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Interaction and biodegradation evaluate of *m*-cresol and quinoline in co-exist system

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ABSTRACT

Environmentally hazardous and toxic chemicals are commonly generated in actual wastewater that the complex compositions in wastewater treatment system need different types of strains to be degraded. The main objective of this research was to understand the effect of extra substrates, phenolic and N-heterocyclic compounds, on the performance of pure cultural and mixed strains under single and dual substrates conditions. Two bacteria, *Lysinibacillus* sp. SC03 and *Achromobacter* sp. DN-06, were acclimated to degrade different concentrations of *m*-cresol and quinoline. The results indicated that *Lysinibacillus* sp. SC03 could completely degrade 100 mg l^{-1} *m*-cresol with no delay time, however, little removal of quinoline was observed; *Achromobacter* sp. DN-06 could degrade 100 mg l^{-1} quinoline in 32 h, but could not remove *m*-cresol, which means *m*-cresol and quinoline is the specific substrate. The degradation rate of *m*-cresol fitted well to the zero-order kinetic equation although the degrading ability of *Lysinibacillus* sp. SC03 was inhibited when less than 100 mg l^{-1} quinoline was added, and the inhibitive effect was confirmed to be a noncompetitive pattern which could be interpreted by the Michaelis–Menten kinetics equation with corresponding parameters V_{max}, K_m, K_1 and K_2 were 13.16 mg l^{-1} h^{-1}, 35.84 mg l^{-1}, 200.0 mg l^{-1} and 285.7 mg l^{-1}, respectively. Moreover, the addition of *m*-cresol-degrading strain (*Lysinibacillus* sp. SC03) could accelerate the removal of quinoline because the metabolites of quinoline could be degraded by *Lysinibacillus* sp. SC03 and the chemical equilibrium moved to more biodegradation of quinoline. Also, this process attributed to less the delay time during the quinoline removal.

1. Introduction

Nowadays, contamination of the environment by hazardous and toxic chemicals is being considered as one of the major problems all over the world. A huge amount of effluents containing organic pollutants from different kinds of factories polluted the rivers and lakes in many developing countries. Phenols are water-soluble compounds which are easily transport and pollute water resources even at very low concentrations (Massalha et al., 2010). They can cause an unpleasant odor and taste to the water. In general, they are considered to be priority pollutants since they are toxic to plants, animals, microorganisms and humans (Wang et al., 2010). N-heterocyclic compounds are produced in coal tar and petroleum industries, and serve as raw materials and solvents in the manufacture of dyes, paints and wood treatment chemicals (Tuo et al., 2012). The existence of these compounds has a great negative impact on human health and environmental quality (Qiao and Wang, 2010; Deng et al., 2011) because of the toxic, mutagenic and carcinogenic properties of these compounds. Phenols and N-heterocyclic compounds are widely found to co-exist in actual wastewater, especially in petrochemical and coking wastewater (Yao et al., 2011a,b). Biological techniques, including activated sludge method, have always been used because of its low cost and minimization of byproducts production (Bajaj et al., 2009; Jiang et al., 2010). Up to date, although many pure strains have been isolated and identified to utilize one or more substrates as carbon and energy sources, it is necessary to investigate the complex relationship between substrates and bacterial consortium for the realistic wastewater in which multiple pollutants are exist.

In an activated sludge system, the relationship between dominant bacteria and substrates is extremely complex and the catabolic pathway for a specific substrate is different (Caspi et al., 2012; Zhao

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et al., 2012). There are three modes (Montagnolli et al., 2009; Trigueros et al., 2010) to describe the influences of substrates on the bacteria's degradation activity: enhancement (Jiang et al., 2010), inhibition (Adav et al., 2007) and co-metabolism (Monsalvo et al., 2009). The interactions (Pedrazzani et al., 2005; Khalid et al., 2009; Machín-Ramírez et al., 2010) among bacteria include positive synergy, negative competition and non-significant effect. For instance, for a complicated substrate degradation system with polycyclic aromatic hydrocarbon (PAH) and N-heterocyclic compounds, the double or multi rings are cleaved gradually by bacteria. Then they were converted into simple ring structural substances (Sun et al., 2009) and at last the benzene ring will be broken to be mineralized. Therefore, to some extent, the compounds with lesser benzene rings might act as the co-metabolism substrates or degrading enzymes' inducers for that with more rings.

In a recent study, we reported that the dual substrates interactions of *m*-cresol and pyridine (Yao et al., 2011a,b) which all have one benzene ring and could be degraded by *Lysinibacillus* sp. SC03, an efficient cresol-degrading bacterium. Also, we acquired a quinoline-degrading bacterium, *Achromobacter* sp. DN-06 (Deng et al., 2011). As an N-heterocyclic compounds with two benzene rings, quinoline is another popular pollutant containing in coking wastewater one of whose degradation intermediates is a single ring product (Fig. 1) (Cui et al., 2004; Peng et al., 2009; Qiao and Wang, 2010; Li et al., 2010; Bai et al., 2010a). Although there are many researches on bacterial consortium's degradation efficiency to similar structural substrates (Kumar et al., 2005; Wang et al., 2010; Bai et al., 2010b), little attention was paid to investigate the interactions between two substrates and two strains in one system. Besides, whether the accompanying cometabolism of simple substrates or the extra additional bacteria accelerate the reaction rate or not is still unknown.

Compared with the biodegradation rate of phenol and pyridine, quinoline is a kind of compounds that hard to be degraded. Based on the metabolize pathway of quinoline, we assumed the addition of phenol-degrading microorganisms could accelerate the degradation of quinoline after the appearance of the intermediate with single ring. The objectives were to analyze the impacts of quinoline on *m*-cresol degradation in dual substrates system, and to identify the interaction type between them. Through the analysis of intermediate metabolites of quinoline, the degrading capacities of the acclimated strains in complicated system were evaluated. The experimental results were aimed to provide more knowledge and support on the possibility and application of dual or more cultured microorganisms for efficient biodegradation.

Table 1
Key characteristics of the strains at different substrates used for isolation.

Bacteria	Growth substrate	Optimal pH	Optimal *T* (°C)	GenBank accession number
Lysinibacillus sp. SC03	*m*-Cresol	6.8–7.3	30–35	EU043375
Achromobacter sp. DN-06	Quinoline	7.0–8.0	30–35	FJ827751

2. Materials and methods

2.1. Chemicals and reagents

Standard samples of *m*-cresol and quinoline were purchased from Dr. Ehrensorfer, Germany. Solvents for HPLC analysis were chromatographic grade, obtained from Fisher Scientific (Pittsburgh, PA). All other chemicals and regents used in the experiments were of analytical grade, and provided by local chemical manufacturers.

2.2. Strains

m-Cresol- or quinoline-degrading microorganisms were originally isolated and enriched independently by plate streaking method from the activated sludge of a coking wastewater treatment plant, which is located in Shaoguan, Guangdong Province, China. Strains were identified in previous studies based on physiological and biochemical tests and 16S rDNA sequence which were named as *Lysinibacillus* sp. SC03 (Yao et al., 2011a,b), an *m*-cresol degrader, and *Achromobacter* sp. DN-06, a quinoline-degrading strain (Deng et al., 2011). Table 1 summarized the key characteristics of the two strains.

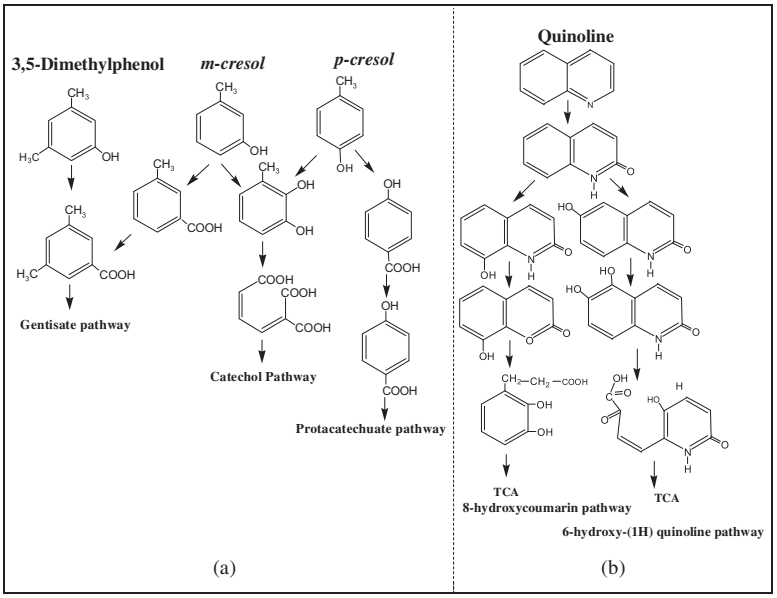

Fig. 1. Aerobic metabolic pathway of the degradation of phenol (a) and quinoline (b) (TCA: Tricarboxylic acid cycle).