Kasetsart J. (Nat. Sci.) 42 : 285 - 293 (2008)

Optimization of Agitation Conditions for Maximum Ethanol Production by Coculture

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ABSTRACT

The coculture of *Saccharomyces cerevisiae* and *Candida tropicalis* was used for batch ethanol production in a synthetic medium containing 20 g/l glucose and xylose as carbon source. After incubation at 30 °C for 18 h at glucose and xylose ratio 1:0, 8:1, 6:1, 4:1, 2:1, 1:1 and 0:1, the yield of ethanol (Y_p/S_s) were 0.35, 0.35, 0.34, 0.32, 0.32, 0.27 and 0.05 g/g, respectively. The yield of cell mass were 0.12, 0.09, 0.08, 0.08, 0.10, 0.09 and 0.10 g/g, respectively. The results indicated that the coculture could produce the maximum level of ethanol at the mixture of glucose and xylose ratio 8:1. Batch fermentation with agitation rate 0-200 rpm was studied using mixed glucose and xylose (8:1) under the same condition. The results showed that the yields of ethanol (Y_p/S_s) were 0.33, 0.38, 0.37, 0.35 and 0.33 while the yields of cell mass were 0.04, 0.04, 0.05, 0.09 at the agitation rate of 0, 50, 100, 150 and 200, respectively. The results suggested that the agitation rate of 50 rpm was suitable for ethanol production by the coculture from the mixed sugars.

Key words: ethanol, coculture, agitation

INTRODUCTION

Saccharomyces cerevisiae and *Candida tropicalis* are facultative microorganisms. Agitating the fermentation broth normally satisfy the oxygen demand of a fermentation process. Among other factors having an impact on the operating conditions during fermentation in bioreactors is agitation and mixing. Agitation is important for uniform mixing of the medium components within the fermentor (dispersion of cells and nutrients) as well as mass transfer phenomena (e.g., oxygen transfer rates). The effect of agitation on ethanol production is important for the successful progress of the fermentation. Agitation is important for adequate mixing, mass transfer and heat transfer. It not only assists mass transfer between the different phases present in the culture, but also maintains homogeneous chemical and physical conditions in the culture by continuous mixing. Agitation creates shear forces, which affect microorganisms in several ways, causing morphological changes, variation in their growth and product formation and also damaging the cell structure (Mittal, 1992). Aeration could be beneficial to the growth and performance of microbial cells by improving the mass transfer characteristics with respect to substrate and product/by-product. The morphology of the microorganism can strongly influence the product formation, since it affects broth rheology and consequently the mass and heat transfer capabilities of the fermentation broth (Atkinson and Mavituna, 1985). The purpose of this study

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was to investigate the growth kinetics of *Saccharomyces cerevisiae* and *Candida tropicalis* and ethanol production at different agitation rates at atmospheric pressure to improve cell mass and ethanol productivity. Agitation is of prime importance in an fermentation process. This study reports the effect of various agitation rate combinations on the mixed culture and mixture of glucose and xylose as a part of the ultimate goal of optimizing growth rate and ethanol yield for *Saccharomyces cerevisiae* and *Candida tropicalis*.

MATERIALS AND METHODS

Saccharomyces cerevisiae 5019 and *Candida tropicalis* 5045 was obtained from (Bangkok MIRCEN) and were maintained at 4°C on slants of Sabouraud agar. Inocula of *S. cerevisiae* and *Candida tropicalis* were grown on glucose and xylose, respectively, in erlenmeyer flasks at 30°C on a rotary shaker at 150 rpm for 24 h. The inoculum medium of *S. cerevisiae* was composed of 20 g/l glucose, 1 g/l yeast extract and 1 g/l $MgSO_4 \bullet 7H_2O$, 2 g/l $(NH_4)_2SO_4$, 0.5 g/l KH_2PO_4., whereas inoculum of *C. tropicalis* was carried out with the liquid medium composed of 20 g/l xylose, 1 g/l yeast extract and 1 g/l $MgSO_4 \bullet 7H_2O$, 2 g/l $(NH_4)SO_4$, 0.5 g/l KH_2PO_4. Sugar solution was autoclaved separately. The batch fermentions carried out in 50 ml of liquid medium in 250 ml erlenmeyer flask. Single culture of *C. tropicalis* and co-cultures of *S. cerevisiae* and *C. tropicalis* were carried out at 30°C in a 250 ml bioreactor containing 50 ml of synthetic medium. The synthetic medium was composed of 1 g/l yeast extract, 1 g/l $MgSO_4 \bullet 7H_2O$, 2 g/l $(NH_4)_2SO_4$, 0.5 g/l KH_2PO_4 and the carbon source (20 g/l glucose or 20 g/l xylose or 20 g/l of mixture of glucose and xylose in the ratio of 1:0, 8:1, 6:1, 4:1, 2:1, 1:1 and 0:1 . The fermentation was carried out at 30°C on a rotary shaker at 150 rpm for 18 h. Ethanol was measured by GC using a 60:80 Carbopack B:5% Carbowax 20 M glass column and a flame ionization detector and nitrogen was the carrier gas.

In order to study the effect of agitation speed on cell and ethanol production of the mixed culture in mixture of glucose and xylose, three different experiments were carried out at rotatory shaker at 0, 50, 100, 150 and 200 rpm for 18 h. For the measurement of dry cell weight (DCW), 5ml of culture broth was centrifuged for 10 min at 10,000 rpm, washed twice with distilled water and dried at 105°C to constant weight. Analysis of glucose was determined by GOD-PAP method and xylose was determined by bromoaniline method (Deschatelet and Yu , 1986).

RESULTS

Effect of mixture of glucose and xylose on fermentation

The study of the mixture effect of glucose and xylose ratio on cell mass and ethanol production yield by mixed culture of *S. cerevisiae* and *C. tropicalis* is shown in Table 1.

The results of the mixture fermentation of glucose and xylose ratio 8:1 by the mixed cultures was shown higher ethanol production than that of the other mixture of sugars ratios.

Table 1 Fermentation parameters for the fermentation of xylose, glucose and mixture of xylose/glucose for ethanol production by mixed cultures of *S. cerevisiae* and *C. tropicalis*.

Ratio of glucose and xylose	$Y_{x/s}$ (g/g)	$Y_{p/s}$(g/g)
0:1	0.10	0.05
1:1	0.09	0.27
2:1	0.10	0.32
4:1	0.08	0.32
6:1	0.08	0.34
8:1	0.09	0.36
1:0	0.12	0.35

S, substrate (glucose); P, product (ethanol); X, cell mass.

Effect of agitation speed on biomass and residual sugars

The biomass dry weight increased with the increase of agitation speed from 0 to 200 rpm (Figure 1- Figure 5).

Agitation could be beneficial to the growth and performance of the microorganism cells by improving the mass transfer characteristics with respect to substrates, products/byproducts and oxygen. Thus, agitation results in a better mixing

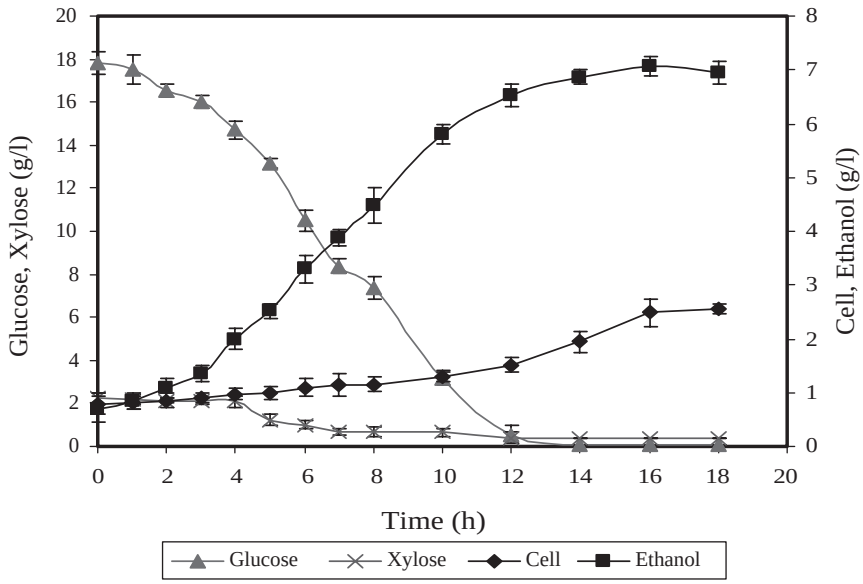

Figure 1 Effect of agitation speed at 0 rpm on kinetics of batch fermentation parameters of glucose and xylose mixture ratio 8:1 with *S. cerevisiae* and *C. tropicalis*.

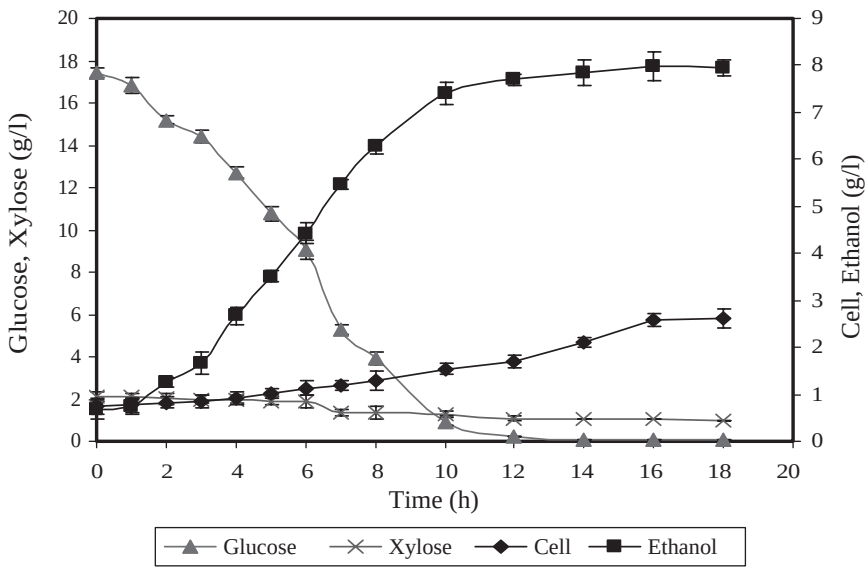

Figure 2 Effect of agitation speed at 50 rpm on kinetics of batch fermentation parameters of glucose and xylose mixture ratio 8:1 with *S. cerevisiae* and *C. tropicalis*.

of the fermentation broth, helping to maintain a concentration gradient between the interior and the exterior of the cells. Such a concentration gradient works in both directions; through better diffusion it helps to maintain a satisfactory supply of sugars and other nutrients to the cells, while it facilitates the removal of gases and other byproducts of catabolism from the microenvironment of the cells. Agitation also favors oxygen supply to the cells that is important for high biomass concentration. In the culture agitated at 0, 50 and 100 rpm, maximum biomass concentration was obtained

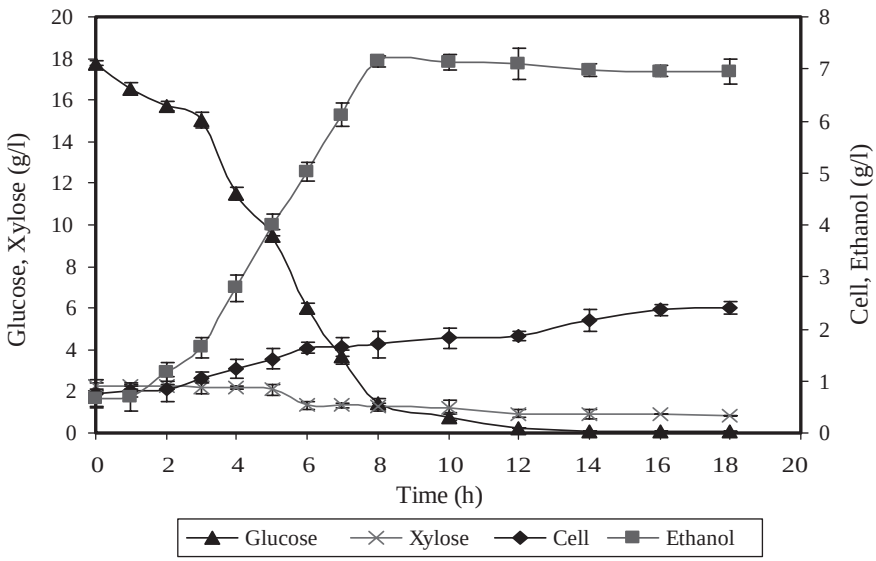

Figure 3 Effect of agitation speed at 100 rpm on kinetics of batch fermentation parameters of glucose and xylose mixture ratio 8:1 with *S. cerevisiae* and *C. tropicalis*.

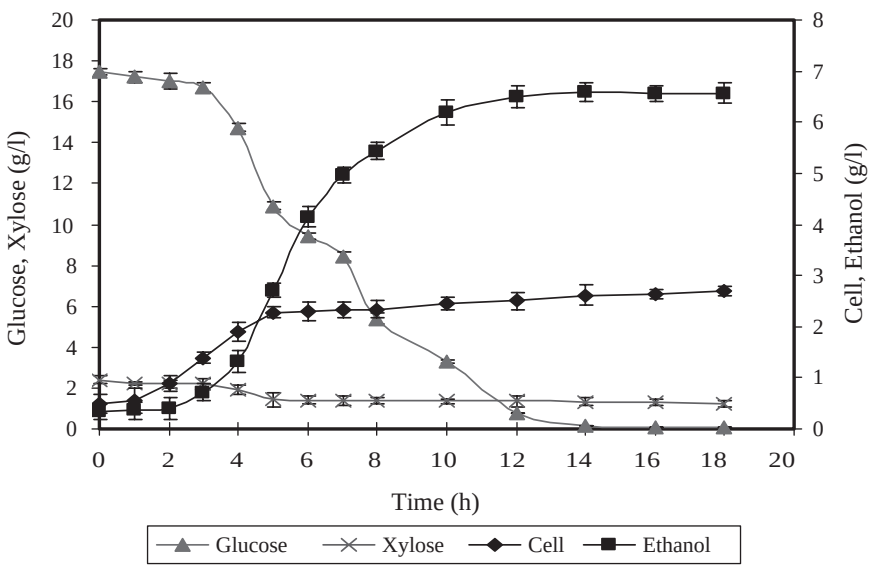

Figure 4 Effect of agitation speed at 150 rpm on kinetics of batch fermentation parameters of glucose and xylose mixture ratio 8:1 with *S. cerevisiae* and *C. tropicalis*.

after 16 h of fermentation, while in cultures agitated at 150 and 200 rpm, maximum biomass dry weight was observed about 2 h earlier. The maximum biomass levels of the cultures agitated at 0, 50, 100, 150 and 200 rpm, were 2.56, 2.62, 2.45, 2.93 and 2.69 g /l, respectively. The yields of cell mass were 0.04, 0.04, 0.05, 0.09 and 0.09 g/g at the agitation rate of 0, 50, 100, 150 and 200, respectively.

The assimilation of sugars also increased with agitation speed. This was in accord with the increase in biomass concentration when the agitation speed was increased. When the maximum concentration of ethanol in the broth was reached for the cultures agitated at 0, 50, 100, 150 and 200 rpm, 99.4, 99.8, 92.1, 97.0 and 83.8 % of glucose were consumed, respectively; while the amounts of xylose utilized were 81.6, 47.6, 45.5, 43.6 and 40.7%, respectively.

The fermentative parameters for the experiments with glucose and xylose as substrate in agitated flasks were shown in Table 2. In order to attain a high yield of fermentation, with a limited biomass production, by keeping the sugar-to-ethanol conversion rate high and constant during all the fermentation time, it is important that the aeration level should remain high during the cell growth stage and low during the production stage (Saha and Bothast 1997).

Dissolved oxygen level in batch production of coculture

The profiles of different level of dissolved oxygen concentration in batch fermentation system of coculture were studied at various agitation speeds ranging from 0 to 200 rpm (Figure 6). Dissolved oxygen concentration decreased rapidly in the early stage and remained nearly constant in the late stage. The lowest dissolved oxygen concentrations, 3, 7, 12, 19 and 23%, were achieved at 18 h, when the agitation speeds were controlled at 0, 50, 100, 150 and 200 rpm. respectively.

Effect of agitation speed on fermentation

Changes of dry cell weight, residue sugar concentration and ethanol concentration at various agitation speeds are shown in Figure1-5. To analyze the kinetic characteristics of these processes, three kinetic parameters, including

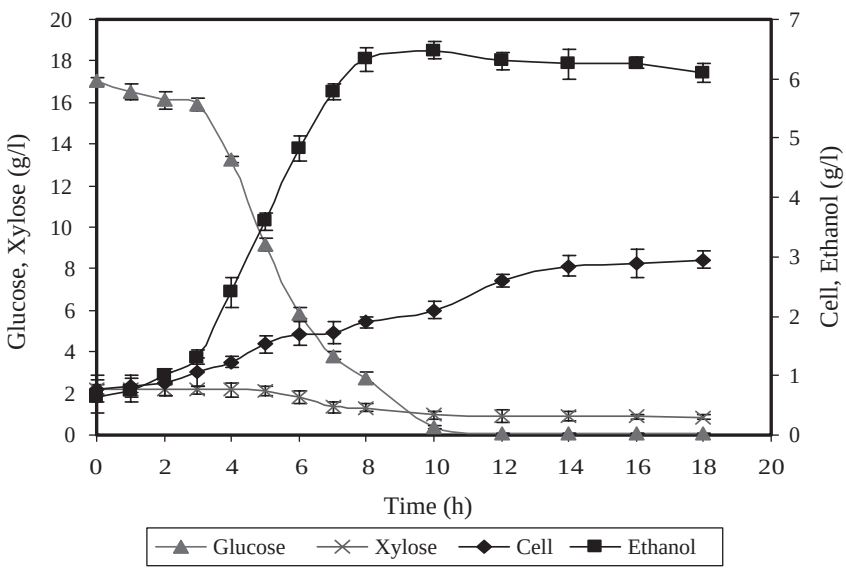

Figure 5 Effect of agitation speed at 200 rpm on kinetics of batch fermentation parameters of glucose and xylose mixture ratio 8:1 with *S. cerevisiae* and *C. tropicalis.*

specific growth rate, specific sugar consumption rate and ethanol productivity were calculated by an interpolation method based on the data of Figure 1-5. Figure (1–5) shows the change of specific growth rate, specific sugar consumption rate and ethanol productivity within the fermentation process. The increase of dissolved oxygen concentration, generated by the increase of agitation speed, resulted in a quick start of growth (shortened lag time) and an increase of biomass formation. The highest dry cell weight (2.94 g/l) was achieved at 18 h at the agitation speed of 200 rpm (Figure 5). Correspondingly, it was found that the higher the agitation speed, the higher the specific growth rate (Figure 5). A relatively high agitation speed was also favourable for sugar consumption. The specific sugar consumption rate shows a similar tendency to specific growth rate,

Table 2 Fermentation kinetic parameters of experiments with using glucose and xylose as substrates for coculture in agitated flask condition.

Parameters	0 rpm	50 rpm	100 rpm	150 rpm	200 rpm
Initial substrate concentration (g/l)	20.00	20.00	20.00	20.00	20.00
Substrate consumed (%)	98.51	95.32	95.24	95.17	97.62
specific glucose consumption rate (g/l h)	3.21	4.16	3.27	3.32	4.26
specific xylose consumption rate (g/l h)	0.71	0.62	0.53	0.52	0.54
Maximum ethanol concentration (g/l)	7.05	8.02	7.16	6.52	6.47
Maximum cell concentration (g/l)	2.52	2.84	2.47	2.71	2.94
Specific growth rate (ln (X/X_0)/h)	0.14	0.09	0.11	0.32	0.14
Ethanol productivity (Q_P, g /l h)	0.51	1.02	0.82	0.84	0.85
Ethanol yield ($Y_{P/S}$, g /g)	0.35	0.40	0.36	0.33	0.32
Cell yield ($Y_{X/S}$, g/g)	0.12	0.14	0.12	0.14	0.15
Ethanol yield ($Y_{P/X}$ in g P/g X)	2.81	2.82	2.90	2.41	2.20

P, product (ethanol); X, cell mass.

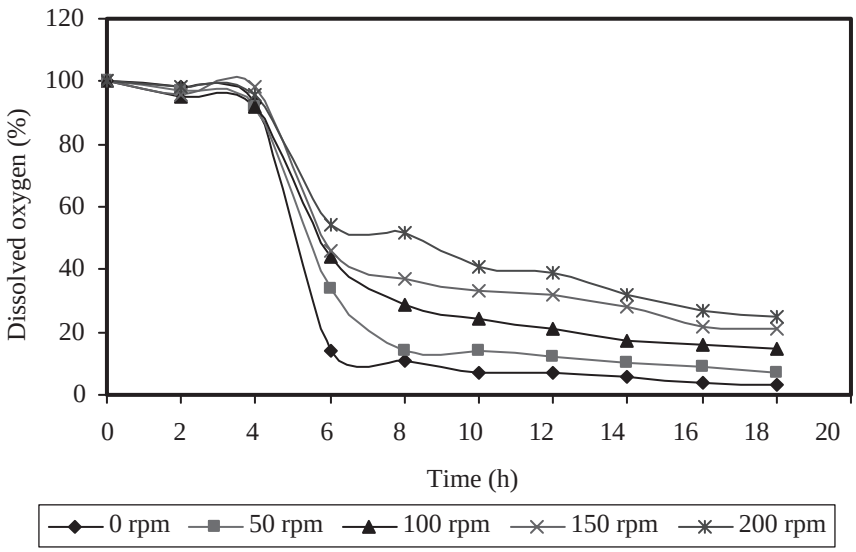

Figure 6 Profile of dissolve oxygen level in batch production by coculture at various agitation speeds of 0 rpm, 50 rpm, 100 rpm, 150 rpm and 200 rpm.

and a relatively high specific sugar consumption rate was achieved at high agitation speed (Figure 5).

However, with respect to ethanol production, the ethanol productivity increased when the agitation speed was 50 rpm, but decreased if the agitation speed was further increased. The highest ethanol productivity (1.02 g /l h) was achieved at 10 h at an agitation speed of 50 rpm (Figure 2) at which the highest maximum ethanol concentration was also attained (Figure 2). The major process parameters, summarized from these processes, are shown in Table 2. A relatively low dissolved oxygen concentration resulting from alternating agitation speed, it may be involve in responsible for the high ethanol activity. The effect of agitation on ethanol production is shown in Figure 2. The ethanol concentration increased significantly with an increase of agitation speed from 0-50 rpm, whereas further increases in agitation speed resulted in a decline of ethanol concentration. The highest concentration of ethanol (8.02 g l^{-1}) was obtained in the culture agitated at 50 rpm, while in cultures agitated at 0, 100, 150 and 200 rpm the maximum concentration of ethanol was 7.05, 7.16, 6.52 and 6.47 g l^{-1}, respectively. The results reported herein demonstrate even higher levels of ethanol (8.02 g l^{-1}) in the broth of a fermentation under conditions of low agitation rates (50 rpm).

The pH decreased during fermentation at the agitation rate at 0 and 50 rpm. The pH decreased from an initial value of 5.5 to 4.7 and 4.9 after 18 h of incubation, respectively. The pH decreased during the first 10 h of fermentation at the agitation rate at 100 and 150 rpm. The pH decreased from an initial value of 5.5 to 4.6 and 5.1 after 10 h of incubation, and then increased at later stages of the fermentation to pH 5.7 and pH 6.1, respectively. The pH decreased during the first 8 h of fermentation at the agitation rate at 200 rpm. The pH decreased from an initial value of 5.5 to 5.3 after 8 h of incubation and then increased at later stages of the fermentation to pH 6.2 (Figure 7).

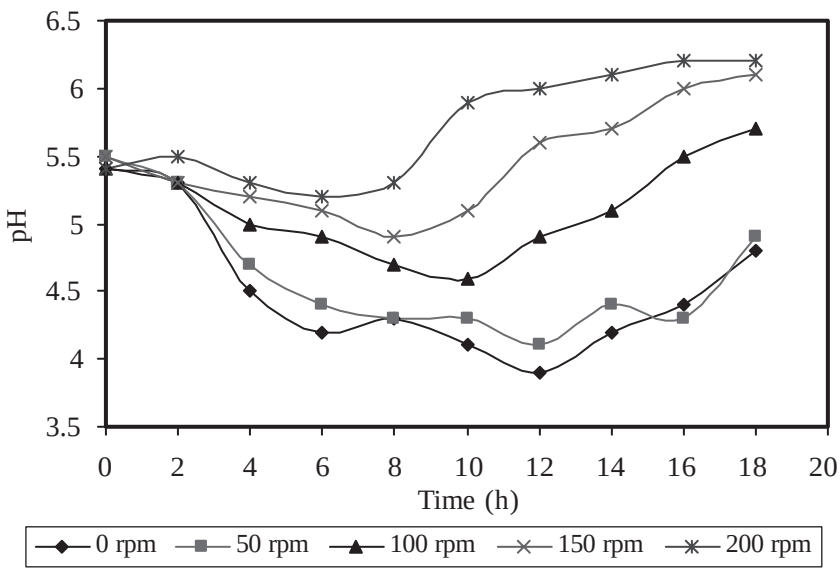

Figure 7 Profile of pH level in batch production by coculture at various agitation speeds of 0 rpm, 50 rpm, 100 rpm, 150 rpm and 200 rpm.

DISCUSSION

The study on effects of operating conditions, such as temperature, agitation speed, type of substrates on ethanol production and other parameters in the process, such as microorganism growth, pH, total and sugars can play an important role in understanding the product production pattern, its influencing parameters, and information for process scale-up. Production of ethanol by yeast is generally believed to be an anaerobic process. The dissolved oxygen concentration has been found to influence the productivity of several compounds (Kempf *et al*., 1997). Oxygen could negatively influence the product kinetics with decreasing values by acting as a direct parameter of product formation. It has also been suggested that agitation could effect to metabolite formation if the reaction of product formation is dependent on oxygen (Barberis and Segovia, 1997).

The biomass dry weight increased with the increase of agitation speed from 0 to 200 rpm (Figure 6). Agitation could be beneficial to the growth and performance of the microorganism cells by improving the mass transfer characteristics with respect to substrates, products/byproducts and oxygen. Thus, agitation results in a better mixing of the fermentation broth, helping to maintain a concentration gradient between the interior and the exterior of the cells. Such a concentration gradient works in both directions; through better diffusion it helps to maintain a satisfactory supply of sugars and other nutrients to the cells, while it facilitates the removal of gases and other byproducts of catabolism from the microenvironment of the cells. Agitation also favours oxygen supply to the cells that is important for high biomass concentration. In the culture maximum biomass concentration was obtained after 18 h of fermentation. The maximum biomass levels of the cultures agitated at 0, 50, 100, 150 and 200 rpm, were 2.52, 2.84, 2.47, 2.71 and 2.94 g l^{-1}, respectively. The assimilation of sugars also increased with agitation speed to 200 rpm. However, this was in accord with the increase in biomass and ethanol concentration when the agitation was 50 rpm. Figure 6 and Table 2 show that relatively high dissolved oxygen concentration was beneficial for cell growth, but low dissolved oxygen concentration would be favourable for ethanol production. It is well known that agitation speed creates turbulence and shear force in the cultivation process which will influence both cell growth and product formation.

The increase in pH after incubation could be due to the deamination of amino acids by the microorganisms and the production of ammonia, which increase the pH of the medium. The concentration of dissolved oxygen also fell rapidly during the first 6 h of fermentation (Figure 7). This may be due to the rapid increase of biomass concentration observed in this period. In all cultures, the concentration of dissolved oxygen from 6 to 18 h fluctuated at 3–24% of the initial saturation level.

It is clear from the data available on ethanol production that polysaccharide concentration, yield and fermentation efficiency are highly dependent on the strain and the fermentation conditions (type and concentration of carbon substrate, other nutrients, aeration, agitation, pH, temperature, fermentor design etc.). This study suggested that the highest yield was achieved at a combined low dissolved oxygen and low shear rate. Both the timing and the level of dissolved oxygen at which it was maintained were critical to obtain high yields under optimum agitation rates.

The results obtained in this work for ethanol production show that further studies, which take into account the bioreactor design, are necessary to optimize the conditions so that the kinetic behavior of the yeast can be improved.

CONCLUSION

The results showed some important aspects of the effect of agitation speed on fermentation parameters during ethanol production by mixed culture of *S. cerevisiae* and *C. tropicalis* in mixture of sugars in a batch fermentor. Ethanol productivity and biomass were significantly influenced by the agitation speed. Low agitation speeds improved the production of ethanol production. In this study, agitation was found to have a significant effect on growth rate and ethanol production because noticeable changes in biomass formation were observed in the experimental studies. The influence of agitation on growth rate can result in changes of bioactive products formation. Dissolved oxygen concentration from agitation was believed to be involved in cell density and ethanol production. By changing agitation in the batch fermentor can be improved, and, therefore, could effect to yeast growth and ethanol production.

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