ethanol
Distillate 2	758	37.9	Water

Fermentation of cheese whey with napa cabbage and molasses addition, for ethanol and the utilization of distillery wastes as organic fertilizer could minimize wastes disposal significantly. First stage distillation was resulted 50.9% of total substrates volume that can used as fertilizer. The second stage distillation was resulted average 11.2% of total substrates volume of ethanol and 37.9% residue in the form of distilled water which can be released to the environment. The results showed that the utilization made an impact on waste minimization up to 61.2% and leaving distilled water residue that can disposed to the environment.

4. CONCLUSION

The fermentation of whey and napa cabbage (1:1) with 10% molasses addition by *Candida lambica* and *Prototheca zopfii* at the temperature of 17-21°C for 72 hours resulted in bes ethanol contents of 11.06%. Two stages distillation resulting in 37.9% distilled water, 11.2% ethanol, and 50.9% distillery waste. The distillery wastes has suited the Indonesian Agriculture Ministerial Decree No.28/2009 of minimum technical requirement for organic fertilizer with N of 0.56%, P of 0.83% and K of 0.35. Ethanol fermentation and utilization of distillery wastes for fertilizer could minimize wastes up to 62.1%.

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