Yeast Cultivation Using Pineapple Juice

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Abstract : Cane or beet molasses is a common raw material for yeast production. Although it supplies enough sugar during the cultivation of yeast, more nitrogen, phosphate, vitamins and minerals need to be supplemented. Since pineapple is inexpensive in Thailand and can be a possible source for growing yeast, pineapple juice was studied as an alternative medium to cultivate yeast. Saccharomyces cerevisiae, as a pure culture, was inoculated into a sterile bioreactor vessel filled with pineapple juice to an initial population of 106 cells/ml. Supplementation was not used. Cultivation was performed at 30°C, air flow 1 vvm and mixing at 200 rpm. The maximum growth rate of the yeast was observed between the 2nd and the 12th hours of the fermentation course. After fermenting for 18 hours, a yeast cell concentration of 7.6x108 cell/mL was achieved, and the sugar content had decreased to 5 g/L. For comparison, cane molasses with 1 g/L of added diammonium phosphate was used as a cultivation medium for S. cerevisiae with the same operating condition and resulted in a lower growth rate of yeast and lower amount of yeast cells. In conclusion, it is promising to produce yeast from pineapple juice. These studies need to be duplicated in a pilot plant scale.

Keywords : yeast cultivation, pineapple juice, molasses

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1. Introduction

Yeasts are single cell microorganisms classified in the kingdom Fungi. Regarding the morphology, yeasts can be differentiated from bacteria by their larger cell size and their oval, elongate, elliptical, or spherical cell shapes. Typical yeast cells range from 5 to 8 □m in diameter, with some being even larger (Barnett et al., 2000; Jay et al., 2005). Production of food grade yeast as an alternative sources of protein for human nutrition has the impressive advantages for single cell protein (SCP) production compared with conventional sources of protein, e.g. soybean and meat (Anupama and Ravindra, 2000; Bekatorou et al., 2006).

Cane or beet molasses is normally used as raw materials for yeast production. Facing the threat of oil depletion and climate change, these biomaterials have been chosen as the feedstock for ethanol production. This biofuel production has an impact on food price increases, leading to recent developments of second-generation biofuels which use non-food residual biomass or non-food crops. Therefore, other possible alternatives for yeast production should be concerned (Mueller et al., 2008). The production of SCP using various strains of yeast and substrates has been studied extensively during the last two decades.

Various types of agricultural products have been optimized for yeast growth media (Paraskevopoulou et al., 2003; Silva et al., 2011; Choi and Park, 2003). Fruit wastes (Khan et al., 2010), papaya extract (Maragatham and Panneerselvam, 2011) and cheese whey (Schultz et al., 2006) were investigated for their suitability as substrates for the production of SCP with various yeast strains. Many researchers reported the potential use of the by-product from pineapple processing for single cell production (Nigam, 1999; Jamal et al., 2009) because it contains sufficient quantities of utilizable nutrients (Nigam, 1998).

Pineapple growing and processing is an established industry in Thailand, the world's production leader (Kengkhetkit and Amornsakchai, 2012). Most of pineapple produced annually is processed, canned, packaged and exported overseas. The fruit is normally available as fresh, canned and juice. The main components of pineapple juice are sugars and amino acids which can be used as a substrate for the growth of microorganisms and production of single cell protein. Since pineapple is inexpensive in Thailand, value addition to pineapple and residues from pineapple processing as an alternative medium to cultivate yeast for food grade SCP is worthwhile. Therefore, this study examined yeast cultivation using pineapple juice as a cheap raw material.

2. Materials and Methods

Yeast strain and fermentation medium

A strain of commercial wine's yeast Saccharomyces cerevisiae produced by Lallemand (Montreal, Canada) was used in the present study. Yeast cultivation was performed in two substrates of pineapple juice and molasses, containing total soluble solid 140 g/L. Diammonium phosphate was added to the molasses (1 g/L). Cultivation liquid media had a pH of 4.16-4.86.

Bioreactor and cultivation

Batch fermentations were performed aseptically in a 5-L bioreactor (Biostat B. Braun Biotech International, Germany). A medium of 2.5 L was placed in the bioreactor. The yeast inoculum was then aseptically transferred to the fermentation vessel to achieve the initial population of 1x106 cells/mL. The cultivation was carried out for 48 hours and the aeration and agitation rates were 1.0 vvm and 200 rpm, respectively. Temperature was maintained at 30 oC.

Enumeration of yeast

The viable yeast count was examined microscopically by a counting chamber slide (Thepkaew and Chomsri, 2013). Cells (450 µL) were added to 50 µL of methylene blue solution (0.4% methylene blue, 10% ethanol and 0.4 M KH2PO4) and mixed. Unstained cells were counted as live cells.

Determination of total soluble solids

The total soluble solids (TSS) was estimated by a hand refractometer (N-1□, Atago, Tokyo, Japan). The results were expressed as degree Brix (°Brix).

3. Results and Discussion

In the present work, cultivation studies on pineapple juice and molasses were carried out to examine a potential use of pineapple juice to produce food grade yeast. The experiment was carried out in a 5-L bioreactor as shown in Figure 1. Saccharomyces cerevisiae was used in this study. The cultivation was carried out for 48 hours.

Figure 1 Methodology of yeast cultivation in bioreactor Visual characteristics

Figure 2 shows yeast cell cultivation in a 5-liter bioreactor. The use of pineapple juice as a medium to grow yeast generated high turbidity which was obviously observed in the vessel compared with a molasses medium. The turbidity of the medium was higher correspondingly to the increase in cultivation time. Propagation of yeast in the medium resulted in turbid medium (Potvin et al., 1997). This visual characteristic during 24 hours of yeast cultivation was agreed with increases in cell concentrations (Figure 3b).

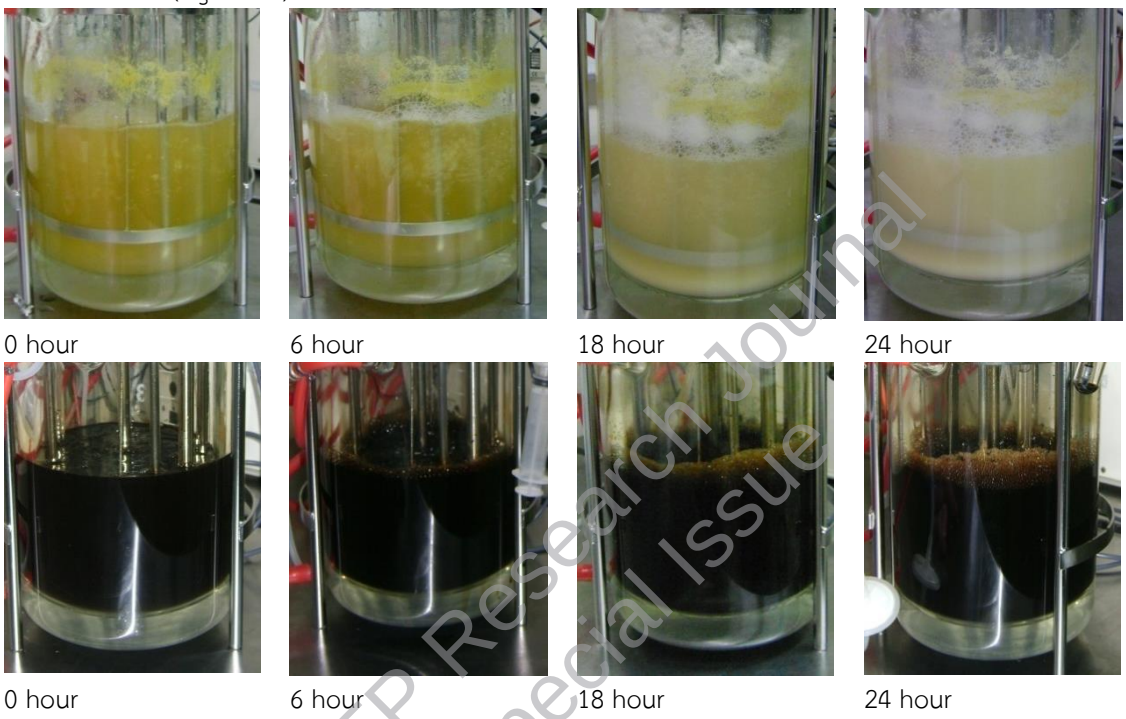

Figure 2 Cultivation of yeast with pineapple juice and molasses in 5-L bioreactor for 48 hours

Cultivation parameters

Total soluble solids and yeast cell concentrations were parameters monitored during cultivation of Saccharomyces cerevisiae in the two media, i.e. molasses and pineapple juice. Under the same experimental condition, pineapple juice as a yeast medium yielded higher maximum cell concentrations than molasses. The maximum cell concentration was produced in molasses medium at 18 and in pineapple juice medium at 24 hours. Yeast cells of 7.8x107 and 7.6x108 cells/mL were achieved and the sugar content decreased from 14 g/L to 10 g/L and 6 g/L in molasses and pineapple juice media, respectively. Substrate consumption rates of 0.17 and 0.33 g/L/h were attained in the pineapple juice and molasses, respectively. Yeast cultivation in the pineapple juice medium (4.2x107 cells/mL/h) expressed higher substrate consumption rate than cultivation in the molasses medium (3.3x106 cells/mL/h). This indicated that pineapple juice could provide a good source of microbial nutrients which yeast was able to uptake for its growth. Similar report was suggested by Nigam (1998) and Thepkaew and Chomsri (2013). The results of this study

showed that the molasses medium supplemented with 1 g/L of diammonium phosphate was not enough as a nitrogen source to support yeast cell propagation (Choi and Park, 2003; Rajoka eta al., 2006).

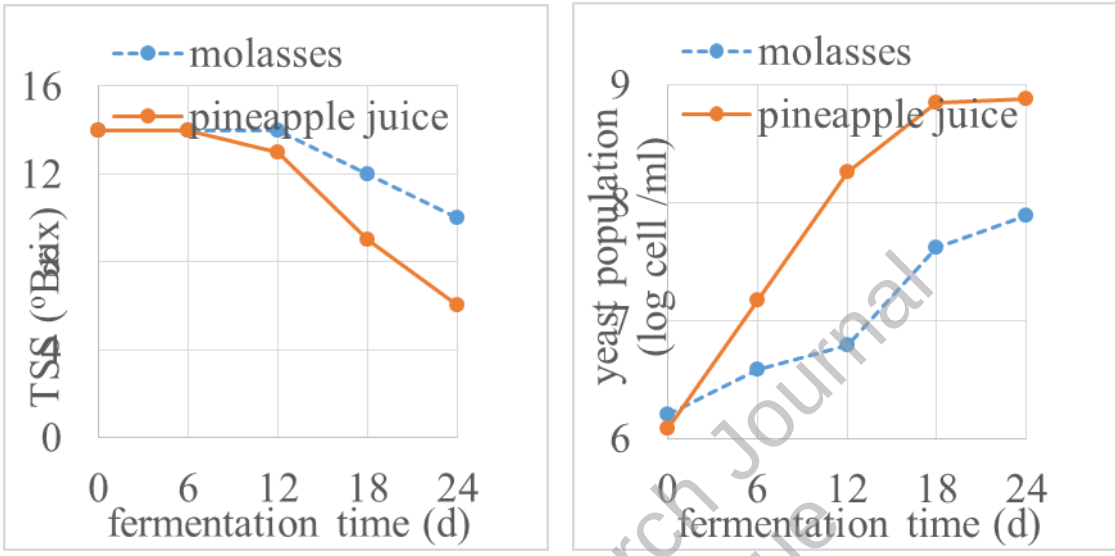

Figure 3 Changes of total soluble solids (a) and yeast cell population (b) during yeast cell cultivation in bioreactor

Finished product

Molasses and pineapple juice produced finished products containing yeast cells of 7.8x107 and 7.6x108 cells/mL and total soluble solids of 10 and 6 g/100 mL, respectively. The higher consumption rate of sugar yielded the higher yeast cell concentration. Higher growth rates gave higher yeast cell concentrations (Ghaly et al., 2005). Lower growth rate of yeast in molasses gave the lower cell concentration in the finish product.

4. Conclusion

The attempt to use pineapple juice for the production of food grade yeast reported here is promising. However, it is noteworthy to investigate other cell-growth parameters influencing yeast growth, like concentrations of assimilable nitrogen, growth factors, oxygen and different yeast strains. In particular results from other growth-substrate sources should be compared under the same conditions. Furthermore, the results from this study need to be duplicated in the pilot plant scale. In progress are studies to determine the SCP produced by other agricultural materials.

5. Acknowledgements

This work was supported by grants from Rajamangala University of Technology Lanna. The authors thank Dr. Rainer Zawadzki for revising the manuscript.

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