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Efficient production of L-lactic acid with high optical purity by alkaliphilic *Bacillus* sp. WL-S20

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ABSTRACT

Highly efficient polymer-grade L-lactic acid production was achieved by an alkaliphilic strain *Bacillus* sp. WL-S20 using inexpensive peanut meal as nitrogen source and sodium hydroxide as neutralizing agent. In multi-pulse fed-batch fermentation of *Bacillus* sp. WL-S20, a L-lactic acid concentration of 225 g/l with a yield of 99.3% was obtained. In single-pulse fed-batch fermentation, a concentration of 180 g/l was obtained with a yield of 98.6%. No D-isomers of lactic acid were detected. The production of a high concentration of optically pure L-lactic acid by alkaliphilic *Bacillus* sp. WL-S20, combined with a low-cost nutrient and environment-friendly NaOH-based process, represent a potentially novel way for L-lactic acid production at an industrial scale.

1. Introduction

Lactic acid finds versatile applications in the food, pharmaceutical, textile and leather industries, and is also a building block for biodegradable plastic, mainly poly(L-lactic acid) (Pang et al., 2010; Wang et al., 2010; Zhao et al., 2010). The microbial production of lactic acid leads to the production of optically pure L- or D- isomers, whereas, chemical synthesis provides only the racemic lactic acid (Karp et al., 2011). Optical purity of lactic acid is important since poly (DL-lactide) is an amorphous polymer, unsatisfactory for industrial applications (Nair and Laurencin, 2007).

Many lactic acid bacteria (LAB) and some fungi are good producers of lactic acid (John, 1996) as they achieve high production titers. However, LAB and fungi grow optimally at about pH 5.5–6.5 and productivities and growth are inhibited when the culture pH is lower. To avoid growth inhibition by lactic acid, $CaCO_3$ is added during fermentation to neutralize lactic acid and maintain the pH around 5–6. During lactic acid extraction and purification, the calcium lactate is acidified with H_2SO_4, resulting in the production of insoluble $CaSO_4$ (Datta and Henry, 2006). Approximately one ton of crude gypsum is produced for every ton of lactic acid (Corma et al., 2007). The large amounts of gypsum have little utility and pose economic and environmental problems (Vaidya et al., 2005). Membrane-based separation and purification technologies do not produce salt wastes (Datta and Henry, 2006), but the bipolar membranes have the fundamental problem of intolerance to multivalent cations such as Ca^{2+} and Mg^{2+} because of the formation of insoluble hydroxides at the critical interface of the bipolar membrane where the ions separate (Datta and Henry, 2006). To make use of bipolar membranes to purify lactic acid, lactic acid production should be performed using monovalent ion such as NaOH. Although NaOH-based L-lactic acid production by a mutant of a *Bacillus* strain has been explored, the final production titer was not satisfying due to the toxicity of Na^+ to the strain (Qin et al., 2010).

Alkaliphiles are defined as microorganisms growing optimally at a pH above 9 (Horikoshi, 1999). They are also tolerant to salt, especially those of monovalent ions, such as sodium ions. Alkaliphilic strains may be promising producers of organic acids (Paavilainen et al., 1994) and their tolerance to high levels of salt and a high pH could also minimize contamination (Calabia et al., 2011). More importantly, NaOH instead of $CaCO_3$ can be used to maintain a neutral environment. *Halolactibacillus halophilus*, an alkaliphilic microorganism isolated from a marine environment, produced L-lactic acid at a concentration of 65.8 g/l in batch fermentation at pH 9, but with a yield and optical purity of only 83% and 98.8%, respectively (Calabia et al., 2011).

The present paper reports on the highly efficient NaOH-based production of polymer-grade L-lactic acid by alkaliphilic *Bacillus* sp. WL-S20 using cheap peanut meal as the nitrogen source to

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