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Original Article

Effect of Tween 80 on production of lactic acid by *Lactobacillus casei*

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Abstract

Batch fermentation was performed to investigate the effect of Tween 80 on the growth and the production of lactic acid of *Lactobacillus casei*. Both, amount and yield of lactic acid, increased by 8% when the fermentation medium was supplemented with 0.1% (w/v) Tween 80 in comparison with the control (without Tween 80 addition) at 48 h. Optimization of Tween 80 concentration in the range of 0.04-0.5% (w/v) showed that 0.07% (w/v) Tween 80 was the optimum for lactic acid production and cell growth. With addition of 0.07% (w/v) Tween 80, yeast extract concentration could be decreased from 1.0% to 0.7% (w/v) while still maintaining high lactic acid yield and sugar conversion.

Keywords: lactic acid, Tween 80, fermentation, *Lactobacillus casei*

1. Introduction

Lactic acid and its derivatives have a wide range of application in pharmaceutical, food, and cosmetics industries (Hujanen and Linko, 1996). Recently, it has gained increasingly interest as a starting material for the manufacturing of poly (lactic acid), a biocompatible and biodegradable plastic. In the polymerization process, the stereospecification of lactic acid is important since highly optically pure lactic acid is necessary to obtain high crystalline poly (lactic acid), which leads to the high strength and chemical and heat resistances of the polymer (Lunt, 1998). Selective production of stereospecific lactic acid has been extensively studied by microbial fermentation, because chemical synthesis resulted in racemic mixture of lactic acid (Hofvendahl and Hahn-Hagerdal, 2000). Only a few lactic acid bacteria, such as *Lactobacillus casei,* produced the predominant $_{L}$(+) form of lactic acid.

Tween 80 (polyoxyethylene sorbitn mono-oleate), a nonionic surfactant, is widely used as an additive in foods, pharmaceutical preparations, and as an emulsifier, dispersant, or stabilizer (Budavavi *et al.*, 1996). Tween 80 can dissolve lipid structure in the cell membrane, thereby improving the membrane permeability and enhancing the release of the intracellular enzyme. Therefore, it has a widespread use in the extracellular enzyme production (Liu *et al.*, 2006; Zeng *et al.*, 2006; Silva *et al.*, 2007; Gonzalez *et al.*, 2008). Tween 80 and related surfactants have also been found to promote the migration of nutritive compounds into cells; therefore, they have been supplemented in the bacterial medium to promote the growth (Reese and Maguire, 1969). Tween 80, served as an essential growth factor, has proved beneficial in the cultivation and fermentation of *Lactobacillus* (Duggan *et al.*, 1959). Oh *et al*. (1995) reported that the growth of *Lactobacillus casei* was strongly affected by Tween 80. Nagarjun *et al.* (2005) further showed that Tween 80 played an important role in the conversion of starch to lactic acid. Several fermentative investigations of lactic acid have supplemented the fermentation medium with 0.1% (w/v) Tween 80 (Nancib *et al.*, 2001; Bai *et al.*, 2003; Kurbanoglu, 2004). However, until today only a few studies relating to the effect of Tween 80 on the fermentation performance of *Lactobacillus* at length have been published.

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In order to expand the market demand for lactic acid, it is important to minimize the bioprocessing cost. Adding more nutrient supplements in the medium would increase the raw material cost and also add to the residual impurities remaining after fermentation, which may have to be removed by costly purification processes during the downstream. This study also investigate how small amounts of Tween 80 and yeast extract were added in the fermentation medium, while still remaining high lactic acid yield and sugar conversion.

2. Materials and Methods

2.1 Microorganism and fermentation

Lactobacillus casei GIM1.159 (originally from ATCC 334) was grown overnight in MRS medium at 37°C to 10^9 c.f.u./ml. The culture broth was transferred to 100 ml of growth medium in a 250 ml Erlenmeyer flask and cultivated at 37°C for 24h with shaking at 150 rpm. Fermentation was carried out in a 100 ml working volume in 250 ml flasks, incubated at 37°C on a rotary shaker operated at 150 rpm (Buyukkileci and Harsa, 2004) after inoculating 5 ml of culture broth in a growth medium. The growth medium consisted of (per liter of distilled water) 0.2 g $MgSO_4 \cdot 7H_2O$, 0.03 g $MnSO_4 \cdot H_2O$, 0.5 g sodium acetate, 2 g triammonium citrate, 1.5 g K_2HPO_4, 1.5 g KH_2PO_4, 50 g glucose, 5 g peptone, 10 g yeast extract , 30 g $CaCO_3$, and varying concentrations of Tween 80 (Polyethylene glycol sorbitan monooleate, Analytic reagent, Shantou Xilong Chemical Factory, Guangdong, China). The fermentation medium was prepared in the same manner, except 90 g glucose was used instead of 50 g glucose.

Three parallel samples were used in all analytic determinations, and data are presented as the mean of three replicates. The standard deviations between replicates are below 5%.

2.2 Analytical methods

Biomass were determined as dry cell weight, five ml of broth was centrifuged at 9, 820 ×g for 10 min by Sigma 3-18K centrifuge (Germany), and the pellet was washed with 5ml of 1 M HCl to remove $CaCO_3$, The mixture was centrifuged again, and the pellet was dried at 80°C to constant weight. Glucose concentration was measured by DNS method (Miller, 1959). $_L$(+) lactic acid was extracted with 1M H_2SO_4 and was estimated by the colorimetric method of Kimberley, *et al.* (1996).

2.3 Statistical analysis

Experimental data were statistically analyzed using OriginPro 7.5 (OriginLab, Northampton, MA). Differences between means were compared using analysis of variance (ANOVA) to determine the optimal fermentation conditions at the 95% confidence level.

3. Results and Discussion

3.1 Beneficial effect of 0.1% (w/v) Tween 80 on the lactic acid production

Fermentation was performed initially with 1.0% (w/v) yeast extract supplemented with 0.1% (v/v) Tween 80, which had been reported in the literature (Nancib *et al.*, 2001; Bai *et al.*, 2003; Kurbanoglu, 2004), in order to verify its beneficial effect on the fermentation. Lactic acid yield and dry cell weight increased by 8% and 29% at 48 h in the presence of 0.1% (v/v) Tween 80, respectively, compared with the control (without Tween 80) (Figure 1a and b).

3.2 Optimization of Tween 80 concentration

Fermentation with different Tween 80 concentrations ranging from 0.04% (w/v) to 0.5% (w/v) was performed to

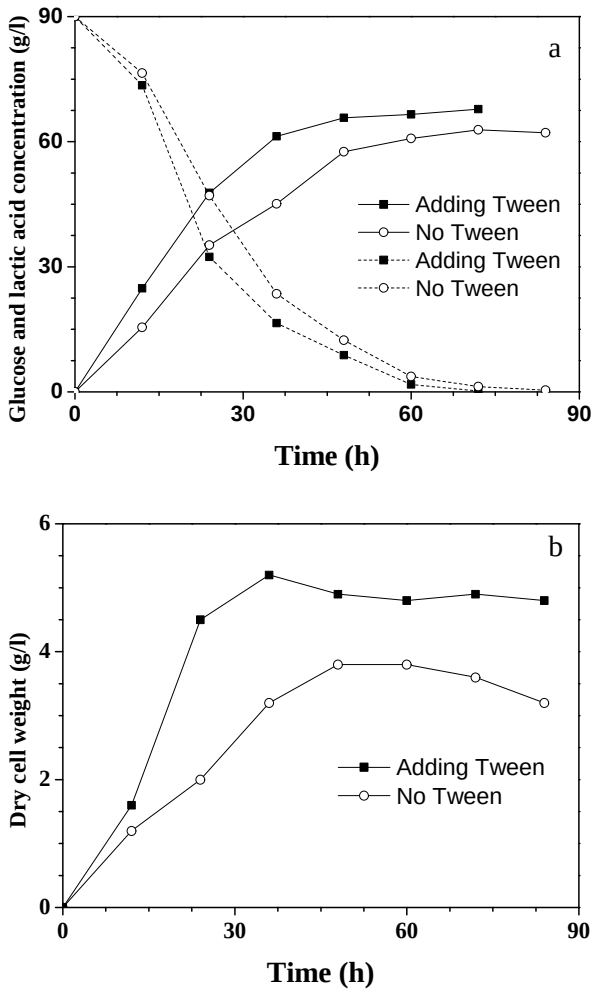

Figure 1. Effect of Tween 80 addition at a concentration of 0.1% (w/v) on glucose utilization, lactic acid production (solid lines represent lactic acid; dashed lines represent glucose) (a) and cell growth (b). Yeast extract supplementation was 1.0% (w/v).

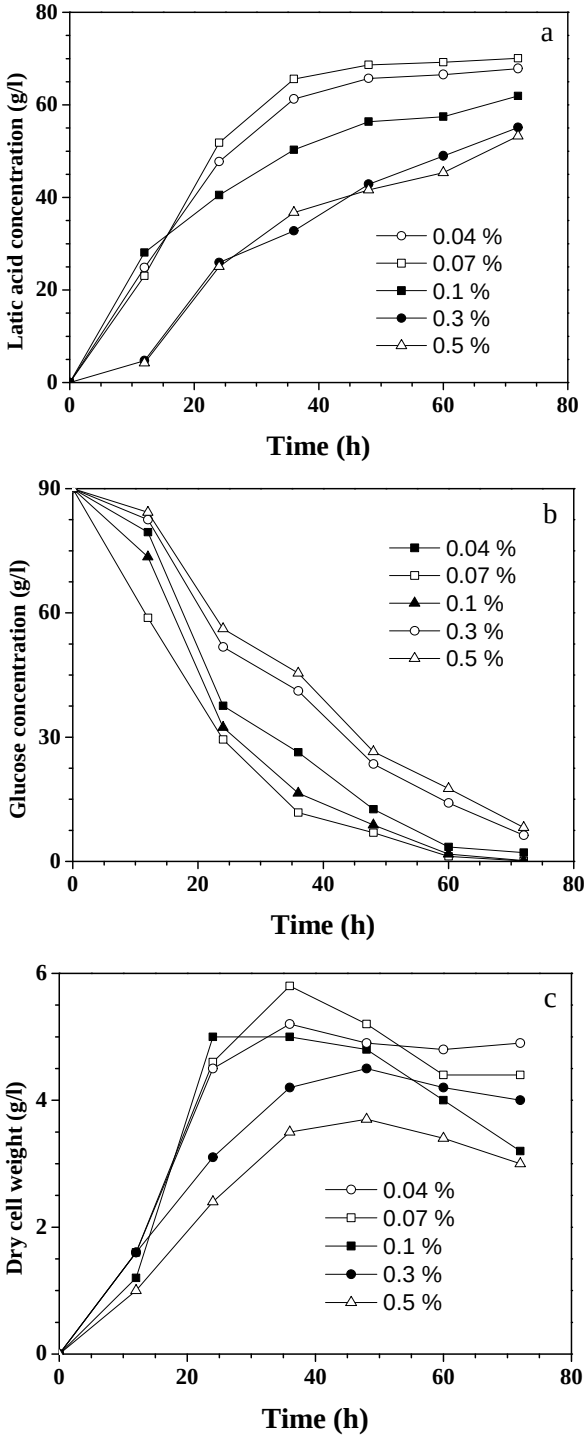

Figure 2. Effect of Tween 80 addition on lactic acid production (a), glucose consumption (b) and cell growth (c). Yeast extract supplementation was 1.0% (w/v).

investigate the optimal amount of Tween 80 (Figure 2a, b, c). The results showed that 0.07% (w/v) Tween 80 was the optimal amount for lactic acid production, sugar consumption, and microbial growth. A higher concentration of Tween 80 (0.5% (w/v)) decreased the lactic acid yield and cell concentration by 16% and 41%, respectively. This was probably

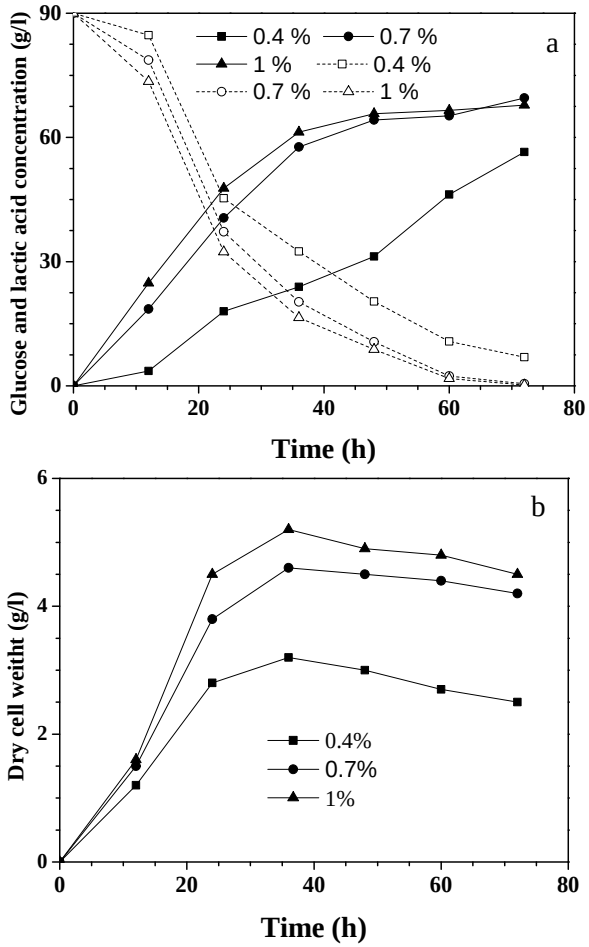

Figure 3. Effect of yeast extract concentration (w/v) on glucose utilization, lactic acid production (solid lines represent lactic acid; dashed lines represent glucose) (a) and cell growth (b). Tween 80 supplementation was 0.1% (w/v).

because Tween 80 became toxic at higher concentration due to the destruction of the cell membrane structure and/or loss of the cell membrane function caused by the solubility of lipid bilayer by Tween 80, a surfactant.

3.3 Effect of Tween 80 addition on reducing yeast extract requirement

Fermentation was also performed to investigate if the amount of yeast extract could be lowered in the fermentation supplemented with 0.1% (w/v) Tween 80. Figure 3a showed that yeast extract could be lowered to 0.7% (w/v) while still maintaining 88% sugar conversion and 81 % lactic acid yield. At 0.4% (w/v) yeast extract concentration, the fermentation performance of *Lactobacillus casei* was poor. Figure 3b showed the beneficial effect of yeast extract on the cell growth. Lactic acid bacteria typically have complex nutritional requirements, due to their limited ability to synthesize their own growth factors, such as B vitamins and amino acids. They require some elements in the yeast extract for

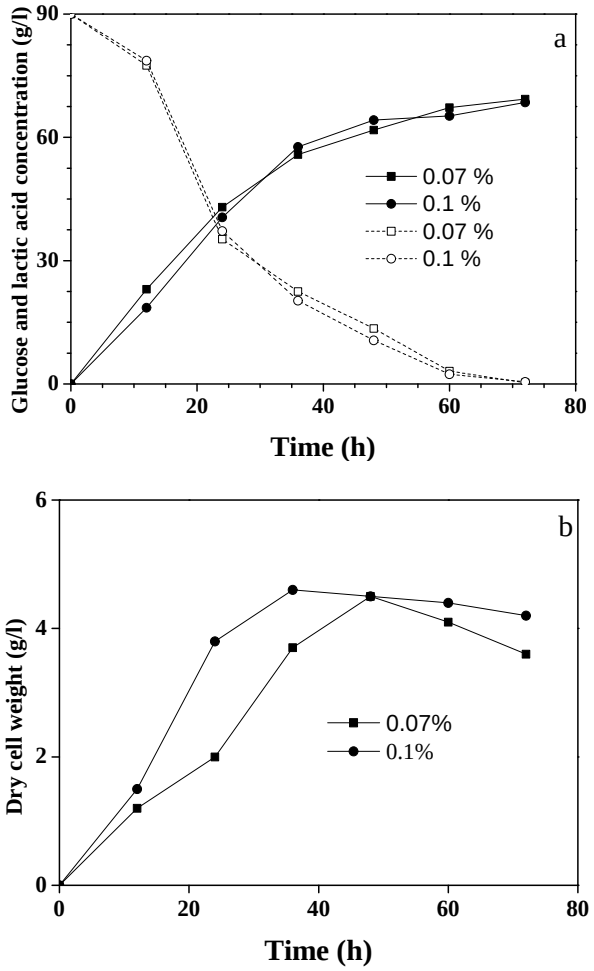

Figure 4. Effect of 0.07% and 0.1% (w/v) of Tween 80 addition on glucose utilization, lactic acid production (solid lines represent lactic acid; dashed lines represent glucose) (a) and cell growth (b) when the fermentation was supplemented with 0.7% (w/v) yeast extract.

growth, such as carbon and nitrogen sources in the form of carbohydrates, amino acids, vitamins, and minerals (Wee *et al.*, 2006).

Finally, fermentation was performed using 0.7% (w/v) yeast extract supplemented with 0.07% (w/v) Tween 80 in comparison to that carried out using the medium supplemented with 0.1% (w/v) Tween 80 and the same amount of yeast extract. Figure 4a and. 4b show that both fermentation systems have similar fermentation performance concerning lactic acid yield, sugar conversion and cell concentration.

4. Conclusions

Tween 80 had a significant effect on the production of lactic acid by *Lactobacillus casei*. Tween 80 at a concentration of 0.07% (w/v) exhibited slightly better fermentation performance compared to 0.1% (w/v) Tween 80. The amount of yeast extract could also be lowered to 0.7% (w/v) from 1.0% (w/v) with addition of 0.07% (w/v) Tween 80, while still maintaining high sugar conversion and lactic acid yield.

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