# EFFECT OF MIXING ON A LAB-SCALE BIOREACTOR PRODUCTIVITY

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## ABSTRACT

In this paper, we study the impact of variable mixing conditions arising from the different sets of aeration rate and stirrer speed on the ethanolic fermentation process, which utilizes the hydrolyzed cassava starch as carbon source. Interestingly, over the ranges of aeration rate and stirrer speed used in the study, the ethanol yield varied from 10% to 85% of theoretical maximum yield. Additionally over these experimental conditions, the selectivity of ethanol over glycerol varied from 3.6 to 12.3. One conclusion that can be drawn from this experimental study is that, the large variations in yield, selectivity and ethanol formation rate were more likely due to the different mixing conditions resulting from different values of aeration rate and stirrer speed, and less likely due to glucose and growth rates as previously reported.

## INTRODUCTION

The production of bio-ethanol as an alternative liquid fuel especially for transportation sector started in the mid 1970s due to serious oil crisis as well as growing environmental concerns, for example global warming (Olsson and Hägerdal, 1996). These ongoing phenomena have lead to a challenge on the search for new technologies beyond that of corn technology to produce biofuels, such as ethanol, from lignocellulosic materials (Starzak *et al.,* 1994). The conversion of lignocellulosic materials to ethanol is very promising and thus, could lead to economical and environmental benefits (Yazdani and Gonzalez, 2007). However, the complete realization of lignocellulosic ethanol technology is currently very slow due to various obstacles where one of them is arising from the very difficult pre-treatment process i.e. to convert lignocelluloses into fermentable sugars. In this regard, the lignocellulosic technology development can be viewed as a way for meeting long-term objective in producing bio-ethanol at large-scale replacing fossil fuels. Thus, a mid-term solution to fill this gap in producing bioethanol is to search for suitable resources other than corn starch and sugarcane e.g., cassava and sago starches are other promising sources for bio-ethanol production.

Cassava is regarded as one of the main raw material to produce ethanol since it can be easily cultivated and has high carbohydrate content. Moreover, cassava is able to yield 3-15 tons/ha in an agricultural environment and even 20-40 tons/ha in an extensive cultivation (Daubresse and Ntibashirwa, 1987). Due to its high drought tolerance and

low demand for nutrients, it can produce acceptable yields even under marginal environmental conditions (Cock, 1982; Stupak *et al.,* 2006).

One of the microorganisms that have been utilized by main industrial ethanol producers is *Saccharomyces cerevisiae* or Baker's yeast (Zaldivar *et al.,* 2001; Cot *et al.,* 2007). Besides that, the *S. cerevisiae* is one of the most vital and most ubiquitous organisms used in the studies of metabolic function and regulation in fermentation processes (Wittmann *et al*., 2005).

Mixing has long been recognized as a critical factor that can strongly affect the reactor performances such as, product yield and productivity. In fermentation process, mixing can be achieved through the use of either mechanical or pneumatic stirrer (i.e. aeration) or combination of both. Application of combined mechanical stirrer and aeration is often adopted in fermentation in which case, the former is mainly to promote mixing and the latter is to improve cell viability and reduce by-product formation. It has been known in ethanolic fermentation that the use of aeration strategy to promote the so-called micro-aerobic condition can significantly reduce the amount of glycerol formation which is undesirable by-product. While high mechanical stirrer speed may lead to more homogeneous mixing, some studies showed that excessive mixing could lead to high turbulent conditions which are detrimental to the living cells – thus leading to poor bioreactor performance. Recent studies have normally focused on the individual impact of either aeration rate or stirrer speed on bioreactor performance. Thus, the significance of their combined impact on performance has frequently been overlooked.

The key aim of the work described in this paper is to study the impacts of aeration rate and stirrer speed on the micro-aerobic fermentation behaviours (e.g. yield, ethanol over glycerol selectivity and fermentation kinetics) in a lab-scale batch bioreactor using the hydrolyzed cassava starch as carbon source. Note that, the application of micro-aerobic fermentation technique is important because it could provide improvement to ethanol tolerance of yeasts and thus, leading to increased yeast cell permeability and overall fermentation rates (Hoppe and Hansford, 1984). Micro-aerobic fermentation will divert carbon from glycerol formation mainly towards biomass formation, whereas an additional nitrogen limitation caused a shift from biomass synthesis towards ethanol formation (Franzen *et al.,* 1997). It is important to point out that the aeration rate and stirrer speed both have strong influence on the mixing conditions in the bioreactor, which further affect the yield, productivity and formation of by-products particularly glycerol.

## MATERIALS AND METHODS

### Materials and Instruments

The fermenter used was the BIOSTAT A plus 2L, MO-Assembly. Industrial Baker's yeast was utilized as the inoculum culture with glucose as the substrate. For the analysis of cassava as medium, 1.5L of fermentation medium was prepared by adding 0.75L of solution medium containing 1.5g yeast extract, 3.75g $NH_4Cl$, 4.37g $Na_2HPO_4$, 4.5g $KH_2PO_4$, 0.38g $MgSO_4$, 0.12g $CaCl_2$, 6.45g citric acid and 4.5g sodium citrate into the

0.75L of hydrolyzed cassava starch in the fermenter. The medium culture was sterilized at 121$^{o}$C for 20 minutes and then cooled down under room temperature. Next, the aeration rate and stirrer speed were set-up at certain values. Then, 40mL of fresh yeast inoculum was added into the fermentation medium in the bioreactor. The fermentation process was kept at 30°C and pH 5 for approximately 72 hours and the samples were taken every 2-3 hours.

### Cassava Starch Hydrolysis

Acid hydrolysis has been used to modify the starch granule structure and to produce soluble products with altered gelatinization behaviour (Ferrini *et al.*, 2008). In this experiment, 150g of fresh cassava starch (powder) was added into 0.75L of 0.1M (i.e. 0.2N) sulphuric acid solution. Both were mixed evenly before sterilization at 121$^{o}$C for 45 minutes in order to break down the starch into fermentable sugars especially glucose. The hydrolyzed cassava starch was then cooled to room temperature.

### Sampling and Analysis

Samples were analyzed for the concentrations of glucose (total reducing sugars), ethanol, glycerol and biomass optical density. The glucose, ethanol and glycerol were analyzed using enzymatic test-kits (R-Biopharm) and UV-VIS spectrophotometer (Perkin Elmer, Lambda 25).

## RESULTS AND DISCUSSION

### Effect of Aeration Rate and Stirrer Speed on Glucose, Ethanol and Glycerol Concentrations

Four different sets of experiments were conducted to study the impact of different aeration rates and stirrer speeds on the production of ethanol in the lab-scale bioreactor. Table 1 displayed the aeration rate and stirrer speed setup for the four different sets of experiments conducted.

Tab. 1: Aeration Rate and Stirrer Speed Setup

| Run | Aeration Rate (LPM) | Stirrer Speed (rpm) |
|---|---|---|
| Set 1 | 1.0 | 150 |
| Set 2 | 1.5 | 150 |
| Set 3 | 1.0 | 250 |
| Set 4 | 1.5 | 250 |

Figure 1 demonstrates the profile of glucose concentrations under different sets of aeration rates and stirrer speeds – i.e. glucose consumption rates. Notice that, the glucose consumption rates under Set 2 and Set 3 were quite comparable. Interestingly, the consumption rate exhibited distinctively different behaviours from that under Set 1 and Set 4. While under the former the consumption rate apparently showed two phases, under the latter the rate showed only single phase. Also the glucose consumption rate under Set 4 seemed to be slowest among the 4 sets of aeration rate and stirrer speed.

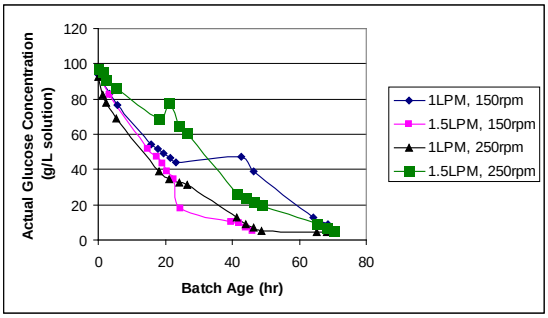


Fig. 1: Graph of Actual Glucose Concentration (g/L solution) vs Batch Age (hr)

With different amount of glucose utilized under different aeration rates and stirrer speeds, different amount of ethanol and glycerol were expected to be produced. Figure 2 showed the ethanol concentration profiles (i.e. ethanol formation rates) under different aeration rates and stirrer speeds. It can be seen that the highest ethanol formation rate and final ethanol concentration achieved was under Set 3, followed by the ethanol formation rate under Set 4, then under Set 1 and the slowest one was under Set 2.

Comparison of rates under Set 1 and Set 3 (i.e. both at 1 LPM) or under Set 2 and Set 4 (i.e. both at 1.5 LPM) reveals that increase in stirrer speed from 150 rpm to 250 rpm led to significant increase in ethanol formation rate and final ethanol concentration. On the other hand, at constant stirrer speed either at 150 rpm or 250 rpm, the increase in aeration rate from 1 LPM to 1.5 LPM led to significant reduction in ethanol formation rate.

But it is interesting to note, although the glucose consumption rates under Set 2 and Set 3 were comparable, the ethanol formation rates under two conditions were very distinctive. In this case, the final amount of ethanol produced varied from 5 g/L under Set 2 to 41g/L under Set 3.

These results suggested that both stirrer speed and aeration rate have very significant effects on ethanol production rate, and thus the different rates were less likely due to glucose consumption rate.

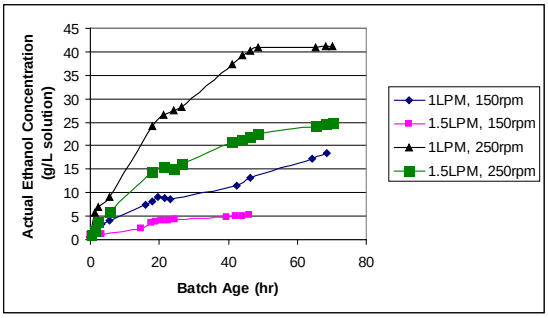


Fig. 2: Graph of Actual Ethanol Concentration (g/L solution) vs Batch Age (hr)

Figure 3 displays the glycerol concentration profiles, which varied significantly with the different experimental setups of aeration rate and stirrer speed. Set 1 produced the highest amount of glycerol and Set 2 produced the least glycerol i.e. lowest final glycerol concentration.

Set 3 and Set 4 produced significantly amount of glycerol with comparable glycerol formation rates which are in between those of Set 1 and Set 2. Unlike glucose and ethanol formation rates, the glycerol formation rates showed inconsistency with respect aeration rate and stirrer speed. Whereas increase in aeration rate from 1 LPM to 1.5 LPM at constant stirrer speed led to reduction in ethanol formation rate, the glycerol formation rate could either decrease or increase depending on the stirrer speed. Thus, at stirrer speed of 150 rpm an increase in aeration rate led to significant decrease in glycerol (i.e. as expected) but at 250 rpm an increase in aeration rate led to unexpected increase in glycerol.

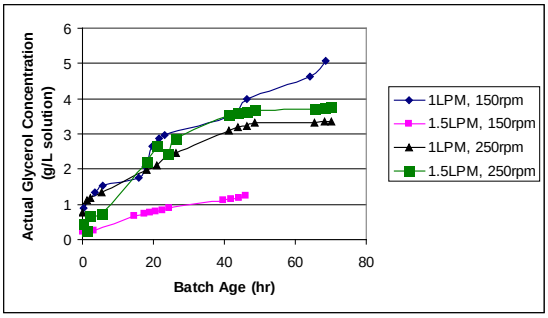


Fig. 3: Graph of Actual Glycerol Concentration (g/L solution) vs Batch Age (hr)

Finally, Figure 4 showed the optical density profiles, where comparable trends (i.e. rates of growth) can be observed. Recall that Set 2 and Set 3 also produced comparable glucose consumption rates (see Figure 1). So, this suggests that the different rates of ethanol and glycerol formations (Figures 2 and 3) were unlikely due to the different glucose consumption and growth rates as suggested previously by Cot *et al* (2007). In this case, the difference in these products formation rates were probably due to the links of growth rate and ethanol production rate to cell viability. Higher rate of ethanol production would lead to lower cell viability. This phenomena could be due to the

inhibition of ATP synthesis or leakage of metabolites from the yeast cells while the yeast cells were metabolically active (Ghareib *et al.*, 1988; Koukou *et al.*, 1990; Alexandre *et al.*, 1994; Cot *et al.*, 2007). Loss of membrane integrity will occur and caused damages to the plasma membrane and will eventually decrease ethanol tolerance and cell death (Koukou *et al.*, 1990; Alexandre *et al.*, 1994; Chi and Arneborg, 1999; Cot *et al.*, 2007).

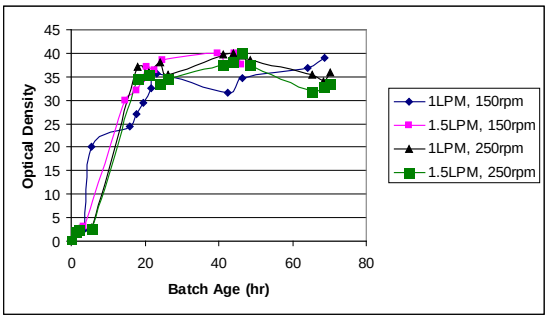


Fig. 4: Graph of Optical Density vs Batch Age (hr)

Based on the results above, it can be observed that different amount of ethanol and glycerol were produced with different aeration rates and stirrer speeds. In general as ethanol increases, glycerol also increases. This statement is validated on the basis of knowledge of the biological role of glycerol by *S cerevisiae*. Glycerol is a byproduct of fermentation to ethanol in a redox-neutral process in *S. cerevisiae* (Wang *et al.*, 2001). Glycerol, not only maintain the redox balance but also maintain the osmoregulation in yeast cells (Wang *et al.*, 2001). Glycerol flux across the plasma membrane of *S. cerevisiae* is controlled either by passive diffusion, by a channel protein (van Aelst *et al.*, 1991; Luyten *et al.*, 1995; Sutherland *et al.*, 1997; Wang *et al.*, 2001) or by an active uptake mechanism (Holst *et al.*, 2000; Wang *et al.*, 2001).

The yeast cells would increase the rate of glycerol productivity in response to decreased extracellular water activity. Under this phenomenon of hyperosmotic stress in the yeast cells, glycerol is conserved within the cells to maintain osmotic equilibrium with the external environment (Wang *et al.*, 2001). Thus, with the increment of ethanol production in the medium, glycerol production would also increase at the same time (but selectivity of ethanol over glycerol depends strongly on aeration rate) in order to overcome hyperosmotic stress within the cells. This statement could be well justified, whereby a decrease in ethanol yield was observed when the glycerol formation is reduced in a micro-aerobic ethanolic fermentation in continuous culture by a carefully controlled oxygenation (Bideaux *et al.*, 2006).

Thus, the above suggestions explain the differences in glucose, ethanol and glycerol concentrations, besides partly due to metabolism being affected by hydrodynamic factors arising from different combination of aeration rate and stirrer speed.

## Effect of Aeration Rate and Stirrer Speed on Ethanol Yield (g.ethanol/g.glucose)

Table 2 showed the summary of maximum ethanol yield achieved for the different aeration rate and stirrer speed. There was an increment of more than two fold (117.6%) of the maximum yield at aeration rate of 1LPM when the stirrer speed was increased from 150rpm to 250rpm. On the other hand, the maximum yield value increased by 378.9% at aeration rate of 1.5LPM when the stirrer speed was increased from 150rpm to 250rpm. Higher stirrer speed would result in higher mass transfer rate within the bioreactor, thus enhanced the ethanol production rate and yield and this result was consistent with the previous reports (Anderasen and Stier, 1953; Nagy *et al.*, 1992; Chabes *et al.*, 2000; Snoek and Steensma, 2007). But at higher aeration rate, the increase in stirrer speed would result in more drastic increase in ethanol yield. On the other hand, the maximum yield decreased when the aeration rate increased from 1LPM to 1.5LPM for both stirrer speeds. From the results, it is suggested that higher yield will be achieved with higher stirrer speed and lower aeration rate. It could be expected that both aeration rate and stirrer speed gave significant influence on the mixing mechanism in the fermentation process, which gave rise to different yield values. Here, Set 3 gave the highest ethanol yield which was 85% of theoretical maximum yield.

Tab. 2: Maximum Yield and Percentage Yield (Maximum Yield with Respect to Theoretical Maximum Yield) under Different Set of Aeration Rate and Stirrer Speed

| Run | Yield (g-ethanol/g-glucose consumed) | Percentage Yield (%) |
|---|---|---|
| Set 1 | 0.215 | 39.1 |
| Set 2 | 0.057 | 10.4 |
| Set 3 | 0.468 | 85.1 |
| Set 4 | 0.273 | 49.6 |

## Effect of Aeration Rate and Stirrer Speed on Selectivity

Table 3 showed the selectivity values for all experimental setups. This shows that selectivity was lower under the influence of stirrer speed of 150rpm. On the other hand, selectivity was higher for stirrer speed under 250rpm. For Sets 1 and 2, the selectivity values were quite comparable but there were many differences between Sets 3 and 4, whereby the differences was around 85%. On the other hand, comparing Sets 1 and 3, Set 1 (with lower stirrer speed) gave lower selectivity. For Sets 2 and 4, Set 2 (with lower stirrer speed) have lower selectivity as well. Just like in the case of ethanol yield, it can be concluded that both aeration rate and stirrer speed also played an important role in determining selectivity.

Tab. 3: Selectivity with Respect to Aeration Rate and Stirrer Speed

| Run | Selectivity (Ethanol Yield/ Glycerol Yield) |
|---|---|
| Set 1 | 3.62 |
| Set 2 | 4.18 |
| Set 3 | 12.34 |
| Set 4 | 6.67 |

## CONCLUSION

In this paper, the simultaneous effects of stirrer speed and aeration rate on the ethanolic fermentation kinetics (i.e. growth, substrate consumption and product formation rates) using hydrolyzed cassava starch were studied i.e. using complex medium. Cassava has large potential to be one of the main raw materials to produce ethanol as an alternative fuel in the future. Results of the study suggests that ethanol yield, selectivity (i.e. ethanol yield/glycerol yield), ethanol and glycerol formation rates are strong functions of both of aeration rate and stirrer speed. A conclusion that can be made from the experimental study is that the variations in yield, selectivity and product formation rate are more likely due to the change in the mixing conditions arising from different values in aeration rate and stirrer speed. In this case, these variations are less likely due to the growth and glucose consumption rates as previously suggested by other investigators. In future, a CFD study will be conducted in order to gain greater insight into how different mixing patterns, which arise from different set of aeration rate and stirrer speed can affect the yeast metabolism – thus the fermentation performance.

## ACKNOWLDGEMENT

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## BRIEF BIOGRAPHY OF PRESENTER

Dr. Samyudia joined Curtin University of Technology, Sarawak Campus in 2005 after spending twelve years as academics and researcher at McMaster University (Canada), Delft University of Technology (The Netherlands), Purdue University (USA), Murdoch University (Australia) and Institut Teknologi Bandung. He received his Ir. (*Ingenieur*) degree from Institut Teknologi Bandung in 1989 and PhD in Chemical Engineering from The University of Queensland, Australia in 1996. He has extensively been involved in industrial research in the area of process systems engineering (e.g. control, modeling and design) by securing a number of competitive research grants and industrial funding i.e. AKZO NOBEL, Shell, DOFASCO. He has supervised thirteen post-doctoral and postgraduate (PhD and Master) students and twenty honors students. He is currently supervising six postgraduate students. He has published more than 90 publications in reputable book series, international journal and conference proceedings.