Malaysian Journal of Microbiology, Vol 2(2) 2006, pp.15-18

Production of *Candida utilis* Biomass and Intracellular Protein Content: Effect of Agitation Speed and Aeration Rate

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ABSTRACT

The effects of agitation speed and aeration rate on the *Candida utilis* biomass and the intracellular protein content were investigated in this study. *C. utilis* inoculum of 10^6 cells/mL (7.8 % v/v) was cultured in 1.5 L pineapple waste medium (3 % Brix) in a 2-L fermentor for 30 h at 30 °C. Agitation speed and aeration rate have significant effects on the dissolved oxygen concentration, which in turn affect the cell growth and the intracellular protein content. The agitation speed of 100, 300, 500, 700 and 900 rpm was employed. The highest yield of protein content (1.2 g/L media) and total biomass (7.8 g/L media) were resulted from yeast cultivation with agitation speed of 900 rpm. Thus, the effects of aeration rate (0.5, 1.0, 2.0 and 3.0 L/min) were studied at agitation speed of 900 rpm. A maximum yield of protein content (1.6 g/L media) and biomass (9.5 g/L media) were attained at aeration rate of 2.0 L/min.

Keywords: *Candida utilis*, agitation, aeration, pineapple waste, intracellular protein

INTRODUCTION

Yeast extract has been recognized for its role as flavor enhancers contributed by its nucleotides and amino acid components. It has been widely used in meat sauces and flavor enhancers (Nagodawithana, 1992). Utilization of sucrose or glucose as carbon source is not economical in production of yeast extract, and less expensive carbohydrate source would be beneficial. Pineapple (*Ananas comosus*) waste is rich in sucrose, the disaccharide most easily utilized by yeast cells (Lee *et al.,* 2001). There is approximately 17,000 hectare of land under pineapple cultivation in Malaysia, producing about 0.25 million tones of fruits. Most of the pineapples are consumed as either fresh products or processed fruit. Of the whole fruits, only 20% is canned, while the remainder, in the form of peeled skin, core, base and crown is discharged as waste (Hutagalung *et al*., 2002). In the processing of pineapple, only high-quality fruits are selected while the low-quality one been left out as the market of the fruit is not so encouraging (Kenji *et al*., 1999). Therefore, it is anticipated that the medium from pineapple waste can be utilized for the production of intracellular protein from *Candida utilis*. Kenji *et al.,* (1999) had investigated the utility of pineapple waste material as low-cost substrate for ethanol fermentation by *Zygomonas mobils* while Nigam (1998) had produced single cell protein from pineapple cannery effluent. To be economically feasible, optimum culture condition for the production of maximum biomass and intracellular protein is necessary. In general, the environmental conditions, such as temperature, dissolved oxygen, pH and so on are important factors which have significant influence on the biomass production. Agitation speed and aeration rate are the two factors affecting dissolved oxygen concentration, which in turn affect the biomass and intracellular protein content (Lee *et al.,* 2001). Therefore, the objective of this research is to study the effect of agitation speed and aeration rate on the *C. utilis* biomass and protein content yields cultivated in pineapple waste material.

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MATERIALS AND METHODS

Microorganism and inoculum preparation

Candida utilis ITM 1017 was obtained from the Culture Collection Centre of Universiti Teknologi Mara, reactivated, maintained on YEPG slants at 4°C and reactivated every 2 months. Loopfuls of *Candida utilis* were used to inoculate 50 mL of pineapple waste medium, incubated at 30 °C, 100 rpm for 18 h, to obtain 10^6 cells/mL.

Pineapple waste medium

Pineapple waste consisting of peels, cores and unwanted parts of the fruit was obtained from a local fruit stall, washed and cut into smaller pieces before the addition of distilled water at a ratio of 1:1 (w:v). The extract was obtained through heat treatment with an autoclave at 121 °C, 15 psi for 15 min. The extract was then filtered using filter papers Whatman No. 3 and Whatman glass microfiber (GF/C). Pineapple waste extract was diluted to 3 % Brix and the pH was fixed at pH 4.5. Every g pineapple waste will produce 1.0 mL medium with a total solid content of 3 % Brix.

Fermentation

About 7.8 % (v/v) inoculum was used as seed culture. Cultivations were conducted using a 2-L benchtop fermentor (B. Braun Biotech International, Switzerland) with the working volume of 1.5 L at 30 °C for 30 h. The pH was automatically controlled at pH 4.5. Foaming was controlled using antifoam 289 (Sigma, Germany). Optimization experiment was carried out with one parameter at a time. Firstly, the effect of agitation speed (100, 300, 500, 700 and 900 rpm) was studied at a fixed aeration rate of 1.0 L/min. The highest agitation speed studied was 900 rpm due to limitation of the fermentor. Secondly, the effect of aeration rate (0.5, 1.0, 2.0 and 3.0 L/min) was studied at an optimum agitation speed.

Analytical methods

Cell growth as dry weight of biomass was determined gravimetrically. A volume of culture was centrifuged and washed twice before drying at 70 °C until constant weight reached. The reducing sugar content of the medium was determined using DNS method (Miller, 1959). Yeast extract was obtained by mechanical rupturing of the cells, according to Loo *et al.* (2002). Yeast cell protein content of the yeast extract was determined by the Biuret method (Robinson and Hodgen, 1940).

RESULTS AND DISCUSSION

Effects of agitation speed

The growth of *Candida utilis* in pineapple waste medium was low in the initial 5-12 h regardless of the agitation speed, possibly due to adaptation of the microorganisms. After 12 h, however, growth varied and much higher biomass was obtained at higher agitation speed (Figure 1). After 30 h of fermentation, the biomass obtained from agitation speed of 700 rpm was 7.93 g/L and that of 900 rpm was 7.83 g/L. At low agitation speed (100 rpm), biomass slightly decreased at the end of the fermentation. This suggested that shortage of oxygen and limitation of substrate (Figure 2) lead to degradation of cells. As the agitation speed increases, the biomass obtained increases and at a higher agitation speed, more oxygen was incorporated into the medium and lead to progressive growth of *C. utilis*.

C. utilis depleted reducing sugar in cultivation media after 5 h of cultivation (Figure 2). About 96 – 98 % of reducing sugar equivalent to glucose in the pineapple waste medium was used-up by the *C. utilis* after 30 h fermentation (Table 1). Calculated yield of biomass was the highest at the agitation speed of 700 rpm, i.e. 0.66 g biomass produced per g glucose-equivalent reducing sugar.

The yeast cell protein content increased slowly from 12 to 30 h with a maximum at 30 h (Figure 3). The intracellular yeast protein was determined to be the highest at 900 rpm, i.e. 15.5 % (w/w of biomass), which corresponded to 1.22 g protein per L medium. A higher agitation speed increased the amount of dissolved oxygen and dispersion of macromolecules in the medium. It might, therefore, contribute to the greater growth and intracellular protein content.

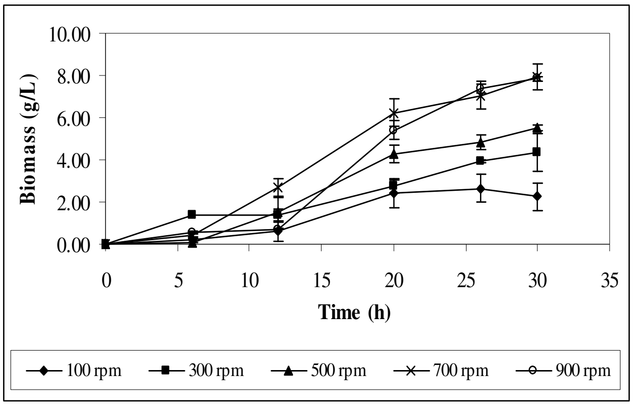

Figure 1: Effects of agitation speed on biomass of *C. utilis* grown in pineapple waste medium. Fermentation was carried out in 1.5 L media at 30 °C with an aeration of 1 L/min for 30 h. Data are mean values ± standard errors of triplicate samples

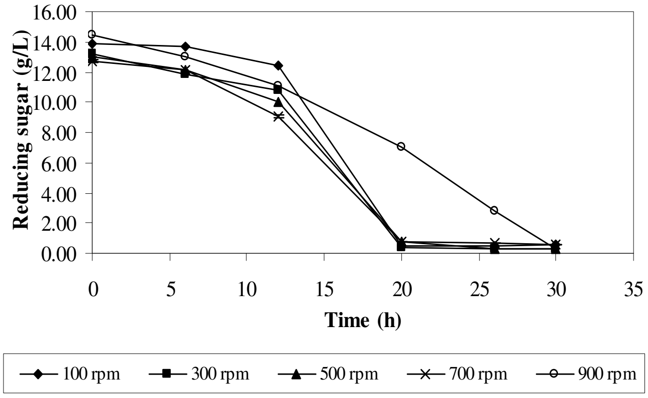

Figure 2: Effects of agitation speed on substrate consumption by of *C. utilis* in pineapple waste medium. Fermentation was carried out in 1.5 L media at 30 °C with an aeration of 1 L/min for 30 h. Data are mean values ± standard errors of triplicate samples

Effects of aeration rate

With operating temperature and agitation speed maintained at 30 ºC and 900 rpm, respectively, the effects of aeration rate were investigated by comparing performance at four aeration rates; 0.5, 1.0, 2.0 and 3.0 L/min. Biomass production increased with the increase of aeration rate, reaching the maximum value of 9.50 g/L media at aeration rate of 2.0 L/min (Figure 4).

Substrate consumption ranges was lowest at the aeration rate of 3.0 L/min, with only 90.7 % of reducing sugar consumed (Table 2 and Figure 5). Calculated yield of biomass was 0.81 g per g glucose-equivalent reducing

Table 1: Effects of agitation speed on total substrate consumption, biomass and yeast cell protein content

Agitation speed (rpm)	Total substrate consumption (%)	Biomass (g/L media)	Yield of biomass [a]	Protein (g/L media)	Yield of protein [b]
100	96.1	2.25	0.17	0.34	0.03
300	98.1	4.35	0.34	0.56	0.04
500	97.8	5.53	0.43	0.67	0.05
700	95.4	7.37	0.66	1.06	0.09
900	97.9	7.83	0.55	1.22	0.09

[a] Yield of biomass: g biomass per g glucose-equivalent reducing sugar
[b] Yield of protein: g protein per g glucose-equivalent reducing sugar

Table 2: Effects of aeration rate on total substrate consumption, biomass and yeast cell protein content

Aeration rate (L/min)	Total substrate consumption (%)	Biomass (g/ L media)	Yield of biomass [a]	Protein (g/L media)	Yield of protein [b]
0.5	97.2	6.38	0.43	0.87	0.06
1.0	97.9	7.83	0.55	1.22	0.09
2.0	94.7	9.50	0.81	1.63	0.14
3.0	90.7	8.70	0.73	1.20	0.10

[a] Yield of biomass: g biomass per g glucose-equivalent reducing sugar
[b] Yield of protein: g protein per g glucose-equivalent reducing sugar

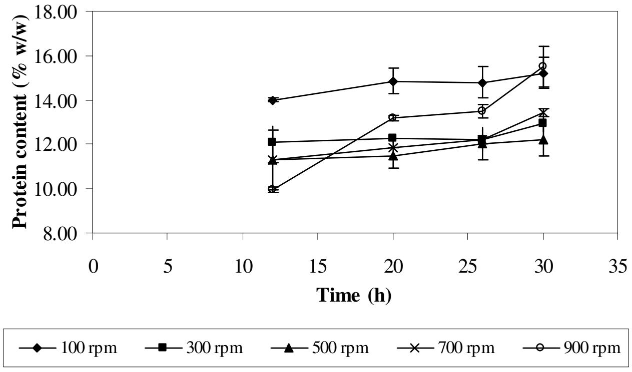

Figure 3: Effects of agitation speed on intracellular protein content of *C. utilis* grown in pineapple waste medium. Fermentation was carried out in 1.5 L media at 30 °C with an aeration of 1 L/min for 30 h. Data are mean values ± standard errors of triplicate samples

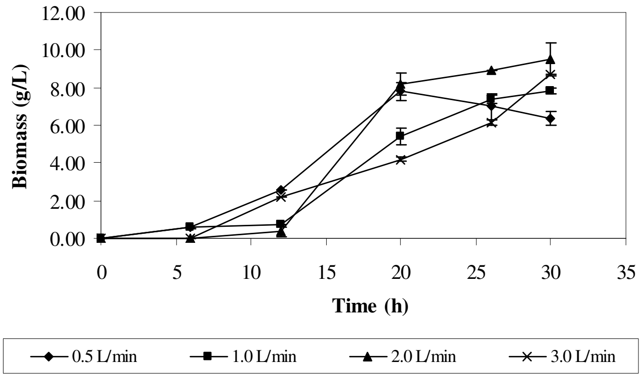

Figure 4: Effects of aeration rate on biomass of *C. utilis* grown in pineapple waste medium. Fermentation was carried out in 1.5 L media at 30 °C with an agitation of 900 rpm for 30 h. Data are mean values ± standard errors of triplicate samples

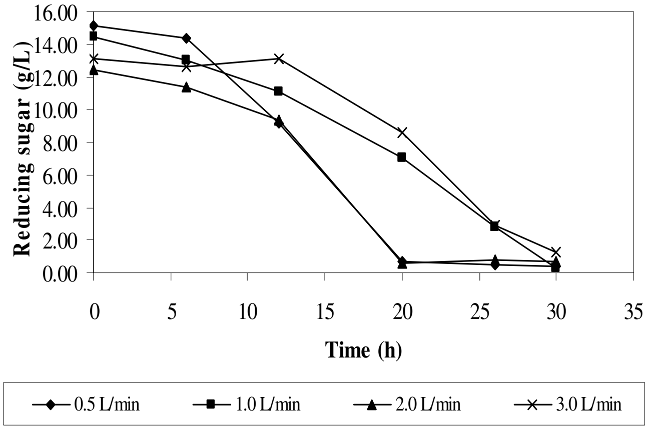

Figure 5: Effects of aeration rate on substrate consumption by of *C. utilis* in pineapple waste medium. Fermentation was carried out in 1.5 L media at 30 °C with an agitation of 900 rpm for 30 h. Data are mean values ± standard errors of triplicate samples

sugar at the aeration rate of 2.0 L/min, which was higher than the yield of *C. utilis* grown in pineapple canning effluent of 0.30 - 0.48 g dry biomass per g carbohydrate consumed (Nigam, 1998). Increase of aeration rate over than 2.0 L/min has caused the yield to decrease by 9.9 %. Substrate consumption at aeration rate of 3.0 L/min (Figure 5) shows that *C. utilis* cannot utilized the reducing sugar in the pineapple waste medium after 20 h of fermentation period. The biomass started to decrease while almost 60 % of initial reducing sugar still remained unutilized in the medium. This suggested that fermentation under the aeration rate of 3.0 L/min is not suitable, as the over-supply of dissolved oxygen might inhibit the growth of *C. utilis*.

The amount of protein produced was the highest at aeration rate of 2.0 L/min, i.e. 1.63 g per L media, with the protein content of 17.14 % (w/w of biomass) (Figure 6). This fermentation condition (900 rpm, 2.0 L/min) has increased the amount and yield of protein produced by 33.6 % and 55.6 % respectively as compared to the non-optimized condition (900 rpm, 1.0 L/min). At the aeration rate of 3.0 L/min, protein content decreased slightly at the end of fermentation. Intensive aeration led to over-supply of dissolved oxygen, influenced the metabolisme of the yeast (Oura, 1974) and thus, resulted in low yield of protein.

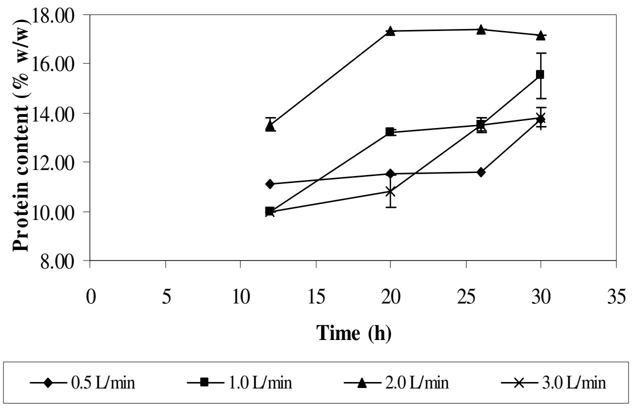

Figure 6: Effects of aeration rate on intracellular protein content of *C. utilis* grown in pineapple waste medium. Fermentation was carried out in 1.5 L media at 30 °C with an agitation of 900 rpm for 30 h. Data are mean values ± standard errors of triplicate samples

CONCLUSIONS

The present results demonstrate the potential of pineapple waste medium as a substrate for the production of biomass and intracellular protein by *C. utilis* ITM 1017. The pineapple waste medium at 3 % Brix can be used to produce single cell protein and microbial protein.

ACKNOWLEDGEMENTS

Authors are grateful to the IRPA grant by Ministry of Science, Technology & Innovation and the Graduate Assistant Scheme for their financial assistances.

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