Production of Yeast Diet for Aquaculture in Batch Fermenters

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Production of yeast diet has been carried out in batch fermenters under optimum culture conditions. The fermentation of *Candida utilis* on a 2% complex medium resulted in 1.22 g/L/h productivity and 65 $\times 10^8$ viable cells/ml, and the addition of 15μM zinc to the medium increased both the productivity and the number of viable cells just a little more. In the case of the fermentation of *Kluyveromyces fragilis*, the highest value of the biomass productivity, 1.94 g/L/h, was obtained on a 2.5% fructose medium with 70×10^8 viable cells/ml, and 1% peptone was found to be a growth factor in this fermentation. When 3.5% NaCl was added to the given medium, both the biomass productivity and the number of viable cells decreased significantly in each fermentation, but this may be considered to preserve yeast diet long without osmotic lysis.

Key words : yeast diet, batch fermentation, *Candida utilis*, *Kluyveromyces fragilis*, biomass productivity, growth factor

Introduction

Although microalgae have been used as an essential food for the larval stages of fish and shellfish (Benemann, 1992), intensive culture and harvesting of them are expensive and labor-intensive. For this reason, yeasts have been considered as an algal substitute for several species of filter-feeders such as rotifers (Fukusho, 1980; Hirayama and Watanabe, 1973), *Artemia* (Johnson, 1980; Nimmannit and Assawamunkong, 1985; Coutteau et al., 1990) and bivalve molluscs (Efifanio, 1979; Urban and Langdon, 1984) because of their small particle size, high protein content as single cell proteins (SCP) and relatively low production costs. Among yeasts, it has been known that some yeasts cause a variety of diseases of the skin (dermatomycoses), systematic mycoses, otomycoses, onychomycoses, etc. mostly by species of *Candida, Cryptococcus, Trulopsis, Trichosporon*, and *Pityrosporum* (Kockova-Kratochvilova, 1990). On the other hand, *Candida utilis* and *Kluyveromyces fragilis* have been proven to be not pathogenic and possible yeast diet as well, since *C. utilis* has been found to have relatively high concentration of essential amino acids in the yeast protein (Lawford et al., 1979) and since *K. fragilis* has been used for long for the SCP production and it has been one of the few microorganisms classified as GRAS (Generally Recognized As Safe for human consumption) (Reed, 1982).

It is becoming increasingly evident that the development of low-cost, high-quality protein feedstuffs is crucial for the future success of the aquaculture industry (Rumsey, 1978). The production of SCP represents an interesting solution to the growing problem of algal substitute. In spite of this, the industrial production of SCP from yeasts has not been extensive mainly due to the thickness of cell wall. To be economically feasible, it is necessary to engineer optimum culture conditions for maximum biomass productivity with efficient utilization of reducing sugars. Yet batch processing represents the predominant production method used in industry. Hence, we present batch fermentations of *C. utilis* and *K. fragilis* in this work in order to obtain high biomass productivity, and the preservation of the yeast feed is also discussed.

Materials and Methods

Microorganisms and Media

Cultures of *Candida utilis* (ATCC 9950) and *Kluyveromyces fragilis* (ATCC 36534) used in this study were maintained on YEPD agar slants which contained : dextrose, 2% ; yeast extract, 0.5% ; peptone, 2% ; and agar, 2%. A single colony was used to inoculate in a shaken tube at 30℃ using a rotary shaker at 180 rpm. The yeast cultures were regularly checked under microscope in order to eliminate any possibility of contamination.

A complex medium and a fructose medium were used for the fermentations of *C. utilis* and *K. fragilis*, respectively. The composition of the complex medium was : dextrose, 20 g/L; K_2HPO_4, 2.8 mM; KH_2PO_4, 12.8 mM; NH_4Cl, 75 mM; Na_2SO_4, 11.5 mM; $MgCl_2$, 125 mM; citric acid, 1.0 mM; and biotin, 4μg/L, and that of the fructose medium was : fructose, 2.5%; yeast extract, 0.5%; and peptone, 1%. The initial pH of each medium was adjusted to 5 with 2N HCl. All media used were sterilized by autoclaving at 121℃ for 20 min.

Equipment and Cultures

Batch fermentations were conducted using 1L-Marubishi fermenter with working volume of 600 ml. The pH, foam, and a dissolved oxygen (DO) were controlled by Labo Controller (Marubishi Co., Japan). The pH was continuously adjusted by using 2N HCl and 2N NaOH, and the foam was controlled using 10% antifoam DB-110A. The aeration and agitation rates were maintained at 1.5 L/min and 600~650 rev/min, in which the DO level never fell below 20% of air saturation for all experiments.

The 5% inoculum (30 mL) was grown to exponential phase in a 250 ml shaken flask for 15 h at 180 rpm and the optimum temperature of 30℃ for *C. utilis* and 37℃ for *K. fragilis*, respectively.

Assays

Samples from the fermenter were analyzed for the concentrations of yeasts. The cell concentration was measured spectrophotometrically at a 620 nm wavelengh using 752 UV Grating Spectrophotometer. Samples of two yeasts were diluted in order to confine the absorbance readings to the range 0.1~0.7 optical densities (OD), as required by the Lambert-Beer law. To relate the measured OD to the dry-weight yeast concentrations (DCW), several samples of the two yeasts were taken at different times over the course of batch cultures. The DCW was determined by weighing the cell pellet after being dried in an oven at 100℃ for 12 h. The cell pellet was prepared by centrifuging a 5 ml sample of broth culture at 5,000 rpm for 10 min and then by decanting the supernatant after washing twice with distilled water. A linear correlation between DCW and OD was obtained. The number of viable cells was counted using a hemocytometer under a light microscope immediately after the cells were stained with Bengal Rose B. Live cells appeared clear and the dead cells appeared pink. All the measurements were performed in triplicates.

Results and Discussion

Batch fermentations of the two yeasts, *K. fragilis* and *C. utilis*, were investigated for mass culture under optimum conditions, where the yield of cell biomass and the biomass productivity on different carbon composition of culture media were compared.

Batch Fermentations

1. *Candida utilis*

A batch fermentation on a 2% YE medium (dextrose, 2%; and yeast extract, 0.5%) was first executed in this study, since YE medium has been generally used for the cultivation of yeasts. The fermentation resulted in 0.53 g/g of yield and 0.90 g/L/h of productivity with 30×10^8 viable cells per ml. To compare these results with those on a different medium, a

Table 1. Batch fermentations of *C. utilis* yeast on different media

Medium \ Measurement	μ_{max} (h^{-1})	OD_{max}	Max. DCW (g/L)	Max. Cell No. (cells/ml)	Yield (g/g)	Productivity (g/L/h)
2% YE medium	0.38 ± 0.04	3.0 ± 0.2	9.0	$(30 \pm 30) \times 10^8$	0.53	0.90
2% Complex medium	0.36 ± 0.01	3.2 ± 0.2	11.6	$(65 \pm 10) \times 10^8$	0.58	1.22
1% Complex medium	0.38 ± 0.07	2.4 ± 0.2	7.0	$(43 \pm 30) \times 10^8$	0.70	0.78

Table 2. Batch fermentations of *K. fragilis* yeast on different media

Medium \ Measurement	μ_{max} (h^{-1})	OD_{max}	Max. DCW (g/L)	Max. Cell No. (cells/ml)	Yield (g/g)	Productivity (g/L/h)
2% YE medium	0.50 ± 0.03	3.4 ± 0.2	9.3	$(15 \pm 20) \times 10^8$	0.47	1.03
2% YE medium	0.61 ± 0.01	3.3 ± 0.1	11.0	$(55 \pm 20) \times 10^8$	0.37	1.47
2.5% Fructose medium	0.74 ± 0.03	3.5 ± 0.1	12.6	$(70 \pm 30) \times 10^8$	0.50	1.94

fermentation was executed on a 2% complex medium. The results in Table 1 shows that the fermentation on the 2% complex medium brought about better results. This means that *C. utilis* needs various types of nutrients to grow in a fermenter. When the fermentation was undertaken on a 1% complex medium, a better yield of cell biomass, 0.70 g/g, was obtained, but the productivity and the number of viable cells were much lower. Hence, the 2% complex medium was selected in this study, and its fermentation profile is shown in Fig. 1, in which the yield of 0.58 g/g and the productivity of 1.22 g/L/h were obtained with 65×10^8 viable cells per ml. As seen in Fig. 1, yeast cells needed lots of oxygen when they grew, and the yield of cell biomass seems to be directly related to the aeration level (Garcia-Gariby et al., 1987).

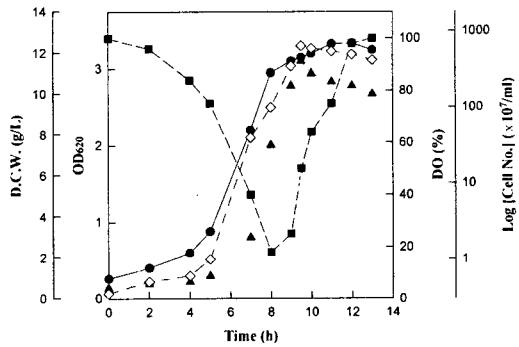

Fig. 1. The batch fermentation of *C. utilis* on 2% complex medium under pH5, 30℃, 1.5 vvm and 600 rpm. −●−, OD; −■−, DO; ···▲···, D.C.W.; ···◇···, Cell No.

2. *Kluyveromyces fragilis*

In a similar way, a batch fermentation of *K. fragilis* was done on the 2% YE medium. The results showed the yield of 0.47 g/g and the productivity of 1.03 g/L/h. On a 3% YE medium, a fermentation gave rise to a better biomass productivity with lower yield of cell biomass, the results of which are tabulated in Table 2. When a fermentation was carried out on 2.5% fructose medium as Cruz-Guerrero et al. (1995) reported, the results were much better than those on the 3% YE medium. With 70×10^8 viable cells per ml, the yield and the productivity were found to be 0.50 g/g and 1.94 g/L/h, respectively in the fermentation. The profile of the fermentation is shown in Fig. 2. Compared to the fermentation of *C. utilis*, this yeast grew faster and depleted the available oxygen in a relatively short time away from the oxygenated zone due to the low solubility of oxygen in the fermenter. Hence, the supply of oxygen is always a concern in this fermentations (Oosterhuis et al., 1985).

The effect of a growth factor on biomass productivity

The productivity and the yield of cell mass could be significantly affected by the presence of a growth

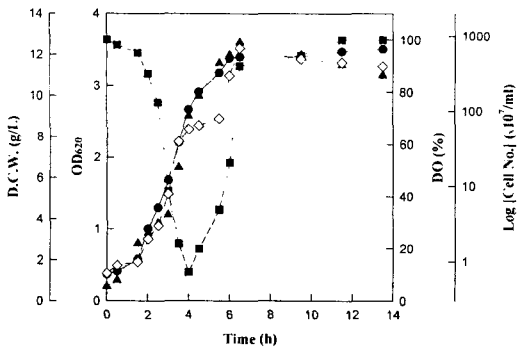

Fig. 2. The batch fermentation of *K. fragilis* on 2.5% fructose medium under pH5, 37℃, 1.5 vvm and 650 rpm.—●—, OD;—■—, DO; ···▲···, D.C.W.; ···◇···, Cell No.

factor. Zinc has known to be a cofactor for a number of metalloenzymes, such as hexokinase, alcohol dehydrogenase, and glutamate dehydrogenase (Lawford et al., 1979). It was also reported that with zinc in the medium the protein content of the culture biomass was significantly increased thereby increasing the efficiency of the single cell protein production process. In this respect, with 15, 25, 45, and 65 μM of zinc added to the 2% complex medium for the fermentation of *C. utilis* yeast, increasing the concentration of zinc decreased the yield and the productivity as shown in Table 3. But, the addition of 15 μM zinc resulted in an increase in the biomass productivity and the number of viable cells from 1.22 g/L/h and 65×10^8 cells/ml to 1.23 g/L/h and 80×10^8 cells/ml, respectively.

In our previous study (Moon et al., 1996), zinc had a somewhat toxic effect on *K. fragilis*, and this yeast could not survive well at the concentrations over 20 μM in flask culture. In this study, 15 μM of zinc, which resulted in better growth yield and the biomass productivity in the fermentation of *C. utilis*, was added to the 2.5% fructose medium and then a fermentation was executed. The fermentation showed lower yield and productivity as the results from the flask culture. When 1% peptone was not supplemented in the 2.5% fructose, poor results seen in Table 3 were obtained. Hence, peptone as amino N-source involved to the cell growth and it must be a growth factor in the fermentation of *K. fragilis*.

Table 3. The effect of growth factor on biomass productivity

Strain	Medium	Measurement					
		μ_{max} (h^{-1})	OD_{max}	Max. DCW (g/L)	Max. Cell No. (cells/ml)	Yield (g/g)	Productivity (g/L/h)
C. utilis	2% Complex medium+15μM Zn	0.36 ± 0.02	3.2 ± 0.1	12.5	$(80 \pm 30)\times10^8$	0.63	1.23
	2% Complex medium+25μM Zn	0.36 ± 0.01	3.3 ± 0.1	12.2	$(65 \pm 30)\times10^8$	0.61	1.22
	2% Complex medium+45μM Zn	0.34 ± 0.02	3.2 ± 0.2	11.3	$(68 \pm 30)\times10^8$	0.56	1.07
	2% Complex medium+63μM Zn	0.34 ± 0.01	3.1 ± 0.1	11.2	$(65 \pm 30)\times10^8$	0.56	0.93
K. fragilis	2.5% Fructose medium+15μM Zn	0.67 ± 0.02	3.3 ± 0.1	10.4	$(52 \pm 30)\times10^8$	0.42	1.49
	2.5% Fructose medium−peptone	0.48 ± 0.02	3.2 ± 0.2	8.5	$(22 \pm 30)\times10^8$	0.34	1.00

Table 4. The effect of 3.5% NaCl on yeast growth

Strain	Medium	Measurement					
		μ_{max} (h^{-1})	OD_{max}	Max. DCW (g/L)	Max. Cell No. (cells/ml)	Yield (g/g)	Productivity (g/L/h)
C. utilis	CONTROL	0.36 ± 0.04	3.2 ± 0.2	11.6	$(65 \pm 10)\times10^8$	0.58	1.22
	CONTROL+3.5% NaCl	0.25 ± 0.05	2.5 ± 0.3	7.8	$(80 \pm 40)\times10^7$	0.39	0.45
K. fragilis	CONTROL	0.74 ± 0.03	3.5 ± 0.1	12.6	$(70 \pm 30)\times10^8$	0.50	1.94
	CONTROL+3.5% NaCl	0.38 ± 0.04	2.8 ± 0.4	6.5	$(25 \pm 35)\times10^7$	0.25	0.59

Preservation of yeast diet

In order to consider the yeast protoplasts used to feed the larval stages of fish and organisms of shellfish that live in about 35 -salinity seawater, the effect of NaCl concentration on the growth of each yeast was studied at 3.5% NaCl (v/v). For both the yeasts, increasing the NaCl concentration increased the length of the lag phase, and 25×10^7 to 80×10^7 viable cells/ml were obtained, even though the yield and the biomass productivity decresed significantly (Table 4). The digestibility of intact yeast cells has been found to be lower than that of cell-wall disrupted cells (yeast protoplasts) when they were used to feed *Artemia* (Coutteau et al., 1990). This may be an important constraint in the use of this SCP as a food source in aquaculture in particular. Besides, the storage of the yeast protoplasts without osmotic lysis is another problem, and it has known that the yeast protoplasts could be stored in seawater at 4℃ for a week without cell lysis (Coutteau et al., 1990). Thus, cultivating these yeasts at 3.5% NaCl may be considered in order to preserve the yeast protoplasts long in an isotonic condition. Otherwise, a good preservative should be developed to maintain the yeast diet without cell lysis by osmosis.

From the all above results, the two yeasts, *K. fragilis* and *C. utilis*, have the advantage of mass production over *Saccharomyces cerevisiae* because they grow faster and produce more single cell proteins than *S. cerevisiae* does, since typical values of the yield and the productivity in an aerobic batch culture of *S. cerevisiae* yeast on 2% glucose were about 0.22 g/g and 0.3 g/L/h, respectively (Scragg, 1991). Problems that arise when feeding a yeast monodiet have often been assigned to nutritional deficiencies of the yeast diet (Urban and Langdon, 1984; Douillet, 1987; Hirayama, 1987). Besides, poor digestibility may be an important constraint in the use of this single cell protein as a food source in aquaculture because yeast cells are known to have a complex and thick cell envelope. Further research should be focussed on this problem.

Conclusions

Mass production of *K. fragilis* and *C. utilis* in batch fermenters was studied to develop the yeasts as an algal substitute in aquaculture because they have known to be not pathogenic and possible yeast diet as well. With 5% inoculum dosage, batch fermentations on the 2% complex medium and the 2.5% fructose medium gave the best results for *C. utilis* and *K. fragilis*, respectively. The yield, the productivity and the viable cell count were found to be 0.58 g/g, 1.22 g/L/h and 65×10^8 cells/ml for *C. utilis* and 0.50 g/g, 1.94 g /L/h and 70×10^8 cells/ml for *K. fragilis*, respectively. For the fermentation of *C. utilis*, better results were obtained by the addition of 15 μM of zinc as a cofactor. In the fermentation of *K. fragilis* yeast cells, better results could not obtained with the addition of zinc, but peptone was found to be a growth factor. Compared to *S. cerevisiae* that had been an established yeast diet for rotifer culture, the batch fermentations of the two yeasts resulted in much higher yield and productivity with higher number of viable cells because they grew faster and produced more single cell proteins than *S. cerevisiae* did.

It was also found that increasing the NaCl concentration increased the length of the lag phase for both the yeasts. In the fermentation, 25×10^7 to 80×10^7 viable cells/ml were obtained, even though the yield and the biomass productivity decreased significantly. This means that cultivating these yeasts at 3.5% NaCl may be considered in order to preserve the yeast protoplasts long in an isotonic condition.

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