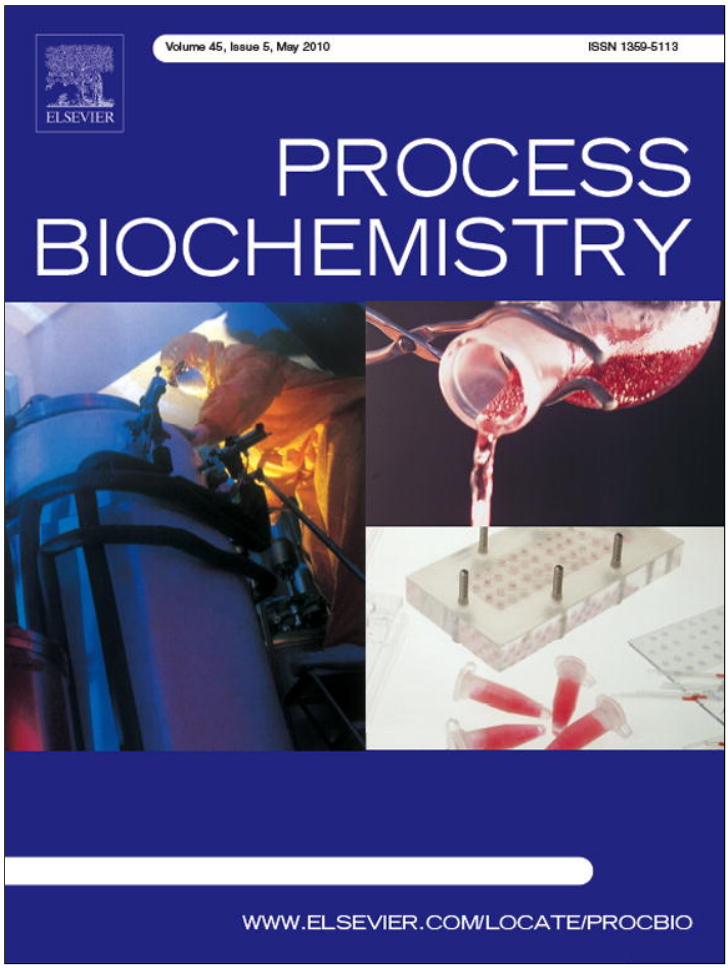

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Short communication

Effects of monorhamnolipid and Tween 80 on the degradation of phenol by *Candida tropicalis*

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ABSTRACT

The effects of the biosurfactant monorhamnolipid (monoRL) and the chemical surfactant Tween 80 on the degradation of phenol by *Candida tropicalis* CICC 1463 were studied. Both surfactants impeded the decay in cell concentrations at the beginning of the fermentation and enhanced the cell growth thereafter. They also increased the degradation efficiencies of 500 mg/L phenol from 86.9% in control to above 99.0% for all test concentrations within 30 h. The monoRL could also be degraded by the *C. tropicalis*. These results indicate that the surfactants could diminish the toxicity of phenol to the yeast, increase cell growth and improve phenol removal. The monoRL is better than Tween 80 because of biodegradability.

1. Introduction

Phenol and its derivatives are widely used as raw materials in many industrial branches, such as petrochemical, chemical, pharmaceutical, pulp, paper, tannery and coal refining industries. The increasing presence of phenols in wastewater coming from these industries represents an important environmental toxicity hazard. Therefore, development of methods for the removal of phenols from industrial wastewater has generated significant interest [1]. Microorganisms able to degrade phenol were isolated as early as 1908 [2]. Since then, biological treatment, which is generally preferred to physical or chemical treatment methods because of lower costs and possibility of complete mineralization [3], has been developed as the most common method to remove phenol from wastewater [1]. Phenol is a recalcitrant compound that exerts toxicity to microorganisms during biological treatment and leads to failure of the whole wastewater treatment if the microbial flora is not adaptable to phenol. Therefore, microbial acclimatization to phenol is necessary to obtain efficient biodegradation [1]. Almost all information about the use of yeast cultures grown in phenols found in the available literature is referred to as *Candida tropicalis* (*C. tropicalis*) [2,4–6].

In industry, many surfactants have been used to improve the removal of phenol from wastewater because of the relative hydrophobicity of phenol [7–13]. The surfactant micelles or vesicles can adsorb phenol to the hydrophobic core and reduce the free phenol in aqueous solutions [14,15]. Therefore, the presence of surfactant may influence the biodegradation of phenol. To our knowledge, however, studies on the role of surfactants in phenol biodegradation, especially by biosurfactants, which possess advantages of biodegradability, low toxicity and ecological compatibility over synthetic counterparts, are lacking [16]. The rhamnolipid biosurfactant is one of the most extensively studied biosurfactants and it causes strong microbial effects [17–20].

In this study, the monorhamnolipid (monoRL, α-L-rhamnopyranosyl-β-hydroxyalkanoyl-β-hydroxyalkanoate [20]) was used as the model biosurfactant, and its effect on phenol degradation by *C. tropicalis* CICC 1463 in aqueous solution was examined. The effect of synthetic surfactant Tween 80 was also tested to provide a comparison. The study may offer some useful information about the mechanism of surfactant function in the biological removal of phenol.

2. Materials and methods

2.1. Chemicals and microorganisms

The biosurfactant, monoRL, was produced, extracted and purified as described by Zhong et al. [20], and its critical micelle concentration (CMC) was 75 μM. Tween 80

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(purity > 99%) was from Sigma–Aldrich (German), and its CMC was 11 μM [21]. Other reagents were of analytical grade. Ultra-pure water with an initial resistivity of 18.2 MΩ/cm produced by Labconco Water Pro PS (Kansas, USA) was used throughout the experiment. *C. tropicalis* CICC 1463 was from the China Center of Industrial Culture Collection (Beijing, China).

2.2. Cultivation conditions

C. tropicalis was enriched in YPD medium with the following ingredients: 1.0 g/L yeast extract, 2.0 g/L peptone and 2.0 g/L dextrose (pH 6.0) [22]. After 12 h, cells in 4 mL broth were harvested by centrifugation at 5600 × *g* for 10 min, washed three times and transferred into mineral salt medium (MSM) containing 500 mg/L phenol and various concentrations of surfactants. The ingredients of MSM were the following: 0.4 g/L $(NH_4)_2SO_4$, 0.4 g/L K_2HPO_4, 0.2 g/L KH_2PO_4, 0.1 g/L NaCl, 0.1 g/L $MgSO_4$, 0.01 g/L $MnSO_4{\cdot}H_2O$, 0.01 g/L $Fe_2(SO_4)_3{\cdot}H_2O$ and 0.01 g/L $Na_2MoO_4{\cdot}2H_2O$ (pH 6.0). For each test, a 1-L Erlenmeyer flask containing 200 mL of medium was cultivated on a rotary shaker at 200 rpm and 30 °C. The blank mediums without cells were placed under the same conditions to evaluate the volatilization and spontaneous transformation of phenol, which were negligible according to the test results. Control experiments, using mediums without surfactants, were also performed.

2.3. Analytical methods

Cell density was monitored spectrophotometrically by measuring the absorbance at a wavelength of 600 nm [22]. The biomass dry-weight concentrations were measured by centrifuging the cell suspension at 5600 × *g* for 10 min and drying the cells to a constant weight at 105 °C for 24 h. The cell-free supernatant was used for further analysis. Phenol concentrations were determined by high performance liquid chromatography (HPLC) [22] using a HP1100 HPLC (Agilent Technologies, California, USA) system with a C18 column (150 mm × 4.6 mm, 5 μm, Agilent). Elution was performed with 400/300 (v/v) methanol/water at a flow rate of 1.0 mL/min. Absorbance was measured at a wavelength of 280 nm, and data analysis was conducted using the Agilent GPC software.

Tween 80 concentration was determined by the modified cobalt thiocyanate active substances method [23]. MonoRL concentration was measured by the phenol-sulfuric acid method after extraction by ethyl acetate [24]. In each method, the detection was carried out on an ultra-variable wavelength UV-2552 spectrometer (Shimadzu Company, Japan).

All tests were performed in triplicate. The results are mean values of triplicates, and the relative standard deviation is lower than 5%.

3. Results

3.1. Phenol biodegradation without surfactant

Fig. 1 shows the degradation of 500 mg/L phenol and cell growth in the culture without surfactant. The cell biomass decreased quickly during the first 6 h. However, 36 h later the cell concentration began to increase. Phenol concentration decreased at the beginning of fermentation. The removal efficiency of phenol was above 99.0% at 48 h. The results indicate that while phenol could be transformed by *C. tropicalis*, it exerted marked toxicity to the yeast cell.

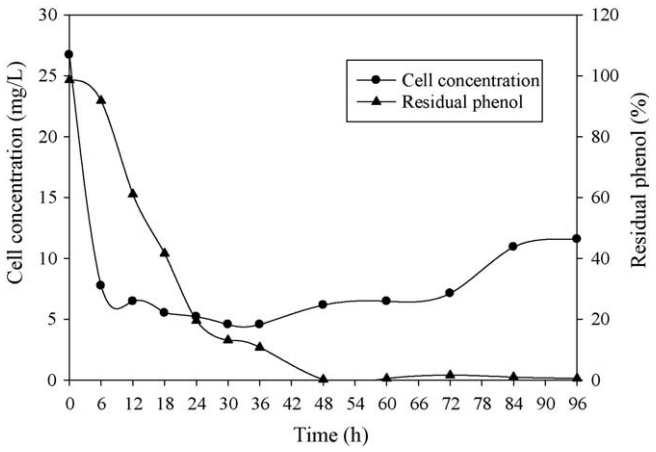

Fig. 1. Cell concentration (●) of *Candida tropicalis* and residual phenol (▲) vs. time.

3.2. Effects of monoRL on phenol biodegradation

Fig. 2 shows the effects of biosurfactant monoRL on the degradation of phenol and cell growth. The presence of monoRL impeded the decay in cell concentrations in the first 6 h and improved the cell growth thereafter. The monoRL at higher concentrations had a stronger effect. However the cell concentrations increased initially, and then decreased (Fig. 2a). MonoRL also enhanced the removal of phenol with increasing concentrations (Fig. 2b). It enhanced the removal efficiencies of phenol from 86.9% in the control condition to above 99.0% for all the test concentrations at 30 h. The change of monoRL concentration during the fermentation was shown in Fig. 2c. The degradation efficiency of monoRL increased with the initial monoRL concen-

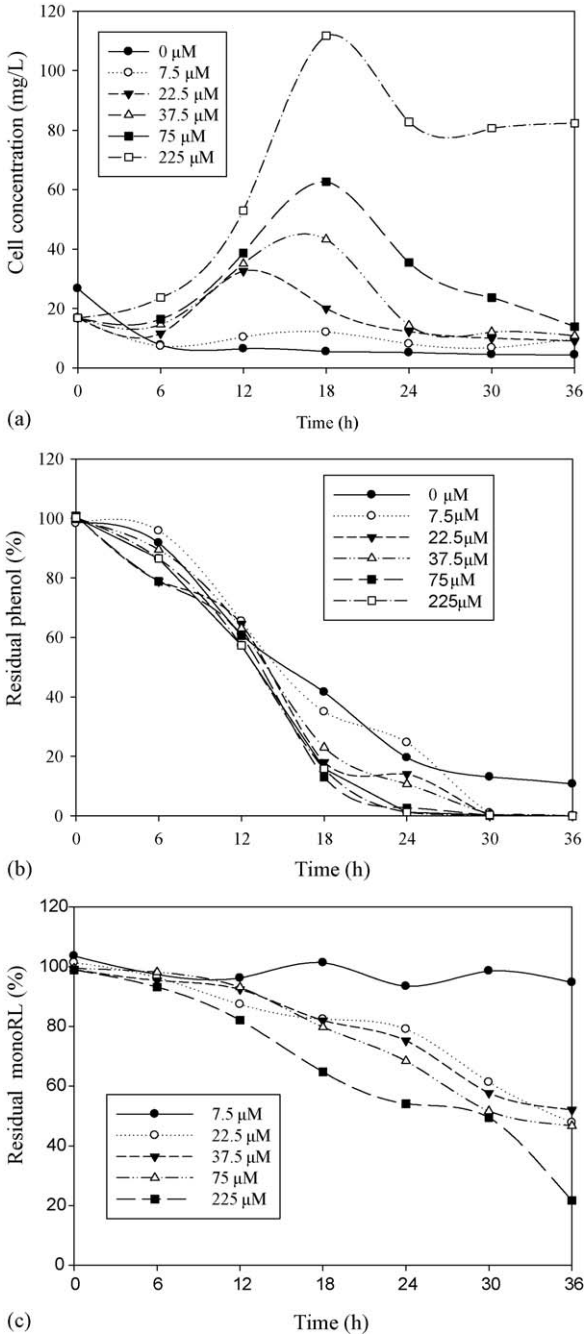

Fig. 2. Degradation of phenol by *Candida tropicalis* in the presence of monoRL. (a) Cell concentration, (b) residual phenol and (c) residual monoRL.

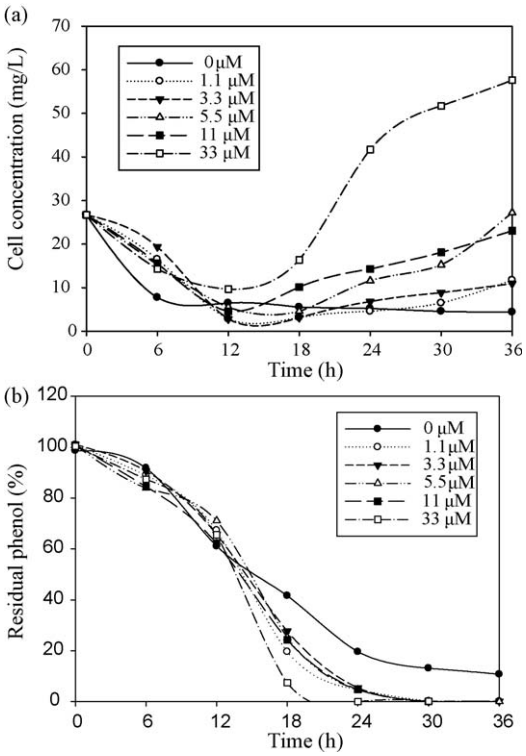

Fig. 3. Degradation of phenol by *Candida tropicalis* in the presence of Tween 80. (a) Cell concentration and (b) residual phenol.

tration. The results suggest that the biodegradable biosurfactant monoRL could diminish the toxicity of phenol to improve the cell growth and the removal of phenol.

3.3. Effects of Tween 80 on phenol biodegradation

Fig. 3 shows the influence of Tween 80 on the degradation of phenol and cell growth. The presence of Tween 80 also impeded the decay in cell concentrations initially before enhancing the cell growth later (Fig. 3a). It also enhanced the removal of phenol (Fig. 3b), and the phenol removal efficiencies reached 99.8% for all the test concentrations at 30 h. The concentrations of Tween 80 in each medium were also tested every 6 h, which did not change during the fermentation period (data not shown). The results show that Tween 80 also had a positive effect on the cell growth and the removal of phenol, but the surfactant was not degradable.

4. Discussion

The cell concentrations decreased as substrate exhausted at the beginning of the fermentation process (Figs. 1–3), which also occurred when *Pseudomonas testosteroni* degraded phenol and 4-chlorophenol simultaneously [25]. *C. tropicalis* is a species of yeast able to degrade phenol and phenol derivatives. However, phenol could cause cellular lysis in some strains of *C. tropicalis* and affect their growth [26]. In this study, the strains were cultivated in the YPD medium without phenol before they were added to the phenol degradation medium. Some of *C. tropicalis* strains might not adapt to phenol and die in the degradation medium during the beginning period. Additionally, phenolic compounds are metabolic uncouplers [27], which can dissipate the mitochondrial electrochemical proton gradient of yeast cells, provoking energy dissipation to inhibit the synthesis of mitochondrial ATP [28]. Thus, the biomass production decreases without reducing the removal rate of phenols from the aqueous solution [29]. The exacerbated energy dissipation was an indication of the toxicity of phenolic compounds. *C. tropicalis* almost degraded phenol after 48 h, but the cell biomass still increased later (Fig. 1). Similar phenomena have also been reported previously [30,31]. *C. tropicalis* degraded phenol by an *ortho* cleavage pathway [32]. Phenol disappeared by phenol hydroxylase converting it into catechol [32]. However, other carbon and energy sources still existed in the medium for cell growth, such as the intermediate products of phenol and the byproducts generated by cell lysis. There were two stages during the degradation process: phenol catabolism and cell growth. During the first stage, *C. tropicalis* catabolized phenol into intermediate products. However, the cell concentration did not increase during this stage. The reason may be that the residual phenol concentration was above the critical concentration to impede cell growth. The turning point of cell growth occurred at 36 h when the residual phenol was 10.8% (53.8 mg/L) (Fig. 1). The available literature also reported that phenol was nontoxic up to 100 μg/mL in *Nostoc linckia* [33]. During the second stage, phenol concentration was low, and cell concentration began to increase.

The presence of monoRL and Tween 80 enhanced the cell growth and the removal rates of phenol (Figs. 2 and 3). The increasing cell biomass could result in faster degradation rates of phenol [34,35]. Surfactant could reduce the free phenol in solution by adsorption [15], which might diminish the cell toxicity of phenol because it was related to the concentration of phenol [33]. Phenol, the low-molecular polarizable compound having an aromatic ring, adsorbs initially to the micelle interface, before

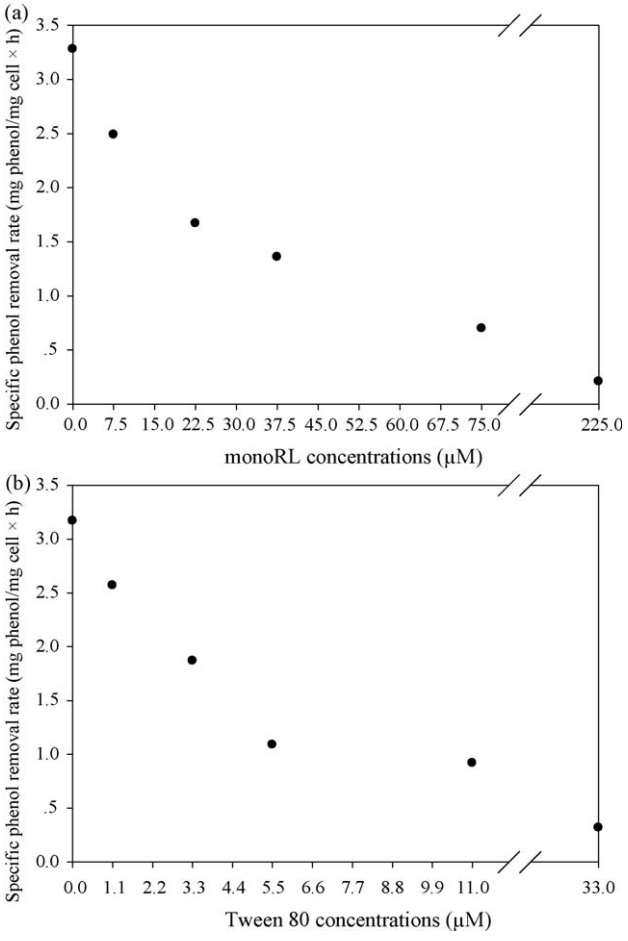

Fig. 4. Specific phenol removal rates in batch cultures with monoRL (a) and Tween 80 (b) at different concentrations at 30 h.

moving deeper in the hydrophobic core [14,15]. Thus, the surfactant molecules form the barrier between phenol and cells. In addition, *C. tropicalis* also used monoRL as the nutrient in the fermentation (Fig. 2c). The simultaneous use of conventional nutrients and phenol possibly enabled the cells to overcome the inhibition effect of phenol [34]. According to the results of Figs. 2 and 3, the specific phenol removal rates (SPRR) (mg phenol/mg cell × h) at 30 h was calculated and shown in Fig. 4. The decreased SPRR with increasing concentrations of each surfactant demonstrated that the presence of surfactant could diminish the cell toxicity of the uncoupling agent. The results also suggest that the effect of surfactant was related to its concentration (Figs. 2 and 3). One explanation for this observation may be that the adsorption of free phenol by surfactant depends on surfactant concentration [14,15]. Because the surfactant aggregation was a stepwise process, the hydrophobic tails collected together and formed aggregates. The large and incompact micelle aggregates turned into the small and compact micelles with increasing surfactant concentration [36].

The cell concentrations increased for the monoRL case. However, the cell concentrations increased initially and then decreased when the phenol was not completely degraded (Fig. 2a and b). To our knowledge, little information is available about this phenomenon. Future studies should seek to understand the underlying mechanisms. A possible reason could be related to the balance of phenol adsorption and desorption by surfactant during the degradation process. After a fermentation period, *C. tropicalis* used most of the initial free phenol (Fig. 2b). If the desorption rate of the residual phenol in the surfactant micelles or vesicles was slower than the degradation rate, the strains might not obtain enough carbon and energy sources, thus leading to a decrease in cell concentration.

5. Conclusion

The effects of two surfactants, monoRL and Tween 80, on the degradation of phenol by *C. tropicalis* in aqueous solution were studied in this paper. The surfactants diminished the cell toxicity of phenol and improved the cell growth and the removal of phenol. The surfactants had a stronger effect at higher concentrations. The results indicate the potential for these two surfactants in the application of biodegradation of phenol in aqueous solution; the biosurfactant is better than the chemical surfactant because of biodegradability.

Acknowledgements

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