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Experimental Investigation on the Impact of Aeration Rate and Stirrer Speed on Micro-Aerobic Batch Fermentation

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Abstract: The impact of aeration rate and stirrer speed on micro-aerobic fermentation is studied at laboratory scale. Result suggests that with Baker's yeast, an increase in aeration rates from very low value can improve bioethanol productivity and yield because it can enhance cell viability. At constant stirrer speed of 150 rpm, the increase in aeration rate from 1 to 1.5 LPM leads to proportional increase in peak Oxygen Uptake Rate (OUR) from 600 to 1000 $\text{mM L}^{-1} \text{h}$. This increase coincides with the increase in yield by 7.5%. Interestingly at constant aeration rate of 1.5 LPM, an increase in stirrer speed from 150 to 250 rpm leads to decrease in peak OUR from 1000 to 457 $\text{mM L}^{-1} \text{h}$ but increase in yield by 64%. In conclusion, both stirrer speed and aeration rate are important factors which have complex impacts on hydrodynamics and microbial metabolism, which in turn affect productivity and yield. Further study using CFD modeling and simulation method is required to gain better insights into how aeration rate and stirrer speed affect metabolism via the mixing mechanisms in a bioreactor.

Key words: Ethanol, fermentation, micro-aerobic, mixing

INTRODUCTION

Growing environmental concerns due to the usage and depletion of non-renewable fuel sources has indeed created vast interest in large scale production of alternative fuels such as ethanol. The production of this energy source from renewable agricultural residues or hardwood species has been predicted to substitute 20% of fossil-based fuels by ethanol within the next 15 years, but significant scientific knowledge and technological investments will be required in order to achieve this objective (Cot *et al.*, 2007).

Traditionally, *Saccharomyces cerevisiae* (Baker's yeast) has been used as the main type of yeasts in the production of alcohol and it has been widely used in industry (Snoek and Steensma, 2007). *S. cerevisiae* has been utilized by the main industrial ethanol producers because it is considered as a model organism, which is generally regarded as safe microorganism that could generate up to 20% ethanol from carbon sources (Cot *et al.*, 2007; Zaldivar, 2001). Previous experimental works suggested that microbes respond differently to an increment in glucose supply, e.g. a variable lag phase after inoculation with an uncontrolled pre-culture (Sonnleitner *et al.*, 1997). Due to the glucose increment

content, the increased flux of sugar entering the yeast cells results in an increased production of NADH, which could not be fully oxidized by the respiratory chain. In order to remove the excess NADH, production of ethanol by fermentation is required (Snoek and Steensma, 2007) i.e., the basis of aerobic alcoholic fermentation.

On the other hand, there are certain limitations which pose serious industrial challenge in the utilization of *S. cerevisiae*. The most recognized challenge to date is the inhibition of fermentation process by accumulation of ethanol (Casey and Ingledew, 1986; Cot *et al.*, 2007). This is due to the reduction of metabolic activity by decreasing glucose and ammonium uptake and the induction of stress responses. Besides that, the production of by-product (i.e., glycerol) and the reduction of cell viability in conventional anaerobic fermentation will lead to low ethanol productivity. In large-scale fermenters, it is impossible to maintain full levels of oxygenation (Snoek, and Steensma, 2007). With this challenge in mind, part of the motivation of the study described the performances of *S. cerevisiae* under different sets of micro-aerobic environmental conditions i.e., with respect to different sets of aeration rates and stirrer speeds in lab-scale batch bioreactor. Micro-aerobic fermentation is of interest in this study since this type of process could provide

improvement to the ethanol tolerance of yeast and thus, leading to increased yeast cell permeability and overall fermentation rates (Hoppe and Hansford, 1984). However, to date the study on the combined aeration and mixing factors in bioreactor have frequently been overlooked. Thus, the key aim of this study is to investigate the combined impact of both aeration and mixing in micro-aerobic fermentation process.

MATERIELS AND METHODS

Materials and instruments: The fermenter used is the BIOSTAT A plus 2 L, MO-Assembly. Industrial Baker’s yeast is utilized as the inoculum culture with glucose as the substrate. 1.5 L of fermentation medium is prepared by adding 75 g glucose, 7.5 g yeast, 3.75 g NH₄Cl, 4.37 g Na₂HPO₄, 4.5 g KH₂PO₄, 0.38 g MgSO₄, 0.12 g CaCl₂, 6.45 g citric acid and 4.5 g sodium citrate. The medium culture is sterilized at 121°C for 20 min and then cooled down to room temperature. Forty milliliter of yeast inoculum is added to the fermentation medium. Temperature and pH conditions are maintained and controlled at 30°C and pH 5, respectively. The batch process is stopped after approximately 72 h and the samples are taken in every 2-3 h analyzed for measuring the optical density, ethanol, glucose and glycerol concentrations. The presence of ethanol, glucose and glycerol are analyzed using R-Biopharm test kits and UV-VIS spectrophotometer.

RESULTS AND DISCUSSION

Effect of aeration rate and stirrer speed on glucose, ethanol and glycerol concentrations: Four different sets of experiments are conducted to study the impact of aeration rates and stirrer speeds on the production of bioethanol in a lab-scale bioreactor. Table 1 shows the aeration rate and stirrer speed setup for the four different sets of experiments conducted.

Generally, similar trends can be identified whereby there is increment in ethanol production and its byproduct, glycerol with increase in aeration rate and stirrer speed.

Figure 1 shows the glucose concentrations under different sets of aeration rates and stirrer speeds. It can be observed that the rates of the glucose consumption are quite comparable for all sets of aeration rate and stirrer speed.

Figure 2 shows the ethanol concentration profiles under different aeration rates and stirrer speeds. Notice that, the ethanol formation rate shows significantly higher value for set 4 than other sets of aeration rate and stirrer speed. The final amount of ethanol produced after 64 h varies from 6.33 to 9.91 g L⁻¹.

Table 1: Aeration rate and stirrer speed setup

Set	Aeration rate (LPM)	Stirrer speed (rpm)
1	1.0	150
2	1.5	150
3	1.0	250
4	1.5	250

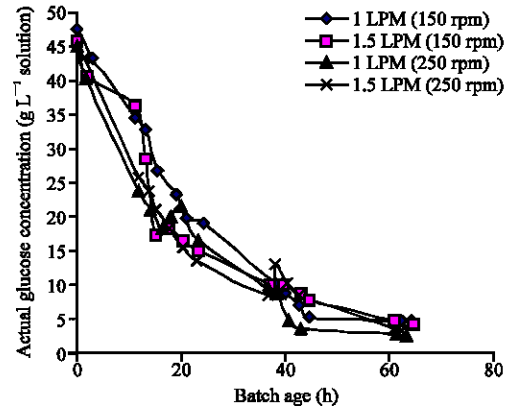


Fig. 1: Graph of actual glucose concentration (g L⁻¹ solution) vs. batch age (h)

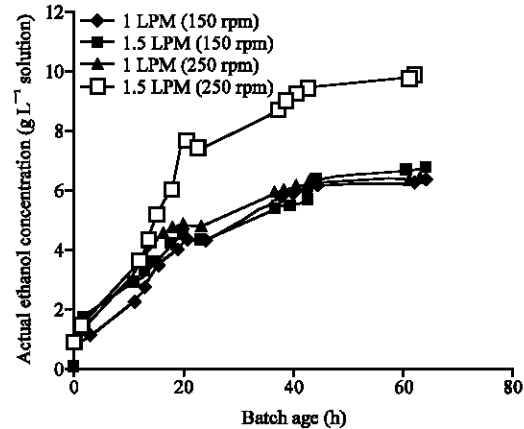


Fig. 2: Graph of actual ethanol concentration (g L⁻¹ solution) vs. batch age (h)

Figure 3 displays how the glycerol concentration profile varies with the different sets of aeration rate and stirrer speed. Interestingly, from Fig. 3, the highest production rate of glycerol seems to coincide with the highest production rate of ethanol i.e., at 150 LPM and 250 rpm (Set 4). A conclusion that can be drawn from the experimental results is that the production of glycerol is highly affected by aeration rate (i.e., stirrer speed has lesser effect). In other words, at 1.5 LPM the rate of glycerol productions at 150 and 250 rpm are almost comparable. Thus, this suggests that glycerol production seems to be dependent on aeration rate more rather than on the stirrer speed.

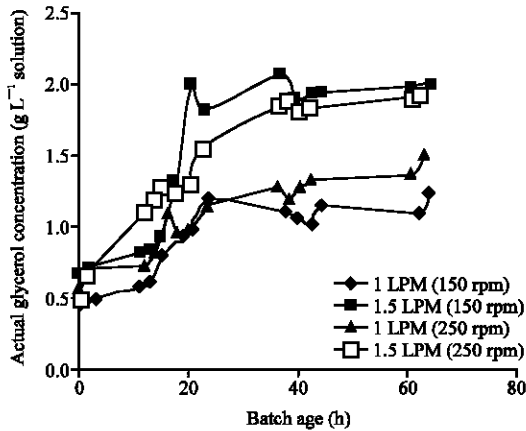


Fig. 3: Graph of actual glycerol concentration (g L^{-1} solution) vs. batch age (h)

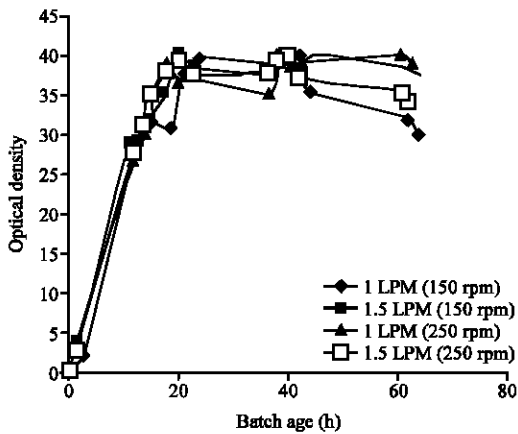


Fig. 4: Graph of optical density vs. batch age (h)

From Fig. 4, it can be seen that the rates of growth under different aeration rate and stirrer speed are also comparable. Thus, this suggests that the different rates in ethanol and glycerol formations are not likely due to the glucose and growth formation rates as previously suggested in (Cot *et al.*, 2007). The more probable explanation in this case could be due to the links of growth rate and ethanol production rate to cell viability. It has been known that the higher the rate of ethanol formation during the fermentation process, the lower is the cell viability which could be due to the inhibition of ATP synthesis or leakage of metabolites from the cells while the yeast cells were metabolically inactive (Ghareib *et al.*, 1988; Koukou *et al.*, 1990; Alexandre *et al.*, 1994a; Cot *et al.*, 2007). This activity will cause the loss of plasma membrane integrity and thus results in the damage of the plasma membrane. The membrane integrity plays an important role in ethanol tolerance, whereby with the loss of membrane integrity, it will lead to the decrease in

phospholipid content and this will affect the level of ethanol tolerance which will eventually cause cell death (Koukou *et al.*, 1990; Alexandre *et al.*, 1994b; Chi and Arneborg, 1999; Cot *et al.*, 2007).

With different stirrer speeds and aeration rates, different amount of glucose will be utilized and thus leads to different amount of ethanol and glycerol to be produced. It is indicated in Fig. 2 that Set 4 gives rise to the highest production of ethanol. On the other hand, Fig. 3 shows that the production on glycerol for Set 2, gives rise to the highest glycerol production rates among all of the experiments carried out. The difference between Sets 3 and 4 is in the value of aeration rate i.e., former has lower aeration rate than the latter. Interestingly, higher aeration rate in Set 4 than in Set 3 leads to higher ethanol concentration but significantly lower glycerol concentration in the former than in the latter i.e., improved selectivity of ethanol over glycerol by increasing aeration rate.

However, in general as ethanol increases with the increment in aeration rate and stirrer speed, the glycerol also increases. This statement is validated on the basis of knowledge of the biological role of glycerol by *S. cerevisiae*. Glycerol is produced during fermentation of glucose to ethanol to maintain the redox balance and osmoregulation in yeast cells (Wang *et al.*, 2001). The yeast cells will increase the rate of glycerol productivity with respect 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 will also increase at the same time (but selectivity 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). Therefore, with higher aeration rate and stirrer speed, higher production of ethanol and glycerol will be observed. So, a conclusion can be drawn from the results that the difference in ethanol produced is not due to glucose consumption rate alone, but also is due to metabolism being affected by hydrodynamic factors- aeration rate and stirrer speed.

Effect of aeration rate and stirrer speed on Oxygen Uptake Rate (OUR): Figure 5 shows the trend of Oxygen Uptake Rate (OUR), whereby when aeration rate is increased from 1 to 1.5 LPM for 150 rpm, it could be

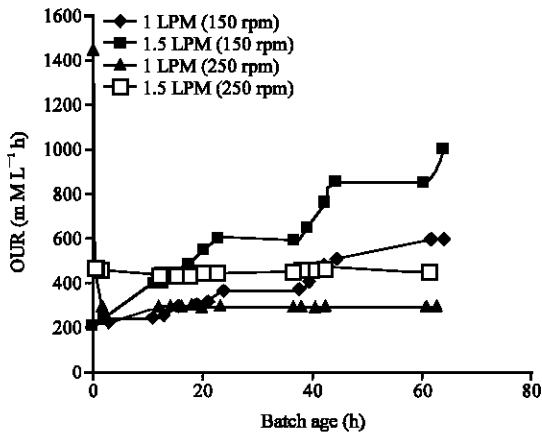


Fig. 5: Graph of OUR vs. batch age (h)

Table 2: OUR with respect to aeration rate and stirrer speed

OUR (m.M.L ⁻¹ .h)	150 rpm	250 rpm
1 LPM	600	300
1.5 LPM	1000	457

Table 3: Yield with respect to aeration rate and stirrer speed

Yield (g. ethanol/g. glucose)	150 rpm	250 rpm
1 LPM	0.13	0.15
1.5 LPM	0.14	0.23

observed that the OUR value increases as the highest OUR value peaks at 1000 mM L⁻¹ h as compared to 1 LPM, which peaks at 600 mM L⁻¹ h. On the other hand, OUR value also increases from 1 to 1.5 LPM for 250 rpm as well.

Table 2 shows the summary results of the highest value of OUR for different aeration rate and stirrer speeds.

Effect of Aeration Rate and Stirrer Speed on Maximum Yield (g.ethanol/g.glucose): Table 3 shows the summary of maximum yield achieved with respect to different aeration rate and stirrer speed. Based on Table 3, there is an increment of around 7.5% on the maximum yield for stirrer speed of 150 rpm when the aeration rate is increased from 1 to 1.5 LPM. On the other hand, the maximum yield value increases by 64% when the stirrer speed was increased from 150 to 250 rpm for constant aeration rate of 1.5 LPM. Higher stirrer speed will result in higher mass transfer rate, i.e., oxygen transfer within the bioreactor. Higher oxygen transfer will enhance ethanol production since several biosynthetic pathways require molecular oxygen, such as sterols, unsaturated fatty acids, pyrimidines and deoxyribonucleotides (Anderasen and Stier, 1953; Chabes *et al.*, 2000; Nagy *et al.*, 1992; Snoek and Steensma, 2007). From the results, it is apparent that higher yield will be achieved with higher aeration rate as well as higher stirrer speed.

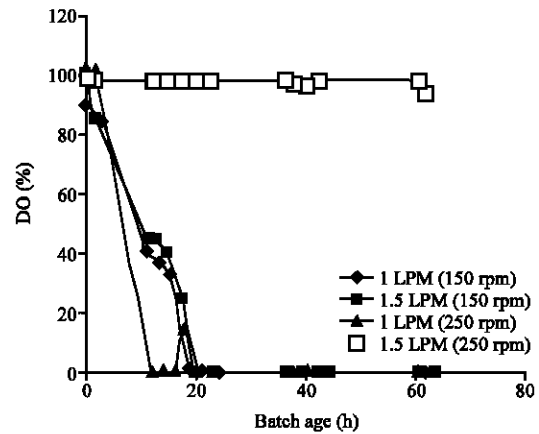


Fig. 6: Graph of DO vs. batch age (h)

Overall, it is indicated that *S. cerevisiae* is able to produce approximately 12.67% ethanol under 1 LPM, 150 rpm, 13.59% ethanol under 1.5 LPM, 150 rpm, 12.78% ethanol under 1 LPM, 250 rpm and 19.81% ethanol under 1.5 LPM, 250 rpm. Thus, with higher aeration rate and stirrer speed, ethanol is able to be produced more efficiently.

Effect of aeration rate and stirrer speed on Dissolved Oxygen (DO): Figure 6 shows the trend of Dissolved Oxygen (DO), whereby the dissolved oxygen tends to decrease with respect to time. As observed, the trend is almost similar for Set 1, 2 and 3. However, different trend is observed for Set 4, whereby the DO does not decrease drastically as compared to other conditions. This difference could be due to conditions that are closed to aerobic fermentation, whereby it is suggested that stirrer speed could lead to drastic shift from micro-aerobic to aerobic condition.

CONCLUSION

As a conclusion, the results suggested that ethanol production is highest for 1.5 LPM and 250 rpm stirrer speed (Set 4) with approximately 20 % ethanol production. It is indicated that ethanol and glycerol productions are highly affected by the aeration rate and stirrer speed in the bioreactor, as both of these parameters will affect the mixing mechanisms between the culture medium and microorganism. Significant differences in productions of ethanol and glycerol could be due to the hydrodynamic factors particularly under different stirrer speeds and aeration rates. Under the range of experimental conditions adopted in this study, this difference is probably not due to glucose consumption rate and growth rate i.e. because growth and consumption rates are comparable for all sets of experiments.

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