# Optimization Of Lachancea fermentati In Bioethanol Production from Sugarcane Molasses

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**Abstrct.** Molasses is a cheap carbon source with an abundance of fermentable sugars as a fermentation substrate. Microorganisms are required for the bioconversion of fermentable sugars in molasses to bioethanol. The main objective of this study is to produce bioethanol from molasses using Lachancea fermentati as the fermenter. The ability of L. fermentati to adapt to the fermentation environment was investigated by physiological characterization. The physiological characteristics consist of a mixture of NaCl, molasses and pH in the test medium. The fermentation is carried out directly by varying the molasses concentration and fermentation time. The test results show that L. fermentati can grow well at a pH of 3 to 6, NaCl concentration of 6% and molasses concentration of 15% at a temperature of 30 °C measured at 600 nm OD. The best bioethanol yield was obtained with 9.4% from 25% molasses and 9.8% at a fermentation time of 48 hours. The observation results suggest that L. fermentati has the potential to produce bioethanol from molasses if the conditions are appropriately adjusted.

**Keywords:** molasses, Lachancea fermentati, physiological characterization, fermentation and bioethanol.

## 1. Introduction

Bioethanol is an alternative and renewable energy. It has special properties, namely good octane number and cetane number, and evaporation with high heat [1]. Moreover, bioethanol as a renewable energy is expected to promote the decarbonization of liquid fuels. The production of second generation bioethanol from sugarcane molasses is an alternative energy production from agro-industrial by-products. This feedstock is sustainable because it is available throughout the year with a high sugar content as a residue, which is beneficial for alcohol production [2].

Sugarcane molasses contains 48-55% sucrose as a cheap carbon source for biorefinery. The results of physicochemical properties, folin-ciocalteu index, tannins, flavonoids and antioxidant capacity of molasses show that sugarcane molasses is a good alternative source for

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bioethanol production [3]. The largest sugarcane molasses suppliers are India, Brazil, and Thailand while Indonesia is in ninth place with 1.3 million tons of production. The Indonesian Ministry of Forestry announced that there is 443,501 hectares of sugarcane plantations in 2021, which is 3.35% more than the previous year. The sugar industry was able to produce 815,488 kL of molasses in 2019, and the Indonesian Agricultural Center expects this amount to continually increase. In conclusion, Indonesia has high potential for bioethanol production as there are abundant molasses sources.

Generally, sugarcane molasses is fermented with Saccharomyces cerevisiae to produce bioethanol. However in this study, Lachancea fermentati is used as the fermentation agent. Lactic acid is produced during molasses fermentation, and L. fermentati can grow well under these conditions [4]. Based on the research conducted by [5], L. fermentati was able to convert the fermentation substrate into 46.4 g/L ethanol with an efficiency of 82% compared to the theoretical yield. Therefore, it can be assumed that L. fermentati can effectively ferment molasses into bioethanol.

The novelty of this study is the use of L. fermentati isolated from coconut water (Cocos nucifera L.) in molasses fermentation to bioethanol. Microorganisms play an important role in fermentation. Therefore, the identification of L. fermentati from coconut water is expected to contribute to the genetic diversity of yeast with significant bioethanol production potential. Accordingly, a physiological characterization of L. fermentati was performed to determine their tolerance to the fermentation environment. This test was performed by observing the growth of L. fermentati in yeast peptone dextrose (YPD) broth containing NaCl, molasses, and pH adjustment. This test is an important point in fermentation to improve yeast performance. In addition, fermentation was carried out with the variations of molasses concentration and fermentation time. Therefore, the main objective of this study is to optimize the production of bioethanol from molasses with L. fermentati through physiological and fermentation variable tests.

## 2. Materials and Methods

### 2.1 Materials

This study was used indigenous yeast that obtained from previous research by Kasmiarti et al (2022) [6], namely Lachancea fermentati strain CNRMA8. 216. Sugarcane molasses is obtained from a sugar industry in Ogan Ilir Regency, South Sumatra.

### 2.2 Methods

#### 2.2.1 Yeast growth

L. fermentati streaked and spread on YPDA (Yeast Peptone Dextrose Agar) media and YPD broth for 24 hours in an incubator for further used.

#### 2.2.2 Physiological characterization

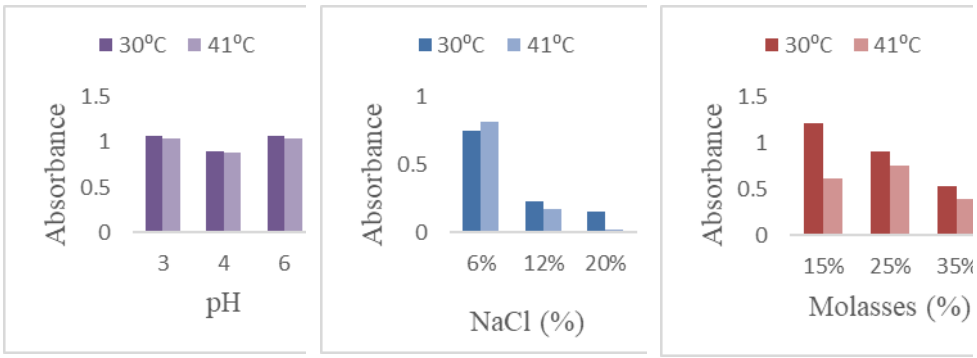
It was modified from Nasir et al (2017) [7] in which the ready yeast was grown on YPD broth with NaCl (6%, 12%, and 20%), pH (3, 4, and 6), and molasses (15%, 25%, and 35%) in incubator at 30oC and 41oC. The cell density was measured at optical density (OD) 600 nm using Orion AquaMate 8000 UV-VIS.

### 2.2.3 Fermentation

Molasses is diluted to a certain concentration to dilute its viscosity. The pH of the diluted molasses was adjusted to 4 by adding H2SO4 and incubated for 24 hours at room temperature. The mixture was centrifuged (6000 rpm) and the resulting precipitate was removed. The molasses was then sterilized by autoclaving at 121°C and 1.2 bar for 15 minutes. Anaerobic fermentation was performed in a 500 mL flask using 300 mL of molasses substrate with adjusted concentration and supplemented by 1.0 g/L KH2PO4, 1.59 g/L (NH4)2SO4, and 0.5 g/L MgSO4.7H2O, and continued by sterilization. 3 mL of yeast inoculum was mixed with the fermentation substrate. Fermentation was carried out at a temperature of 30ºC with different concentrations of 5%, 10%, 15%, 20% and 25%. Then the time was varied from 24, 30, 36, 42 and 48 hours. Bioethanol yield was measured using Orion AquaMate 8000 UV-VIS at 598.5 nm by reacting 0.5 mL of the sample with acid dichromate reagent [8].

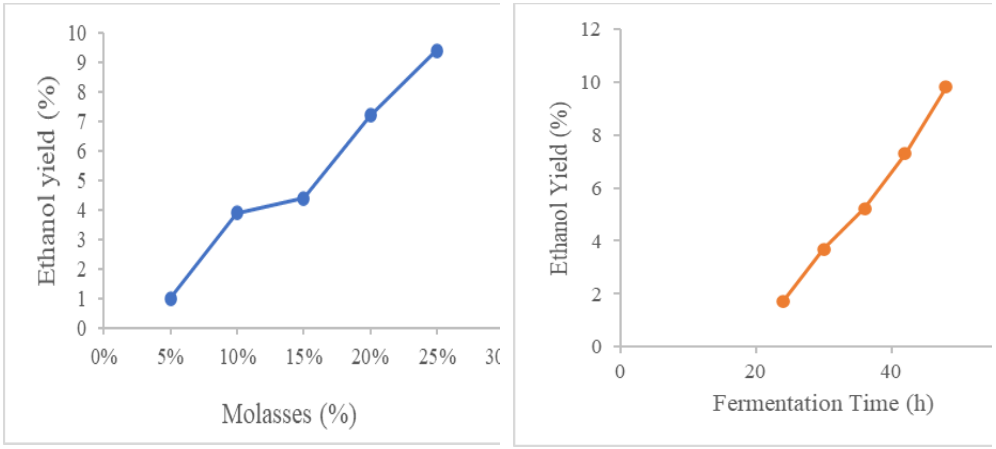
## 3. Results and Discussion

80% of the world's molasses is used to produce alcohol through biochemical processes. Molasses is generally composed of 45-60% total sugar, 20-25% reducing sugar, 25-35% sucrose, 10-16% ash, 0.4-0.8% calcium, 0.1-0.4% sodium, 1.5-5% potassium, and a pH of 5-5.5. In addition, molasses does not contain furfural compounds, which are toxic to most fermenting microorganisms [9]. Sucrose content in molasses was converted to reducing sugar by yeast during fermentation by the enzyme invertase. Minerals such as Ca, K, and Mg are also present in molasses and affect the fermentation result. The effect of calcium ions on the production of ethanol from molasses by yeast showed that a high content of metal ions can inhibit the activity of the enzyme invertase. The reduction of mineral content, especially calcium and sodium, in molasses can be done by decalcification with H2SO4 [10]. Therefore, physiological characterization was performed to investigated the yeast growth in these conditions.




(a) (b) (c)

**Figure 1.** The growth of Lachancea fermentati in YPD broth with different variations of (a) pH, (b) NaCl, and (c) molasses.




(a) (b)

**Figure 2.** The presentation of ethanol produce: (a) molasses concentrations and (b) fermentation time.

L. fermentati has good growth ability at different pH (3-6), with Figure 1(a) showing no significant differences. The addition of NaCl at a concentration of 6-20% had a strong effect on the measured density of L. fermentati cells. In Figure 1(b), L. fermentati has an osmotolerance limit of 6% NaCl content in the medium. Moreover, L. fermentati grew best at a concentration of 15%. From Figure 1(a-c), it can be seen that L. fermentati is not a thermotolerant yeast, which was growing well only at 30ºC.

The yield of bioethanol produced decreases with increasing substrate concentration (Figure 2(a)). This may be due to the ability of L. fermentati to convert sugar sources to alcohol at sufficient concentrations. The variation of fermentation time is 24 hours, 30 hours, 36 hours, 42 hours and 48 hours at the same molasses concentration of 25%. This time variation was done with the aim of seeing the effect of fermentation time on the ethanol content produced. From Figure 2(b), it can be seen that the ethanol content increased with increasing fermentation time. According to [8], the longer the fermentation time, the higher the activity of yeast in converting simple sugars to ethanol. The highest ethanol content was obtained after 48 hours of fermentation at 9.8%. The highest bioethanol content was obtained at 9.4 % at a molasses substrate concentration of 25 %.

Substrate concentration affects yeast performance in fermentation. This is because each yeast has a tolerance for sugar and alcohol content in its growth. In addition, yeast has an optimal growth period consisting of adaptation, exponential, stationary, and death phases. In this study, L. fermentati was able to ferment molasses at a concentration of 25% for 48 hours. This indicates that L. fermentati has tolerance at this value.

## 4. Conclusions

Lachancea fermentati has the physiological properties at pH 3to 6, NaCl concentration of 6% and molasses concentration of 15% at 30ºC. L. fermentati can ferment molasses to bioethanol, with a yield of 9.4% at 25% fermentation substrate and 9.8% within 48 hours of fermentation.

## 5. Acknowledgement

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