The Journal of Microbiology, March 2002, p.82-85 Vol. 40, No. 1

NOTE

Growth of *Issatchenkia orientalis* in Aerobic Batch and Fed-batch Cultures

Hyung Tai Shin[1], Yoo Beom Lim[2], Jong Ho Koh[2], Jong Yun Kim[2], Soon Young Baig[1], and Jae Heung Lee[1]*

[1]Department of Food and Life Science, Faculty of Life Science and Technology, Sungkyunkwan University, 300 Chunchun-dong, Jangan-gu, Suwon 440-746, Korea
[2]NEL Biotech Co., Ltd, 800-15 Duksan-ri, Samjuk-myon, Ansung 451-882, Korea

(Received December 17, 2001 / Accepted February 25, 2002)

The aerobic batch growth of *Issatchenkia orientalis* DY252 with glucose and fructose medium was investigated at 32°C and pH 5.0. Aerobic ethanol production was evident with yeast *I. orientalis*. A diauxic lag of about 1 h between growth on glucose and growth on ethanol during batch culture was observed. However, no diauxic growth occurred with fructose. As the incubation temperature was increased from 32 to 39°C, viability at the end of each batch culture declined significantly, from 93 to 43%. Unlike the effect of temperature, viability was not greatly affected by incubation pH, and cell yield values in a range of 0.45-0.48 were obtained. In order to overcome overflow metabolism, a fed-batch culture under glucose limitation was carried out. Compared with aerobic batch culture, about 10% improvement in cell yield was achieved with a fed-batch culture in optimal conditions.

Key words: diauxic lag, direct-fed microbials (DFMs), *Issatchenkia orientalis*, overflow metabolism, viability

Direct-fed microbial feed additives (previously called probiotics) have been used for many years as feed additives to foster good animal health and optimal production (Kung, 1988; Lee *et al.*, 2002). Many previous studies have already documented the positive effects of feeding direct-fed microbials (DFMs) to animals (Callaway and Martin, 1977; Kung *et al.*, 1977; Harris and Webb, 1990; Martin and Nisbet, 1992; Reynolds, 1998). Yeast culture using *Saccharomyces cerevisiae* is one of the DFMs available on the market, and the mode of action of the yeast culture (i.e. the main effect of yeast culture comes from the respiratory activity of yeast) has been reported (Newbold *et al.*, 1996; Wallace, 1996).

In a previous work, it was found that yeast *Issatchenkia orientalis* DY 252 may be a potential candidate for use as a microbial feed additive (Lee *et al.*, 2002). However, so far only limited information on *I. orientalis* is available (Tamaki *et al.*, 1989; Seiler and Busse, 1990). The aim of the current research was to study the fermentation kinetics of *I. orientalis* DY252 in aerobic batch and fed-batch cultures, and to determine the optimal conditions for production of a novel DFM in view of cell yield and viability of *I. orientalis* DY252.

I. orientalis DY252 (Lee *et al.*, 2002) isolated at this laboratory was used throughout the course of this work. It was maintained by transferring to fresh YM agar (Difco) plates biweekly and was stored at 4°C. The composition of fermentation medium per liter was as follows: 80 g glucose; 5 g yeast extract (Difco); 4 g KH_2PO_4; 0.36 g Na_2HPO_4; 14 g $(NH_4)_2SO_4$; 2 g $MgSO_4 7H_2O$; 50 g corn steep liquor. When fructose medium was employed, only the glucose was replaced.

Single yeast colonies were taken from a stock culture plate and transferred to a sterilized Erlenmeyer flask containing 100 ml of 2% glucose medium. Shaking incubation of the flask was carried out for 16 h at 30°C. Unless otherwise specified, the yeast was transferred to a 2.5 L jar fermentor (KoBioTech, Korea) containing 900 ml of fermentation medium. The fermentor was flushed with N_2 gas before inoculation in order to calibrate the dissolved oxygen (DO) probe (Mettler-Toledo GmbH, Germany). The pH was controlled by automatic addition of 4 N KOH or 4 N H_2SO_4. For a fed-batch culture of *I. orientalis* DY252, 100 ml of inoculum was transferred to 400 ml of the fermentation medium to give 500 ml (initial glucose concentration was 40 g/L) at the beginning. After 6 h cul-

* To whom correspondence should be addressed.
(Tel) 82-31-290-7893; (Fax) 82-31-290-7884
(E-mail) jaeheung@skku.ac.kr

tivation when the glucose was almost exhausted, a constant feeding of glucose solution (100 g/L glucose) at a flow rate of 35 ml/h was carried out for 14.3 h (viz. total feeding volume was 500 ml).

Dry cell weights of biomass were determined for 10 ml samples, washed with 10 ml of 0.85% NaCl solution and once with distilled water, and then dried at 105°C for 20 h. Growth of cultures was also measured by following the increase in absorbance at 640 nm in a spectrophotometer (Spectronic 20, Baush & Lomb) to make a calibration curve between absorbances and dry cell weights. Total yeast populations were counted by means of a microscope (Olympus, Japan) using a Hawksley haemocytometer of 0.1 mm depth. Viable cell counts were measured by plating 0.1 ml of appropriately diluted samples on agar plates which were incubated for 24 h at 30°C. Estimation of glucose was performed using the glucose oxidase/peroxidase method (Sigma catalog number 315-100). Fructose concentrations in batch culture kinetic studies were estimated using the dinitrosalicylic acid method (Miller, 1959). For ethanol estimation, samples were analyzed by a gas chromatography (FID, N_2 gas flow rate=30 ml/min. H_2=30 ml/min, Air=300 ml/min, injector 150°C, column 100°C, detector 200°C) using an HP-Innowax column (30 m× 0.32 mm).

Batch culture kinetics on glucose medium

The uptake of glucose, the production of biomass and ethanol on glucose medium at 32°C and pH 5.0 is shown in Fig. 1. Also shown in Fig. 1 is the dissolved oxygen tension (DOT) profile during the batch culture. During the early period of fermentation (~7h) there was an accumulation of ethanol. Following this period, the ethanol concentrations declined due to the uptake of ethanol by *I. orientalis*. Similar results were also obtained with *S. cerevisiae* under aerobic conditions (Barford, 1981; van Dijken and Pronk, 1995; Pham *et al.*, 1998). As can be seen from Fig. 1, growth was complete after 9.2 h and the final biomass concentration was 33.5 g/L; therefore, the cell yield Yx/s was estimated to be 0.46. This compares well with previous results (Patkar and Seo, 1992; Pham *et al.*, 1998) but is higher than that reported by Barford (1981) with *S. cerevisiae*. As shown in Fig. 1, complete utilization of glucose resulted in an abrupt increment of DOT. A diauxic lag of about 1 h between growth on glucose and growth on ethanol during the batch culture was evident (see DOT profile). A diauxic growth on glucose and ethanol with *S. cerevisiae* has also been reported (Barford, 1981; Pham *et al.*, 1998). Fig. 2 shows the total and viable cell counts of *I. orientalis* DY252 with fermentation time. As shown in Fig. 2, viability of up to 93% was maintained towards the end of batch fermentation at 32°C.

Batch culture kinetics on fructose medium

Fig. 3 shows the batch culture kinetics on fructose medium at 32°C and pH 5.0. It is clear that the batch culture kinetics of *I. orientalis* DY252 with fructose was very similar to that with glucose. Unlike the fermentation with glucose

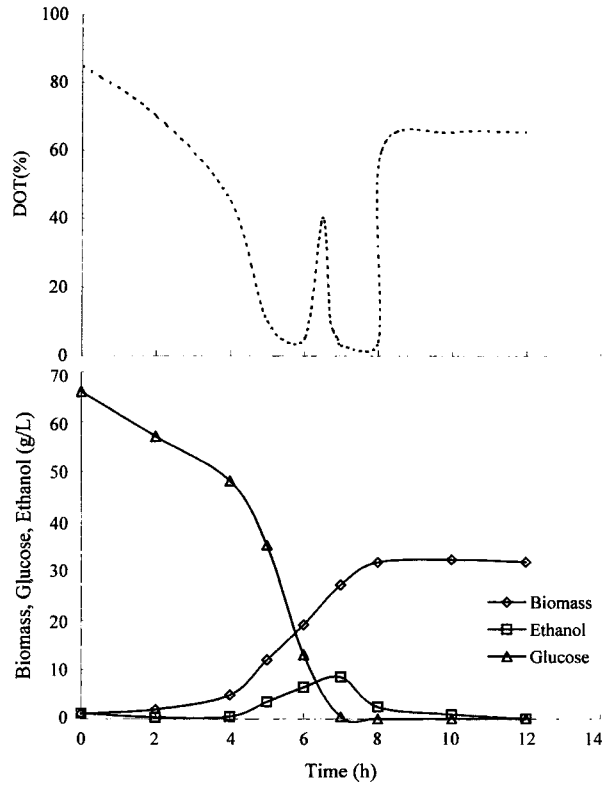

Fig. 1. Batch culture kinetics of *I. orientalis* DY252 with glucose medium at 32°C and pH 5.0.

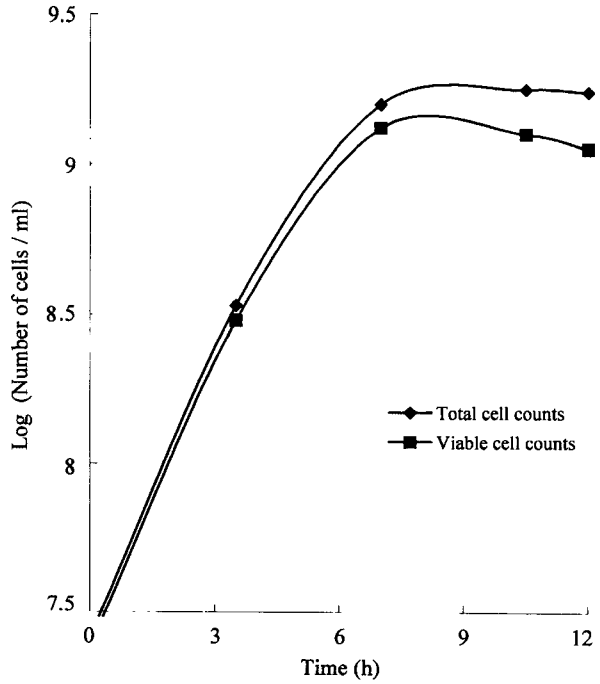

Fig. 2. The total and viable cell counts of *I. orientalis* DY252 with fermentation time at 32°C and pH 5.0.

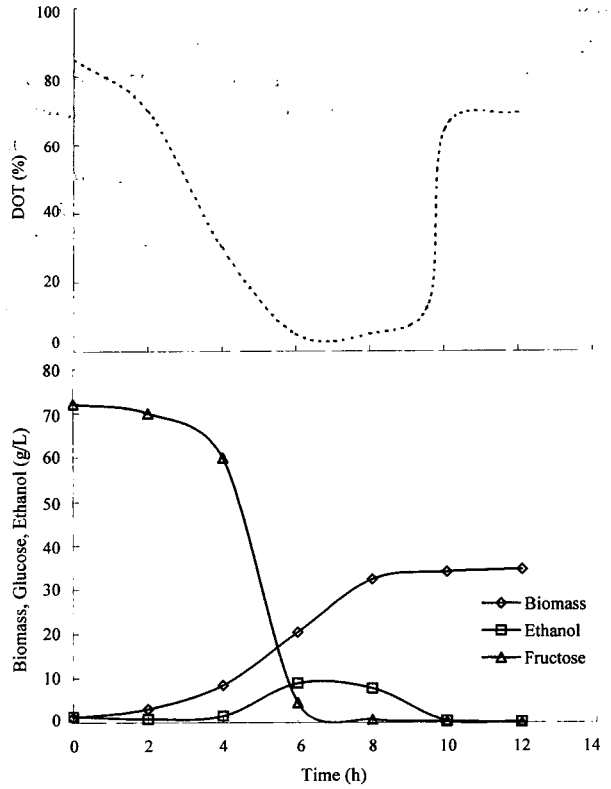

Fig. 3. Batch culture kinetics of *I. orientalis* DY252 with fructose medium at 32°C and pH 5.0.

Table 1. Effect of temperature on kinetic parameters of *I. orientalis* DY252 at pH 5.0.

	Temperature (°C)		
	32	35	39
Viability (%)	93	88	43
Biomass (g/L)	33.5	27.5	27.5
Cell yield (g/g)	0.46	0.38	0.38
Fermentation time (h)	9.2	10.7	10.2

Table 2. Effect of pH on kinetic parameters of *I. orientalis* DY252 at 32°C.

	pH		
	3.0	4.0	5.0
Viability (%)	92	92	93
Biomass (g/L)	32.5	34.5	33.5
Cell yield (g/g)	0.45	0.48	0.46
Fermentation time (h)	9.6	9.8	9.2

medium, however, no diauxic lag was observed with fructose medium. The detailed study of this mechanism was not attempted in the present study and will be the subject of a later publication.

Effects of temperature and pH on batch culture kinetics

In Table 1, the kinetic parameters determined at each fermentation temperature at pH 5.0 are given. As shown in Table 1, although cell yield and fermentation time were slightly affected, viability was significantly affected by temperature. This means that prolonged exposure of this strain to high temperatures resulted in an increased rate of cell death. Table 2 shows the effect of pH on the kinetic parameters at 32°C. Fermentation time was of the same order of magnitude, and overall cell yield Yx/s together with viability for *I. orientalis* DY252 appeared to be unaffected by pH over the range of 3.0-5.0. A maximum cell yield of 0.48 was achieved at pH 4.0. With regard to environmental effects on the fermentation kinetics, the viability of *I. orientalis* DY252 at the end of batch culture was significantly affected by temperature but not by pH. We concluded that optimum environmental conditions in view of cell yield and viability were 32°C and pH 4.0.

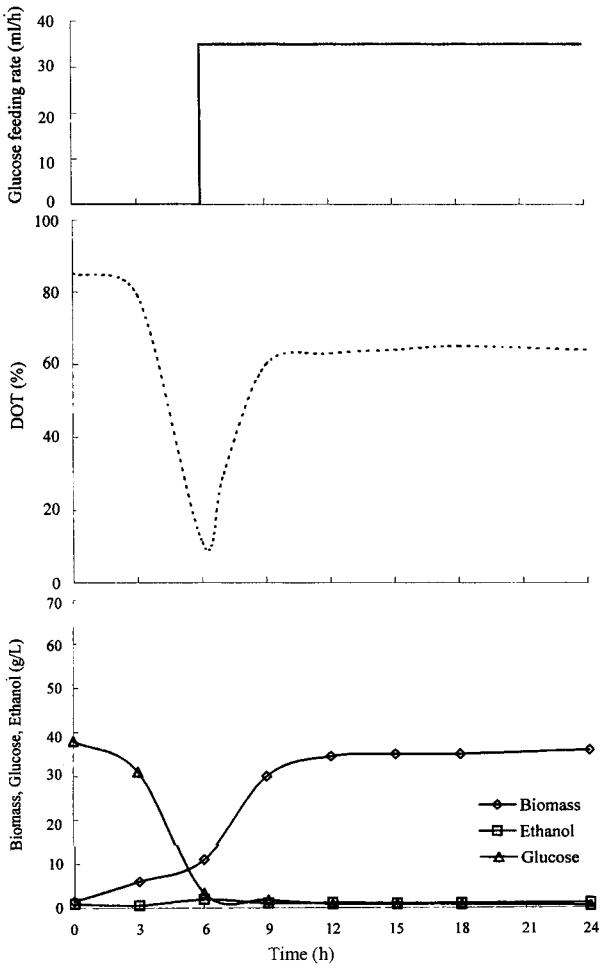

Fig. 4. Fed-batch culture kinetics of *I. orientalis* DY252 at 32°C and pH 4.0.

Fed-batch culture of I. orientalis DY252 under glucose limitation

Fig. 4 shows typical fed-batch culture kinetics of *I. orientalis* DY252. After 6 h of batch cultivation, glucose was

almost exhausted and thereafter the glucose feeding was carried out. Instead of exponential feeding of glucose, the feeding rate was kept constant in order to avoid O_2 limitation. As can be seen from Fig. 4, DOT was maintained above 60% saturation. During the feeding period, glucose was undetectable, indicating that the fed-batch culture was under glucose limitation. Therefore, the so-called the Crabtree effect or overflow metabolism (Barford, 1981; Pham *et al.*, 1998; Postma *et al.*, 1989) could be overcome. It is well known that some yeast species produce ethanol even in aerobic cultures at high sugar concentrations, i.e., more than about 0.04 g/L glucose (Postma *et al.*, 1989). This aerobic ethanol production is called the overflow metabolism. Compared with aerobic batch culture results, about 10% improvement in cell yield was achieved with a fed-batch culture of *I. orientalis* DY252 at 32°C and pH 4.0.

References

Barford, J.P. 1981. A mathematical model for the aerobic growth of *Saccharomyces cerevisiae* with a saturated respiratory capacity. *Biotechnol. Bioeng.* 23, 1735-1762.

Callaway, E.S. and S.A. Martin. 1977. Effects of a *Saccharomyces cerevisiae* culture on ruminal bacteria that utilize lactate and digest cellulose. *J. Dairy Sci.* 80, 2035-2044.

Harris, B. and D.W. Webb. 1990. The effect of feeding a low inclusion yeast product of lactating dairy cows. *J. Dairy Sci.* 73 (Suppl.) 266 (Abstr.).

van Dijken, J.P. and J.T. Pronk. 1995. Regulation of carbon metabolism in chemostat cultures of *Saccharomyces cerevisiae* grown on mixtures of glucose and ethanol. *Yeast* 11, 407-418.

Kung, L. 1998. Direct-fed microbial and enzyme feed additives, p. 15-19. *In* S. Muirhead (ed.), 1998-99 Direct-fed microbial, enzyme & forage additive compendium, vol. 4, The Miller Publishing Company, Minnesota.

Kung, L., E.M. Kreck, R.S. Tung, A.O. Hession, A.C. Sheperd, M.A. Cohen, H.E. Swain, and J.A.Z. Leedle. 1977. Effects of a live yeast culture and enzymes *in vitro* ruminal fermentation and milk production of dairy cows. *J. Dairy Sci.* 80, 2045-2051.

Lee, J.H., Y.B. Lim, J.H. Koh, S.Y. Baig, and H.T. Shin. 2002. Screening of thermotolerant yeast for use as microbial feed additive. *J. Microbiol. Biotechnol.* 12, 162-165.

Martin, S.A. and D.J. Nisbet. 1992. Effect of direct-fed microbials on rumen microbial fermentation. *J. Dairy Sci.* 75, 1736-1744.

Miller, G.L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.* 31, 426-428.

Newbold, C.J., R.J. Wallace and F.M. McIntosh. 1996. Mode of action of the yeast *Saccharomyces cerevisiae* as a feed additive for ruminants. *Brit. J. Nutr.* 76, 249-261.

Patkar, A. and J.H. Seo. 1992. Fermentation kinetics of recombinant yeast in batch and fed-batch cultures. *Biotechnol. Bioeng.* 40, 103-109.

Pham, H.T.B., G. Larsson, and S.O. Enfors. 1998. Growth and energy metabolism in aerobic fed-batch cultures of *Saccharomyces cerevisiae*: simulation and model verification. *Biotechnol. Bioeng.* 60, 474-482.

Postma, E., C. Verduyn, W.A. Scheffers, and J.P. van Dijken. 1989. Enzymic analysis of the Crabtree effect in glucose-limited chemostat cultures of *Saccharomyces cerevisiae*. *Appl. Environ. Microbiol.* 55, 467-477.

Seiler, H. and M. Busse. 1990. The yeasts of cheese brines. *Int. J. Food. Microbiol.* 11, 289-303.

Tamaki, H., H. Kumagai, and T. Tochikura. 1989. Purification and properties of glutathione transferase from *Issatchenkia orientalis*. *J. Bacteriol.* 171, 1173-1177.

Reynolds, D.L. 1998. An overview of basic microbiology, p.9-14. *In* S. Muirhead (ed.), 1998-99 Direct-fed microbial, enzyme & Forage additive compendium, vol.4, The Miller Publishing Company, Minnesota.

Wallace, R.J. 1996. The mode of action of yeast culture in modifying rumen fermentation, p. 217-232. *In* T.P. Lyons and K.A. Jacques (ed.), Proceedings of the 12th annual symposium, Nottingham University Press, Nottingham.