CHEMICAL ENGINEERING TRANSACTIONS Volume 18, 2009
Editor Jiří Klemeš
 ISBN 978-88-95608-04-4 ISSN 1974-9791
DOI: 10.3303/CET0918090

Single-step bioconversion of starch to bioethanol by the coculture of ragi tapai and *Saccharomyces cerevisiae*

[1,2*]Azlin Azmi, [1]Masitah Hasan, [2]Maizirwan Mel, [1]Cheng Ngoh
[1]Department of Chemical Engineering, University of Malaya,
50603 Kuala Lumpur.
[2]Biotechnology Engineering, Kulliyah of Engineering,
International Islamic University Malaysia, Jalan Gombak, 53100 Kuala Lumpur.
*Corresponding author email: azlinsu76@iiu.edu.my

The objective of this preliminary experiment was to test the hypothesis that coculturing commercialised ragi tapai with S. cerevisiae might have improved the ethanol production and reduce the accumulation of inhibitory concentration of reducing sugar and this would enhance the amylolytic activity. Coculture of a commercialised ragi tapai with *S. cerevisiae* using unhydrolysed raw starch in single step-fermentation produced a high ethanol concentration. A 35.26 g/L of ethanol was produced when the starch was inoculated with ragi tapai and cocultured it with *S.cerevisiae* 1 h later. This was 48% higher than the yield obtained when the starch was inoculated with ragi tapai only (23.79 g/L). The glucose concentration was maintained at low concentration in the coculture medium compared to the medium with ragi tapai only. However, stachyose concentration profile was higher in cocultured medium as compared to the one with ragi tapai only. In conclusion, coculture ragi tapai with *S. cerevisiae* enhanced the ethanol production and prevented the inhibitory effect of reducing sugars on amylase activity.

Keywords: Cassava starch; bioethanol; glucose; ragi tapai; *Saccharomyces cerevisiae*, single-step bioconversion.

1. Introduction

The use of bioethanol has several advantages. It is not only mitigate the air pollution and reduce the green house gas emissions, but also can reduce imported oil and refined gasoline thus creates the energy security and varied energy portfolio. Furthermore, bioethanol industry will provide a range of economic especially in agriculture sector and social benefits.

One of the alternative ways in producing bioethanol is by production from starchy materials. Using cassava starch as substrate in bioethanol production will reduce cost of the production of bioethanol since cassava plants are abundant, cheap and easily

Please cite this article as: Azmi A.S., Hasan M., Mel M. and Cheng N.G., (2009), Single-step bioconversion of starch to bio-ethanol by coculture of tapai ragi and saccharomyces cerevisiae, Chemical Engineering Transactions, 18, 557-562 DOI: 10.3303/CET0918090

planted. Cassava based fuel ethanol is reported to be more energy efficient than gasoline, diesel fuel and corn based fuel ethanol but less efficient than biodiesel (Du Dai et al., 2005). Malaysia harvests about 400,000 ton per year cassava roots and most of it is for starch production, only small quantity is processed into chips for animal and negligible for direct human consumption (Onwueme, 2002).

For ethanol production, direct fermentation of starch using amylolytic yeasts offers an alternative to the conventional multistage using commercial amylases for liquefaction and saccharification followed by fermentation with yeast (Abuzeid and Reddy, 1986; Verma et al., 2000; Knox et al, 2004). However, the amylolytic yeasts capable of efficiently hydrolysis starch are very few (Knox et al, 2004). Ragi tapai or ragi tape serve as an alternative for this setback. Ragi tapai is a dry-starter culture prepared from a mixture of rice flour, spices and water or sugar cane juice/extract (Merican and Quee-Lan, 2004). It is usually used to ferment cassava/ tapioca or glutinous rice into tapai or tape, a popular Malaysian delicacy, normally consumed as desert. In Hesseltine et. al. (1988) study, from 41 starter samples from seven Asian countries, in every sample of the dry starter, at least one yeast and one Mucoraceous mold (*Mucor, Rhizopus,* and *Amylomyces*) were present with one or two of bacteria of types of cocci were present. Merican and Quee-Lan (2004) presented cell count of ragi from different origin and showed that the fungal counts is 8×10^7 cell/g to 3×10^8 cell/g, yeast counts of 3×10^6 to 3×10^7 cell/g, and bacterial count of less than 10^5 cell/g. Although wide range of organisms has been found in ragi, only a few genera were found present in the tapai or tape. This indicated that most of the other organisms are contaminants. Merican and Quee-Lan (2004) listed the yeasts and mucorale that were present in tapai which is the shot listed from ragi tapai's list.

In this study, bioconversion of unhydrolised raw cassava starch into bioethanol in a single step process by coculture of commercial ragi tapai and *Saccharomyces cerevisiae* was investigated. The amount of sugar alcohols, glucose and ethanol produced were analysed.

2. Materials And Methods

2.1 Microorganisms and culture conditions

In this experiment, the cultures used were commercialised *ragi tape* obtained from local market and industrial yeast, *Saccharomyces cerevisiae*. The culture medium containing 0.1% (w/v) peptone and distilled water with no other nutrient was added. The medium was autoclaved at 121°C for 15 minutes. The dry starter and yeasts, each was then placed into the medium and incubated at 37°C at 200 rpm for 25-30 minutes before inoculation.

2.2 Batch fermentation

Cassava or tapioca flour used in this experiment is from one brand and was obtained from local market. Ten percent of cassava flour was mixed for 5 minutes in prewarmed water (60°C) in a sterile jacketed fermenter. After 5 minutes, the temperature was raised to 70°C and mix for 1 h. After that, the temperature was maintained at 30°C. The fermenter was then inoculated with ragi tapai only, or ragi tapai and *S. cerevisiae* (for 0 h coculture), or ragi tapai first followed by *S. cerevisiae* 1 h later (for 1 h coculture).

Each (ragi tapai and *S. cerevisiaei*) contains 5% (w/w) of inoculation. The agitation was maintained at 50 rpm.

2.3 Analytical method

Data were collected every 2 hours for the first 12 hours and every 6 hours onwards for the next 60 hours (16 data collection for each batch). The concentration of ethanol, glucose and oligosaccharides were determined by centrifuging at 5000 rpm for 30 minutes and the supernatant was analysed using SUPELCOGEL C-610H column on an HPLC (WATERS) equipped with a refractive index detector. The column was eluted at 30°C with 0.1% H_3PO_4 at 0.5 ml min^{-1}.

3. Results And Discussion

This preliminary experiment was to test the hypothesis that coculturing dry starter ragi tapai with *S. cerevisiae* might have improved the ethanol production and reduced the accumulation of inhibitory concentration of reducing sugar and this would result in an enhancement of amylolytic activity. Ragi tapai was chosen based on its ability to produce high glucose and ethanol yields direct from starch observed from previous study (Azmi et al., 2008). Even though ragi tapai was mixed with several microorganisms, at the end of fermentation only the presence of yeast and mold were observed in the medium. However the type of mold is yet to identify. When co-culture with *S. cerevisiae*, the dominant microorganisms was yeasts and no mold was observed (figure 1).

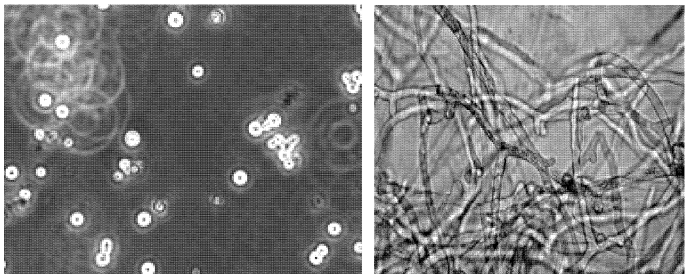

Figure 1: (Left) Image of yeast at 42 h of fermentation from 1 h coculture medium with no presence of mold. (Right) Image of mold captured from 72 h of fermentation medium with ragi tapai only.

From ethanol concentration profile as shown in figure 2, it shows that the maximum ethanol concentration from ragi tapai inoculation only produced 23.79 g/L ethanol. When simultaneously cocultured (at 0 h coculture), the ethanol production increased to 31.79 g/L at 10 h and the ethanol concentration was 27.41 g/L at 72 h. The ethanol lost might be due to ethanol converted into organic acids. Among all three batches, the highest ethanol concentration was obtained from coculture of ragi tapai with *S. cerevisiae* inoculated 1 h later. From this coculture the maximum ethanol production

was 35.26 g/L. This was 48% higher than the yield obtained with monoculture of ragi tapai (based on 23.79 g/L).

The most prominent type of reducing sugar observed from the HPLC result was glucose. The highest glucose concentration (figure 3) obtained was 31 g/L as early as 30 h after inoculation with ragi tapai only. At this time 21 g/L ethanol was produced. When coculture with *S. cerevisiae* simultaneously or after 1 h, the glucose concentration obtained was less than 6 g/L and it was maintained at that low level. This shows that *S. cerevisiae* utilised glucose immediately and produced ethanol before it can accumulate and reduce the glucose concentration. Correspondingly this inhibits the fermentation process by osmotic pressure on the cells (Bai et. al., 2008).

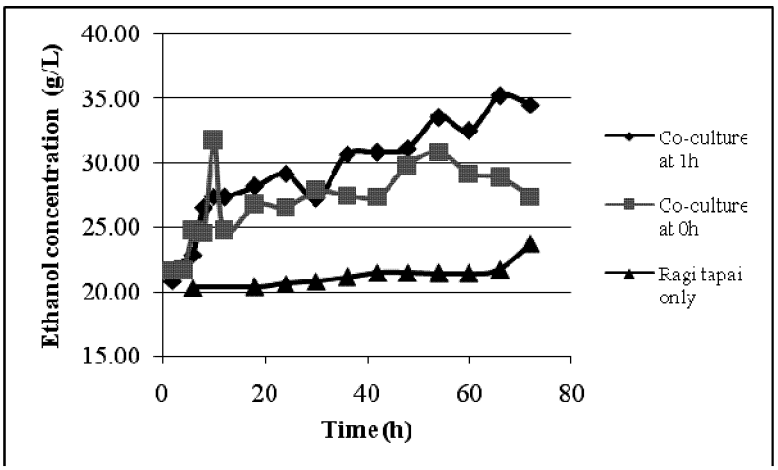

Figure 2: Ethanol concentration.

Other sugar alcohols such as maltoheptaose, maltohexaose, maltopentaose, maltotetraose, stachyose and isomaltotriose were also observed and analysed. Maltoheptaose is an oligosaccharide with the highest sugar monomer whereas isomaltotriose is the lowest sugar monomer. Out of these six sugar alcohols, only stachyose was consistently produced during all three fermentations as shown in figure 3. In the fermentation with ragi tapai without coculture with *S.* cerevisiae, the stachyose concentration was the lowest. However, this concentration profile increased when cocultured with *S.cerevisiae*. This phenomenon may be due to high cell number from extra 5% (w/w) of *S. cerevisiae.* The yeast strain alone could have degraded starch into stachyose. It might also due to low glucose concentration of coculture medium and this enhanced the amylolitic activity and thus results in more stachyose production. There was no maltoheptaose, maltohexaose, maltopentaose and maltotetraose observed from the medium inoculated with ragi tapai only. On the other hand, maltopentaose was produced after 54 h of fermentation at about 7 – 9 g/L in both cocultured medium with

no production of maltoheptaose and maltohexaose. Maltotetrasoe and isomaltotriose had been produced inconsistently throughout the fermentation in the cocultured medium.

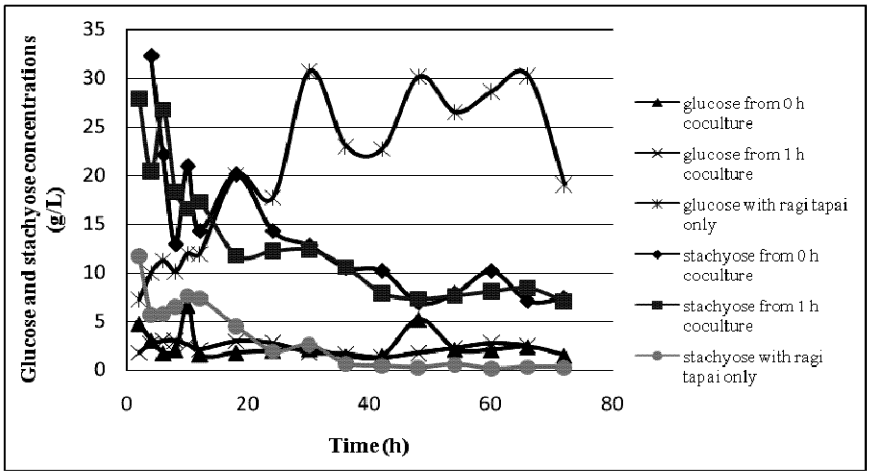

Figure 3: Glucose and stachyose concentrations.

4. Conclusion

The production of ethanol directly from fermentation of starch can be carried out using ragi tapai. This production can be enhanced by coculturing ragi tapai with *S. cerevisiae* which gave rise to ethanol yield by 48% higher than that without coculture. The glucose concentration was lower in the coculture and had prevented the inhibitory effect of reducing sugars. Stachyose concentration profile shows that the process enhanced the amylolytic activity to produce more sugar while maintaining the sugar concentration at low level.

Simultaneous single step bioconversion from unhydrolised raw cassava starch into ethanol will not only reduce the cost of enzyme that is used in liquefaction and saccharification steps but it will also reduce the substrate inhibition especially on yeast cells. The sugar that released from fermentation of starch would be consumed immediately by yeast cells before it could accumulate and reduce sugar concentration, and correspondingly inhibited the fermentation process by osmotic pressure on the cells.

5. Acknowledgment

Sincere thanks to Ministry of High Education, Malaysia for the research fund (FRGS 0207-48).

References

Abuzied, M. M., Reddy, C.A., 1986. *Direct fermentation of potato starch to ethanol by cocultures of Aspergillus niger and Saccharomyces cerevisiae.* Applied and Environmental Microbiology, pp. 1055-1059.

Azmi, A.S., Hasan, M., Mel, M., 2008. *Screening of microbes for producing ethanol from cassava starch.* 15th Regional Symposium on Chemical Engineering (RSCE) in conjuction with 22nd Symposium of Malaysian Chemical Engineers (SOMChE), Vol I, pp.177-181.

Bai, F.W., Anderson, W.A., Moo-Young, M., 2008. *Ethanol fermentation technologies from sugar and starch feedstocks.* Biotechnology Advances 26, pp. 89-105.

Du Dai, Zhiyuan Hu, Gengqiang Pu, He Li and Chengtao Wang, 2005. *Energy efficiency and potential of cassava fuel ethanol in Guangxi region of China*, Energy Conversion and Management, Vol 47, Issues 13-14, pp 1689-1699.

Hesseltine, C.W., Rogers, R., Winarno, F.G., 1988. *Microbiological studies on amylolytic oriental fermentation starters.* Mycopathologia 101, pp. 141-155.

Knox, A.M., du Preez, J.C., Kilian, S.G., 2004. *Starch fermentation characteristics of Saccharomyces cerevisiae strains transformed with amylase genes from Lipomyces kononenkoae and Saccharomycopsis fibuligera.* Enzyme and Microbial Technology 34, pp. 453–460.

Merican, Z., Quee-Lan, Y., 2004. *Tapi processing in Malaysia: A technology in transition.* Industrialization of Indigeneous fermented foods, pp.247-270, Marcel Dekker Inc., New York.

Onwueme I. C., 2002, *Cassava in Asia and the Pasific*, CAB International 2002. www.ciat.cgiar.org/downloads/pdf/cabi_07ch4.pdf (15/5/2008)

Verma, G., Nigam, P., Singh, D., Chaudhary, K., 2000. *Bioconversion of starch to ethanol in a single-step process by coculture of amylolytic yeasts and Saccharomyces serevisiae 21.* Bioresource Technology, pp. 261-266.