The Combination of Mozzarella Cheese Whey and Sugarcane Molasses in the Production of Bioethanol with the Inoculation of Indigenous Yeasts

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Objectives of the research were to determine the best combination of mozzarella cheese whey and sugarcane molasses as agro-industrial wastes in producing bioethanol with the inoculation of indigenous yeasts. The research has been done experimentally by Completely Randomized Design (CRD) $3 \times 3$ factorial pattern, and the data was analyzed with ANOVA then continued with Tukey test. Treatments consisted of two factors, i.e. addition of sugarcane molasses (10%, 15%, 20%) and indigenous yeasts combination $[C.\ tropicalis\ 1 + Blast.\ capitatus + C.\ tropicalis\ 2\ (1:1:1),\ C.\ tropicalis\ 1 + Blast.\ capitatus\ (1:1),\ C.\ tropicalis\ 1 + C.\ tropicalis\ 2\ (1:1)]$ that were replicated three times. Substrates fermented at ambient (25-27°C) for 24 hours, then the bioethanol contents tested with chromium dichromate oxidation. Results show that the treatment combination of sugarcane molasses concentration and indigenous yeasts combination and single treatments of indigenous yeasts combination have shown significant effects towards the bioethanol contents, meanwhile the treatments of molasses concentration has shown non-significant effects. The best bioethanol contents (8.49%) was gained by the treatment combination of 15% sugar cane molasses concentration with the inoculation of $C.\ tropicalis\ 1 + Blast.\ capitatus\ (1:1)$.

Key Words

Bioethanol, Mozzarella cheese whey, Sugarcane molasses, Indigenous yeasts

1. Introduction

Mozzarella is one type of cheese that is now widely produced in Indonesia. Its production increases along with the increasing popularity of pizza and other similar food products. However, along with the rapid increase in production capacity, problems arise in the management of byproducts that produce mozzarella cheese whey liquid.

Mozzarella cheese whey is often discharged directly into the environment and allegedly becomes one of the causes of pollution. The disposal may also disrupt the balance of the soil microflora, potentially cultivating pathogenic microorganisms and at risk of emitting CO and CH$_4$. Mozzarella cheese whey has a high acidity level, so it will be problematic if discharged into the waters. The disposal of whey into the surrounding waters is also potentially disruptive given the high BOD, COD and low water discharge. Meanwhile, the organic material remaining in mozzarella cheese whey still has the potential to be utilized.

Lactose is a specific sugar or carbohydrate contained in mozzarella cheese whey, and its content can reach 4 - 5%. Lactose can act as a major carbon source for lactic microorganism’s growth. Lactose as a carbon source will be synthesized by lactic microorganisms into glucose and galactose, then utilized through glycolysis to produce energy, organic acids and ethanol.

Ethanol is one of the derivative products of mozzarella cheese whey that has the potential to be developed. Besides being used as a disinfectant, ethanol can also be used as fuel. Ethanol can act as a chemical solvent and include an important element in the development of cosmetics and pharmaceutical fields.

However, some research shows that the use of mozzarella cheese whey as a source of ethanol production still resulting in low yield. Bioconversion of cheese whey...
by *Kluyveromyces marxianus* yielded 0.86% ethanol\(^7\). Meanwhile, the conversion of Neufchatel cheese whey by *Kluyveromyces lactis* was only able to produce ethanol levels of 1.79-1.94%\(^8\)\(^9\).

The effort in increasing the level of bioethanol yield continues to be done. One of them is through the addition of cellulose-based biomass. Fermentation of pineapple skin and cheese whey (1:1) by *S. cerevisiae* was able to increase bioethanol level up to 2.25%\(^10\). The combination of cheese whey with sweet sorghum which fermented by *S. cerevisiae* yielded 3.5% bioethanol\(^11\).

Other cellulose-based biomass that potential for bioethanol production is sugarcane molasses. The yielded bioethanol from sugarcane molasses could reach 60.32%, with high conversion yield of 94.26% and \(Y_{\text{ethanol}} = 0.6\) g/g with using Egyptian yeast isolate *Candida tropicalis* strain HSC-24\(^12\).

The use of microorganisms in fermenting bioethanol also plays an important role in increasing the yield. Indigenous yeast from mozzarella cheese whey also needs to be explored, that has potential in fermenting lactose to bioethanol. Moreover, mozzarella cheese whey is the natural habitat. *Candida spp.* has been discovered being one of ethanol and glucose-tolerant indigenous yeasts that is isolated from mozzarella cheese whey which can resulting in high bioethanol\(^13\).

Objectives of the research were to determine the best combination of mozzarella cheese whey and sugarcane molasses as agro-industrial wastes in producing bioethanol with the inoculation of indigenous yeasts.

### 2. Experimental

Indigenous yeasts were isolated using a modified medium with the addition of 3% Yeasts Extract/YE (Kraft Foods) and 10 ppm amoxicillin into Potato Dextrose Agar/PDA (Oxoid Ltd.)\(^14\). Three yeast-like microorganisms were isolated from mozzarella cheese whey. Then, it was purified and tested with RapID Yeasts Plus System and analyzed by ERIC (Electronic Code Compendium) http://www.remel.com/eric\(^15\).

Bioethanol production was obtained from the treatments which consisted of two factors, i.e. the addition of sugarcane molasses concentration [10% (M1), 15% (M2), 20% (M3)] and combination of indigenous yeasts [Y1/Yeast A + Yeast B + Yeast C (1:1:1), Y2/Yeast A + Yeast B (1:1), Y3/Yeast A + Yeast C (1:1)] which is added until it reaches 3% (v/v). The treatment has three replications. Incubation holds at ambient (25-27 °C) for 24 hours, then the bioethanol contents were tested with chromium dichromate oxidation\(^16\).

The design of experimental was done by Completely Randomized Design (CRD) 3 × 3 factorial pattern, and the data was analyzed with ANOVA then continued with Tukey test.

### 3. Results and Discussion

#### 3.1 Indigenous yeasts identification

Three indigenous yeasts were isolated from mozzarella cheese whey and identified macroscopic and microscopically (Fig. 1).

Macroscopic identification shows similar characteristic between Yeast A and C, which consist of round-shape, white and flat colony, while the characteristic of Yeast B has irregular-shape, yellowish and texturized colony. The microscopic characteristics of Yeast A, B and C were round with pseudo-mycelium, the cell length of Yeast A and C were \(\pm 700\) µm, while Yeast B was \(\pm 500\) µm. Three indigenous yeasts isolates were identified, and the results

<table>
<thead>
<tr>
<th>Yeast Name</th>
<th>C. tropicalis (1)</th>
<th>Blast. capitatus</th>
<th>C. tropicalis (2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeast A</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Yeast B</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Yeast C</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

### Table 1 Indigenous Yeasts Identification

![Fig. 1 The isolates of Yeast A (A), Yeast B (B) and Yeast C (C) on plates, with the image of Yeast A (D), Yeast B (E) and Yeast C (F) under the microscope](image-url)
Table 1) show Yeast A and C have similarity with Candida tropicalis and Yeast B is similar with Blastoschizomyces capitatus.

Several indigenous yeasts have been isolated from cheese whey and others milk-based products. Candida tropicalis was isolated from milk products such as Serro Minas cheese from Brazil, Kumis from Colombia and Amasi from Zimbabwe. Geotrichum capitatum other names for Blastoschizomyces capitatus, has been found in milk, cheese curd and cheese brine. Both yeasts have proved to utilize the remaining milk protein in cheese whey by using their proteolytic activity.

3.2 Bioethanol production with the inoculation of indigenous yeasts and the various of sugarcane molasses concentration

Results of the treatments combinations are shown in Table 2. The production of bioethanol with the addition of 15% sugarcane molasses concentration with the inoculation of indigenous yeasts combination of C. tropicalis (1) and Blast. capitatus with ratio 1:1 (M2Y2) gave the highest bioethanol contents of 8.488%.

Bioethanol production rate is related to the cultural activities, nutrition and sugar concentration available on the substrate. The use of mixed culture could reach the optimum condition for bioethanol production from agro-industrial wastes with the addition of molasses. At the low molasses addition, bioethanol productivity was low, while the higher molasses concentration resulting in secondary effects of catabolite repression of the oxidative pathways, so that the 15% molasses addition was the maximum for the production of bioethanol. Bioethanol production kinetics inhibited if the sugar concentration was 15% or more.

The results analyzed with ANOVA and interaction between treatments of various sugarcane molasses concentrations and the combination of indigenous yeasts with the single treatment of yeasts combination have a significant effect. Meanwhile, the single treatment of molasses addition has shown non-significant effects (Table 3).

The Tukey test was conducted to determine the significance between treatments. Table 4 shows that every treatment was significantly different with others, and M2Y2 treatment is the best treatment for bioethanol production from mozzarella cheese whey with 15% sugarcane molasses addition that was inoculated with C. tropicalis + Blast. capitatus.

Table 3 Analysis of Variance of bioethanol production with the treatment of various sugarcane molasses concentration and indigenous yeasts combination

Table 4 Tukey test result for the combination of treatments of sugarcane molasses concentration and indigenous yeasts combination

Note: Sugarcane molasses addition: 10% (M1), 15% (M2), 20% (M3), and indigenous yeasts combination: Y1 = C. tropicalis 1 + Blast. capitatus + C. tropicalis 2 (1:1:1), Y2 = C. tropicalis 1 + Blast. capitatus (1:1), Y3 = C. tropicalis 1 + C. tropicalis 2 (1:1).

Note: Sugarcane molasses 10% (M1), 15% (M2), 20% (M3), and indigenous yeasts combination [Y1/ C. tropicalis 1 + Blast. capitatus + C. tropicalis 2 (1:1:1), Y2/ C. tropicalis 1 + Blast. capitatus (1:1), Y3/ C. tropicalis 1 + C. tropicalis 2 (1:1)].
4. Conclusion

Results show that the treatment combination of sugarcane molasses concentration and indigenous yeasts combination, and single treatments of indigenous yeasts combination have significant effects towards the bioethanol contents, meanwhile the treatments of molasses concentration has shown non-significant effects. The best bioethanol contents (8.49%) was gained by the treatment combination of 15% sugarcane molasses concentration with the inoculation of \textit{C. tropicalis} 1 + \textit{Blast. capitatus} (1:1).

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References

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Table 5 Tukey test result for the single treatment of indigenous yeasts combination

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Bioethanol(%)</th>
<th>Sig. (0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y2</td>
<td>7.384</td>
<td>a</td>
</tr>
<tr>
<td>Y1</td>
<td>7.112</td>
<td>b</td>
</tr>
<tr>
<td>Y3</td>
<td>6.782</td>
<td>c</td>
</tr>
</tbody>
</table>

Note: Y1 = \textit{C. tropicalis} 1 + \textit{Blast. capitatus} + \textit{C. tropicalis} 2 (1:1:1), Y2 = \textit{C. tropicalis} 1 + \textit{Blast. capitatus} (1:1), Y3 = \textit{C. tropicalis} 1 + \textit{C. tropicalis} 2 (1:1)

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