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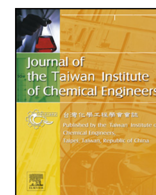

Sodium benzoate stimulates xylitol production by *Candida mogii*

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ABSTRACT

Xylitol is an important commercial sweetener that can be produced by fermentation. Previous studies of xylitol production have not been able to combine high average productivity and yield in a single process. Benzoate is a highly chaotropic stressor and has been found to stimulate the fermentation metabolism of yeasts at low concentrations. Therefore, in the current work, it was hypothesized that benzoate increases xylitol production, because it is a concurrent kosmotropic/compatible solute under aerobic conditions. Shake flask experiments without control of dissolved oxygen revealed that sodium benzoate added at a concentration of up to 150 ppm stimulated the fermentation of xylose to xylitol by *Candida mogii*. Sodium benzoate at a concentration of >200 ppm had a clear inhibitory effect on the xylose metabolism. Controlled batch fermentations carried out in bioreactors with and without sodium benzoate (150 ppm) were used to further assess its potential for improving the xylitol production. Under highly aerobic conditions (dissolved oxygen concentration >75% of air saturation), the presence of sodium benzoate (150 ppm) increased both specific xylitol productivity and yield. The specific xylitol productivity increased by >2-fold and the yield increased by ~30%, relative to control. However, benzoate increased the xylitol yield only slightly under oxygen limiting conditions. The volumetric xylitol productivity previously obtained for most potential strains was highest under microaerobic conditions. However, in the current study, the xylose consumption was remarkably enhanced under aerobic conditions in the presence of sodium benzoate, which makes an increase in the volumetric productivity of xylitol feasible for industrial applications. This method is readily applicable to previously developed xylitol processes by simply adding a suitable amount of sodium benzoate. The findings of this study devise new interventions for microbial processes in industrial reactors to expand the microbial tolerance of chaotropic stressors and, hence, the biotic windows for such processes.

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1. Introduction

Xylitol is a natural five-carbon sugar alcohol that is widely used as a sweetener in the food industry [1]. Xylitol for commercial use is produced in an expensive chemical process [1,2]. An alternative is to convert D-xylose derived from plant material to xylitol by yeast fermentation. Such processes have been extensively studied [3–5], but remain expensive. Earlier studies revealed that the yeast *Candida mogii* is a promising producer of xylitol from xylose [6]. In general, the yeast converts xylose to xylitol, which is then consumed for cell growth and maintenance. Accumulation of xylitol occurs if its consumption is reduced or prevented. Benzoate, a well-known growth inhibitor, offers the possibility of enhancing xylitol accumulation by suppressing its consumption and xylose fermentation.

Sodium benzoate is a highly chaotropic stressor [7,8], similar to other aromatics such as phenol and benzyl alcohol, which have a comparable log *P* and chaotropicity-mediated mode of action, inhibit cellular systems, and induce stress responses to protect macromolecular systems against the macromolecule-disordering effects of the aromatic solute [9,10]. These include the synthesis of kosmotropic/stabilizing compatible solutes such as polyols, including xylitol [7], which can reorder membranes, proteins, and other structures in the presence of a kosmotropic substance or compatible solute, as demonstrated in various studies [10–13].

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Nomenclature

C_{O_2}	oxygen concentration (μmol/L)
C_P	maximum xylitol concentration (g/L)
C_{Na}	sodium benzoate concentration (ppm)
q_{O_2}	specific oxygen uptake rate (mmol/(g h))
q_P	specific xylitol production rate (g/(g h))
q_S	specific xylitol consumption rate (g/(g h))
$Y_{P/S}$	xylitol yield (g/g)
$Y_{X/S}$	biomass yield (g/g)

Greek letters

μ	specific growth rate (d^{-1})

This has also been demonstrated via studies of the protective effects of compatible solutes, including polyols, on the activity of enzymes and other macromolecular systems [10,12,14,15].

Benzoate is a widely used food preservative. At low concentrations, benzoate has been found to stimulate the fermentation metabolism of certain yeasts [16–19]. Warth [19] showed that a low concentration (0.5 mM) of benzoic acid reduced the growth but stimulated the fermentation rate of *Saccharomyces cerevisiae*. The increased fermentation rate was explained by the energy required by the yeast to transport benzoic acid out of the cell [19]. Up to a benzoic acid concentration of 0.5 mM, the ATP levels and the intracellular pH remained relatively high. At higher concentrations of benzoic acid, the fermentation was inhibited, leading to a decrease in ATP levels and intracellular pH. In further studies, benzoic acid was found to inhibit glycolysis at the levels of pyruvate kinase and phosphoglycerate kinase [19]. A similar protective mechanism reduces the energy available for growth in the presence of sorbic acid in *S. cerevisiae* cultures [16].

Verduyn et al. [17] showed that low concentrations of benzoic acid stimulated yeast respiration. Low amounts of benzoate decreased biomass yield but increased the specific oxygen uptake rate [17]. High concentrations of benzoate reduced the specific oxygen uptake rate and promoted alcoholic fermentation [17]. Francois et al. [20] reported increased concentrations of glucose-6-phosphate and fructose-6-phosphate in yeast cells in a medium supplemented with benzoate. A decreased concentration of fructose-1,6-diphosphate indicates an inhibition of 6-phospho-fructo-1-kinase. Krebs et al. [21] found that phosphofructokinase was inhibited to a greater extent than hexokinase at acidic pH. Benzoate was found to reduce the ATP levels [21].

No information is available on the effect of benzoate on xylitol production. In view of the above mentioned effects of benzoic acid on yeast metabolism, we hypothesized that supplementation of the culture medium with benzoic acid has the potential to enhance the xylitol yield and productivity by affecting the metabolism in two possible ways (Fig. 1). Firstly, a possible inhibition of phosphofructokinase could divert the metabolic flux through the pentose phosphate pathway to trigger a greater regeneration of NADPH (Fig. 1). This, in turn, may lead to an increased activity of the enzyme xylose reductase and, therefore, an enhanced specific xylitol production rate. Secondly, an increased ATP demand may lead to an increased xylose uptake rate with a consequential increase in the production of NADH and increased production of ATP through respiration. Therefore, the addition of benzoic acid is expected to force the cells to produce more ATP to transport the benzoate out of the cell. An increase in the ATP demand should initiate more xylose uptake, more NADH production, and an increased productivity. An excess of NADH may contribute to an increased xylitol yield by inhibiting the xylitol dehydrogenase. The effect of sodium benzoate on xylitol production has not been examined, yet. This work focused on assessing the hypothesized effects.

2. Materials and methods

2.1. Microorganism, culture media, and inoculum preparation

Candida mogii ATCC 18364 (TISTR 5892) was maintained on YM agar slants at 4 °C. The agar slant medium contained (per L) 3 g yeast extract, 3 g malt extract, 5 g peptone, 10 g glucose, and 20 g agar. A newly sub-cultured slant was incubated at 37 °C for 24 h. A loopful of this culture was inoculated into a minimal medium to produce the preculture for the experiments. The minimal medium had the following composition (per L of medium): 18.75 g KH_2PO_4, 6 g $(NH_4)_2HPO_4$, 1.13 g $MgSO_4{\cdot}7H_2O$, 0.1 g $CaCl_2$, 36.5 mg myo-inositol, 18.2 mg calcium pantothenate, 3.66 mg thiamine-HCl, 0.9 mg pyridoxal-HCl, 0.018 mg biotin, 9.1 mg $FeCl_3$, 6.4 mg $MnSO_4$ H_2O, 5.46 mg $ZnSO_4{\cdot}7H_2O$, 1.46 mg $CuSO_4{\cdot}5H_2O$, and 20 g glucose [6,22]. For preparing the inoculum, 1 mL of the yeast suspension with an optical density of 0.8 at 620 nm (~0.4 g/L dry cell weight) was transferred to each of two 250-mL Erlenmeyer flasks. Each flask contained 20 mL of the minimal medium described earlier. The yeast was grown aerobically at 250 rpm, 30 °C, for 24 h. The contents of each flask were then transferred to 500-mL Erlenmeyer flasks, each containing 180 mL of the minimal medium. These flasks were cultivated under the conditions specified above.

2.2. Shake flask fermentations

Inocula were grown on a rotary shaker at 250 rpm for 24 h. The first pre-cultures were grown in test tubes containing 10 mL of the minimal medium. This preculture (2.5 mL) was used to inoculate a 250-mL Erlenmeyer flask containing 22.5 mL of the minimal medium (10% inoculum). Xylitol production was carried out in 500-mL Erlenmeyer flasks containing 250 mL of the minimal medium without glucose and initially 10 g/L of xylose. Eight shake flasks were simultaneously cultured on a rotary shaker (250 rpm, 30 °C). The flasks had different concentrations of sodium benzoate: 0, 100, 150, 200, 300, 400, 500, and 600 ppm. The pH was manually controlled at pH 6.0 by adjusting every 2 h as necessary. The flasks were sampled every 2 h. The samples were analyzed for concentrations of biomass, xylose, and xylitol.

2.3. Bioreactor batch fermentations

2.3.1. Effect of sodium benzoate under aerobic conditions

A laboratory stirred-tank bioreactor (B.E. Marubishi, Japan) was used for aerobic cultures. The yeast was grown for 24 h in 3.7 L of minimal medium initially containing 10 g/L glucose and 5 g/L xylose as carbon sources. The dissolved oxygen (DO) concentration was kept above 75% of air saturation by operating the bioreactor at an aeration rate of 1 vvm and an agitation speed of 600 rpm. The pH was maintained at 4.5 by automatic addition of 6 M NaOH, as needed. The temperature was controlled at 30 °C. The xylitol production phase was started by adding 300 mL of a solution of D-xylose to achieve a xylose concentration of 10 g/L in the culture medium. The pH was then manually adjusted to 6.0 by adding 6 M NaOH. Once all xylose and xylitol had been consumed, the next experiment began by adding 250 mL of a xylose solution and 50 mL of a sodium benzoate solution so that the initial concentrations were 10 g/L for xylose and 150 ppm for sodium benzoate. All other conditions remained as specified above. The fermentation was run until the carbon source was depleted.

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