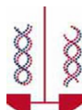

Journal of Bioscience and Bioengineering
VOL. 108 No. 4, 344–347, 2009

www.elsevier.com/locate/jbiosc

TECHNICAL NOTE

Enhancement of L(+)-lactic acid production using acid-adapted precultures of *Rhizopus arrhizus* in a bubble column reactor

Zhan Ying Zhang,[1] Bo Jin,[1,2,3,*] and Joan M. Kelly[4]

School of Chemical Engineering, The University of Adelaide, Adelaide, SA 5005, Australia[1] *School of Earth and Environmental Sciences, The University of Adelaide, Adelaide, SA 5005, Australia*[2] *Australian Water Quality Centre, Bolivar, SA 5095, Australia*[3] *and School of Molecular and Biomedical Science, The University of Adelaide, SA 5005, Australia*[4]

Received 9 December 2008; accepted 10 April 2009

88 g/L lactic acid was produced from waste potato starch (equivalent to 100 g/L glucose) in a bubble column reactor using appropriate acid-adapted precultures of *Rhizopus arrhizus*. Further experiment showed that repeated dilution of cultures caused the decrease of lactic acid concentration and productivity due to formation of large fungal pellets.

© 2009, The Society for Biotechnology, Japan. All rights reserved.

[**Key words:** Lactic acid; *Rhizopus arrhizus*; Morphology; Pellet; Acid-adapted precultures; Bubble column reactor]

Cultivations of filamentous fungi in submerged cultures for production of organic acids, antibiotics and enzymes have been investigated widely over the past several decades. Filamentous fungi are morphologically complex microorganisms with morphological forms varying from fluffy mycelia, clumps to pellets in submerged cultures (1). It has been demonstrated that small pellets are a desirable morphological form in industrial fermentation processes as pellet biomass improve rheology of fermentation broths, and consequently favor mixing and mass transfer (1).

Lactic acid producers from *Rhizopus arrhizus* (*syn. oryzae*) have attracted a great interest in the last two decades because of their amylolytic ability, low requirements on nutrients and ability to produce optically pure L(+)-lactic acid (2, 3). The main disadvantage of lactic acid production by *Rhizopus* is the low yield due to formation of undesirable morphological forms such as clumps. Efforts have been made to enhance lactic acid yield in reactors by inoculation of pelletized *Rhizopus* obtained in shake flasks (4–7). However, with these pelletizing strategies the lactic acid yields are still lower than 80%.

In a previous study, we successfully developed an inoculation strategy using acid-adapted precultures to control the morphology of *R. arrhizus* in pellet forms in a stirred tank reactor (STR) (8). The lactic acid yield in the STR reached 86%, which was 6%–45% higher than those using other common pelletizing strategies (8). In this study, we applied this inoculation strategy with a bubble column reactor (BCR) system and investigated the effect of acid-adapted precultures on fungal morphology, and production of lactic acid and by-products. Furthermore, we studied the effect of repeated dilution of cultures on lactic acid production.

R. arrhizus DAR 36017, obtained from the Orange Agricultural Institute (Australia) was used in this study. This strain was maintained and grown for spore production on potato dextrose agar slants at 30 °C for 7 days and stored at 4 °C.

The composition of preculture and production media has been described previously (8). Preculture medium contained (g/L): soluble starch, 10; peptone, 5.0; yeast extract, 5.0; KH_2PO_4, 0.2; $MgSO_4 \cdot 7H_2O$, 0.2. pH of preculture medium was adjusted to an initial value ranged from 2.5 to 5.5 before sterilization. Production medium used in the reactor consisted of waste potato starch (equivalent to 100 g/L glucose), 3.0 g/L $(NH_4)_2SO_4$, 0.25 g/L KH_2PO_4, 0.15 g/L $MgSO_4 \cdot 7H_2O$ and 0.04 g/L $ZnSO_4 \cdot 7H_2O$. The preculture and production media were autoclaved at 121 °C for 20 min.

The preculture was prepared in a 250 mL shake flask containing 100 mL preculture medium. The procedures for preparation of acid-adapted precultures have been described elsewhere (8). Spores were harvested from slants using a platinum loop and suspended in sterilized water. The 1st precultures with an inoculum size of 10^5 spores/mL were incubated for 18 h at a designated pH of 2.5–5.5. The 2nd precultures at a designated pH were inoculated with 5 mL of the 1st preculture and grown for 12 h. In this paper, pH of the precultures refers to the initial pH unless otherwise stated. The biomass concentration varied from 0.7 g/L to 2.2 g/L in 18 h cultivation of the 1st acid-adapted precultures and from 0.7 g/L to 1.8 g/L in 12 h cultivation of the 2nd acid-adapted precultures which were adapted at a pH from 2.5 to 5.5 (8). All the precultures were cultivated at 30 °C and 150 rpm in an orbital shaker.

Production of lactic acid was carried out in an 11.5 L stainless steel BCR with a working volume of 7.5–8.5 L. The BCR was equipped with a sintered stainless steel sparger (pore size, 50–70 μm) at the bottom.

* Corresponding author. School of Chemical Engineering, The University of Adelaide, Adelaide, SA 5005, Australia. Tel.: +61 8 8303 5996; fax: +61 8 8303 4308.
E-mail address: bo.jin@adelaide.edu.au (B. Jin).

1389-1723/$ - see front matter © 2009, The Society for Biotechnology, Japan. All rights reserved.
doi:10.1016/j.jbiosc.2009.04.009

7.5 L production medium in the BCR was sterilized at 121 °C for 20 min. After sterilization, the volume of production medium was about 7.1–7.2 L because of water evaporation. The inoculation was conducted by transferring 375 mL of acid-adapted preculture into the BCR. The temperature and aeration rate in the BCR were maintained at 30 °C and 0.4 vvm (air volume aerated per medium volume per min) throughout cultivations. The cultivation pH was controlled at pH 6.0 with the addition of 10 M NaOH solution. 0.1% antifoam (v/v) (Dow Corning® 1510, BDH, UK) was added to the reactor before sterilization. A few more drops of antifoam were added in cultivations if necessary. All the cultivations were stopped in 60 h.

When the 375 mL of acid-adapted preculture was transferred from shake flasks to BCR with 7.1–7.2 L production medium, the preculture was diluted about 20 times. Cultivation was carried out for 18 h with 6.75 L culture drained at an aeration rate of 1.0 vvm (to keep the culture as homogenous as possible) through sampling port and approximate 0.75 L culture retained in the reactor. The other 6.75 L fresh production medium was added into the BCR which resulted in 10 times dilution of the culture. This cultivation was further carried out for 60 h. The other cultivation was further carried out for 18 h and 10 times dilution of the culture was repeated again as described. The diluted culture was also cultivated for 60 h. Aeration rate, cultivation temperature and pH in all cultivations were controlled at 0.4 vvm, 30 °C and 6.0 respectively.

30 to 50 mL samples were taken from the BCR in a 6 h or 12 h interval. The morphology of *R. arrhizus* DAR 36017 was photographed using a digital camera (PowerShot A95, Canon, Japan). For pellet distribution analysis, a series of sieves with an aperture of 1.0 mm, 1.4 mm, 1.7 mm, 2.0 mm and 2.8 mm were used to collect the pellets with a diameter less than 1.0 mm, between 1.0 mm and 1.4 mm, between 1.4 mm and 2.0 mm, and above 2.8 mm. Biomass was harvested after filtration and washed three times using tap water. Biomass weight was determined after drying at 60 °C for 72 h. All organic compounds, including glucose, lactic acid, fumaric acid and ethanol were analyzed by high performance liquid chromatography (HPLC) (8). The results presented were the means of at least duplicate experiments.

Fig. 1 shows the representative morphological forms of *R. arrhizus* in the BCR using different acid-adapted precultures. It was observed that precultures inoculated significantly affected the morphology of the fungal biomass in the BCR. The morphology of *R. arrhizus* varied from coalesced fluffy mycelia to freely dispersed small pellets in the BCR inoculated with the 1st precultures. Fluffy mycelia (Fig. 1B1 and C1) were formed in the reactor which was inoculated with the 1st precultures adapted at pH 3.5–5.5 whereas the freely dispersed small pellets appeared in the reactor using the 1st precultures at pH 2.5 and 3.0 (Fig. 1A1). The use of the 2nd precultures at pH 4.0–5.5 resulted in the formation of freely dispersed small pellets as the dominant morphological form in the BCR (Fig. 1C2). Freely dispersed compact and large pellets (Fig. 1A2) were formed with the 2nd precultures at pH 2.5 and 3.0 while fluffy mycelia were observed in the reactor using the 2nd preculture at pH 3.5 (Fig. 1B2).

Analysis of pellet distribution of dispersed pellets in 48 h shows that the total pellets smaller than 2.0 mm made up 93% of the total biomass (w/w) while pellets over 2.0 mm were only 7.0% when the 1st preculture at pH 2.5 was used. The use of the 2nd preculture at pH 2.5 and pH 3.0 resulted in a significant reduction in the percentage of small pellets and an increase in the numbers of large pellets. Approximate 90% small pellets (<2.0 mm) were also formed in the BCR using the 2nd precultures at pH 4.5 to 5.5. It was found that fluffy mycelia dominated in the BCR with the 1st preculture at pH 5.0 (Fig. 1). In summary, using the 1st precultures pH 2.5 and 3.0, and the 2nd precultures at pH 4.5 to 5.5 led to the formation of small pellets (<2.0 mm) whereas using the 1st and 2nd precultures at adapted at other pH resulted in the formation of fluffy mycelia in the BCR.

Table 1 shows the results of production of lactic acid and by-product using different acid-adapted precultures as inoculum. The lactic acid concentration in the BCR was significantly enhanced from 45.9 g/L to 88.7 g/L with the 1st precultures adapted at pH from 5.5 to 2.5. Lactic acid concentrations of 87.9–88.5 g/L were achieved using the 2nd precultures at pH 4.5–5.5. The maximum lactic acid productivity reached 2.1 g/L/h with small pellets, higher than those with large pellets or fluffy mycelia. In summary, high lactic acid concentration and productivity were associated with freely dispersed

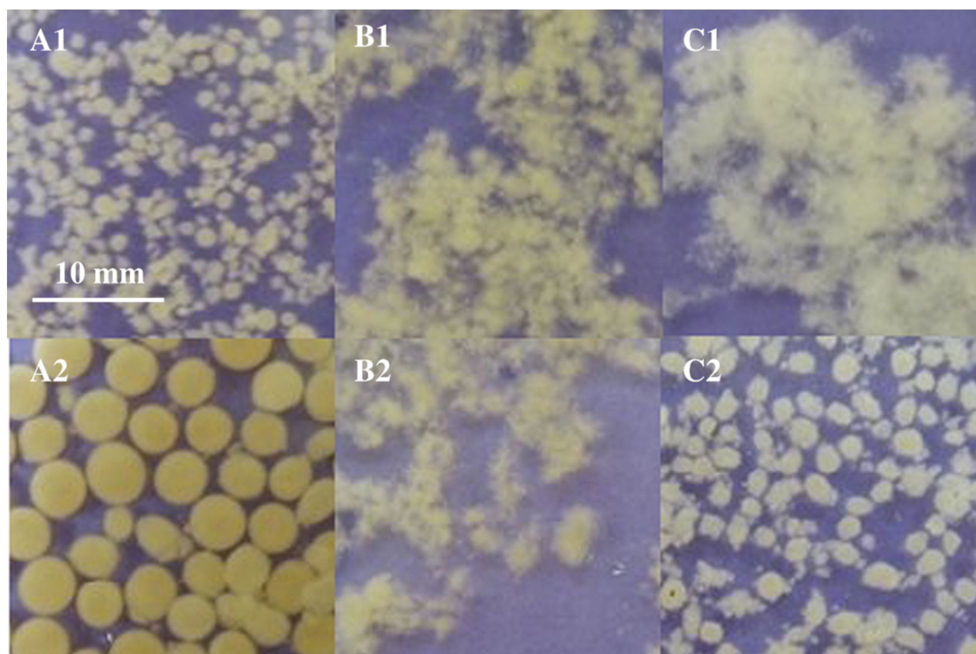

FIG. 1. Effect of precultures on the morphology of *R. arrhizus* in the BCR. The precultures used were the 1st precultures adapted at pH 2.5 (A1), pH 3.5 (B1), pH 5.0 (C1), the 2nd precultures adapted at pH 2.5 (A2), pH 3.5 (B2) and pH 5.0 (C2). Photos were taken in 48 h from a 9.0 cm petri-dish.

Download English Version:

https://daneshyari.com/en/article/21698

Download Persian Version:

https://daneshyari.com/article/21698

Daneshyari.com