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Biosynthesis of benzoylformic acid from benzoyl cyanide by a newly isolated *Rhodococcus* sp. CCZU10-1 in toluene–water biphasic system

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ABSTRACT

Benzoylformic acid was synthesized from the hydrolysis of benzoyl cyanide by a newly isolated *Rhodococcus* sp. CCZU10-1. In this study, an aqueous–toluene biphasic system was developed for highly efficient production of benzoylformic acid from the hydrolysis of benzoyl cyanide. In the aqueous–toluene biphasic system, the phase volume ratio, buffer pH and reaction temperature were optimized. Using fed-batch method, a total of 932 mM benzoylformic acid accumulated in the reaction mixture after the 10th feed. Moreover, enzymatic hydrolysis of benzoyl cyanide using calcium alginate entrapped resting cells was carried out in the aqueous–toluene biphasic system, and efficient biocatalyst recycling was achieved as a result of cell immobilization in calcium alginate, with a product-to-biocatalyst ratio of 14.26 g benzoylformic acid g^{-1} dry cell weight (DCW) cell after 20 cycles of repeated use.

1. Introduction

Nitrile compounds are important chemical building blocks because they are readily generated and could be transformed into high-value acids and amides (Brenner, 2002; He et al., 2011; Kiziak and Stolz, 2009; Kumar et al., 2010; Ma et al., 2008). Recently, there is a considerable industrial interest in enzymatic hydrolysis of nitriles owing to the desirability of conducting such conversions under mild conditions that would not alter other labile reactive groups (Banerjee et al., 2002; Brady et al., 2004; Brenner, 2002; Gröger, 2001; He et al., 2007; He et al., 2011; Zhang et al., 2010; Zhang et al., 2011b). There are two different enzyme systems involved in the biocatalytic hydrolysis of nitriles. The first process is a two-step reaction (He et al., 2011; Ma et al., 2008). A nitrile hydratase (EC 4.2.1.84) (Asano et al., 1982; Kaufmann et al., 1999; Lin et al., 2011; Ma et al., 2008, 2010; Raj et al., 2007) catalyzes the hydration of nitriles followed by the biotransformation into the corresponding acids with the aid of an amidase (EC 3.5.1.4) (Vaidya et al., 2009). The second process involves direct hydrolysis of the nitrile to corresponding carboxylic acids with the release of ammonia by a nitrilase (EC 3.5.5.1) (He et al., 2010b; Kaplan et al., 2006; Layh et al., 1992; Mauger et al., 1990; O'reilly and Turner, 2003; Wu et al., 2008).

Benzoylformic acid is an important intermediate for the drugs of thromboxane synthetase inhibitors as well as antihypertensive agents. It has been chemically prepared by hydrolysis of benzoyl cyanide (Winfried et al., 1986), dehydrogenation of mandelic acid (He at al., 2008), acylation of phthalic acid (Liron and Yoel, 1994), oxidation of styrene (Nongkynrih and Mahanti, 1993). It is well-known that nitrile-metabolizing biocatalysts are powerful tools used for industrial production of some valuable acids under mild conditions (Banerjee et al., 2002; Brady et al., 2004; Brenner, 2002; Gröger, 2001; He et al., 2007, 2011; Kaul et al., 2004; Zhang et al., 2010). In order to effectively synthesize benzoylformic acid from benzoyl cyanide, it is necessary to screen for high activity of benzoyl cyanide-hydrolyzing biocatalysts.

However, a potential substrate inhibition may be observed in the aqueous monophase reaction media in the hydrolysis of benzoyl cyanide. Therefore, it is necessary to build an appropriate reaction system, and the water–organic biphasic media provide a very attractive alternative (Gong and Xu, 2005; Zhang et al., 2011a). In water–organic biphasic media, the hydrophobic substrate nitrile will be mainly retained in the organic phase, which can act as a reservoir for the toxic substrate, thus regulating the substrate concentration around biocatalysts and lowering the substrate inhibition (Zhang et al., 2011a). A low water system could be used for nitrile-hydrolyzing reaction (Layh and Willetts,

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