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EFFECT OF THE SURFACTANTS TWEEN 20 AND CTAB ON FLUORANTHENE AND ANTHRACENE DEGRADATION BY *P. PUTIDA*

A. C. Rodrigues, A. G. Brito and L. F. Melo

Centro de Engenharia Biológica – Instituto de Biotecnologia e Química Fina, Universidade do Minho, 4700 Braga, Portugal

ABSTRACT
The effect of a nonionic and a cationic surfactant, Tween 20 and CTAB, on the biodegradation rate of fluoranthene and anthracene by a *P. putida* strain was investigated. Tween 20 had a positive effect on the biodegradation rate of anthracene. However, it did not affect significantly fluoranthene oxidation. The cationic surfactant CTAB inhibited the oxidation of both PAHs, fluoranthene and anthracene.

KEYWORDS
Bioavailability; polycyclic aromatic hydrocarbons; surfactants

INTRODUCTION

It has been proposed that the presence of nonionic surfactants at concentrations above the critical micelle concentration (CMC) can increase the rate of biodegradation of polycyclic aromatic hydrocarbons (PAHs) by solubilization or emulsification (Edwards *et al*., 1991). On the other hand, surfactants may reduce the adhesion of cells to the hydrocarbon-water interface (Rosenberg and Rosenberg, 1995).

The ability of surfactants to enhance the bioavalability of PAHs will depend on the mechanisms used by bacteria to get access to these hydrophobic substrates.

Therefore, the objective of this study is to investigate the effect of the nonionic surfactant Tween 20 and the cationic surfactant cetyltrimethyl ammonium bromide (CTAB), on the biodegradation rate of fluoranthene and anthracene by a *Pseudomonas putida* strain.

METHODS

250-mL Erlenmeyer flasks containing 50 mL of a mineral medium with fluoranthene or anthracene as sole substrate (100 mg/L, aproximately), with and without added surfactant, were incubated in the dark, at room temperature and 150 rpm. The inoculum was a culture of *P. putida* pregrown on the respective PAH.

Cell growth was assessed by measurements of optical density at 540 nm. A respirometric technique was used to measure the activity of the cells. Three different concentrations of Tween 20 were tested, 100, 500 e 1000 mg/L. The effect of the cationic surfactant CTAB was tested at a concentration of 100 mg/L.

RESULTS AND DISCUSSION

Figures 1 and 2 show the results obtained in the respirometric assays.

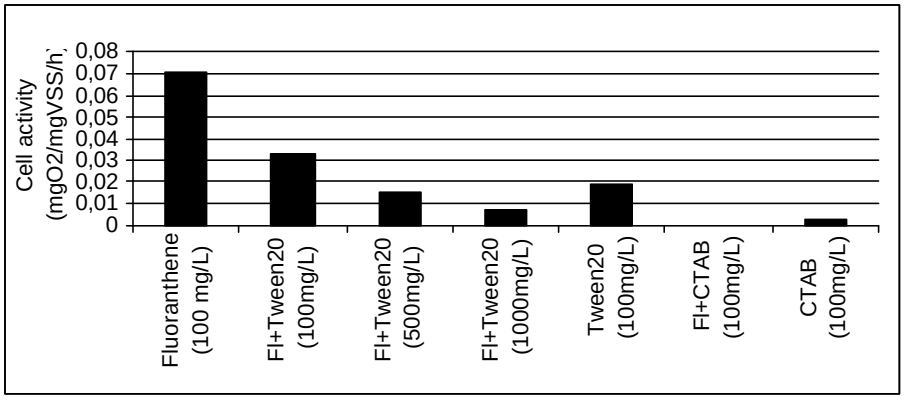

Figure 1 – Activity of *P. putida* cells using fluoranthene as substrate in the presence of surfactants.

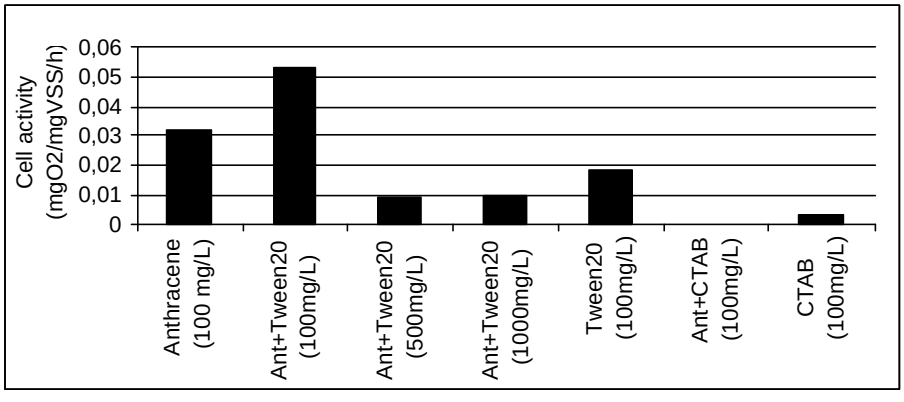

Figure 2 – Activity of *P. putida* cells using anthracene as substrate in the presence of surfactants.

As can be seen, an increase in the concentration of Tween 20 resulted in a decrease in the activity of the *P. putida* cells. This result shows that PAHs are less acessible to microorganisms when higher concentrations of the nonionic surfactant are present. It was also observed that growing either on fluoranthene or anthracene, the cell activity at Tween 20 concentrations of 500 and 1000 mg/L was even lower than the one obtained when this surfactant was used as sole carbon source (100

mg/L). Consequently, this result may indicate a toxic effect of Tween 20 at concentrations higher than 500 mg/L.
In the case of fluoranthene, the activity of the cells using this PAH as sole carbon source was higher than the one obtained in the presence of surfactant. For anthracene (whose solubility in water is 3.6 times lower than the one of fluoranthene), the opposite was observed. Indeed, the cell activity was higher when Tween 20 (100 mg/L) was added.
Concerning the cationic surfactant CTAB, an activity of the *P. putida* cells of 0.0031 mgO_2/mgVSS/h was registered using this surfactant as sole carbon source. However, no cell activity was detected using either fluoranthene or anthracene as substrate in the presence of CTAB. A possible explanation for this phenomenon can be the fact that, being a quaternary ammonium compound, CTAB binds by chemisorption to the cell surface of bacteria preventing the direct contact between the PAH and the bacteria.
The results of the batch experiments are presented in Figures 3 and 4.

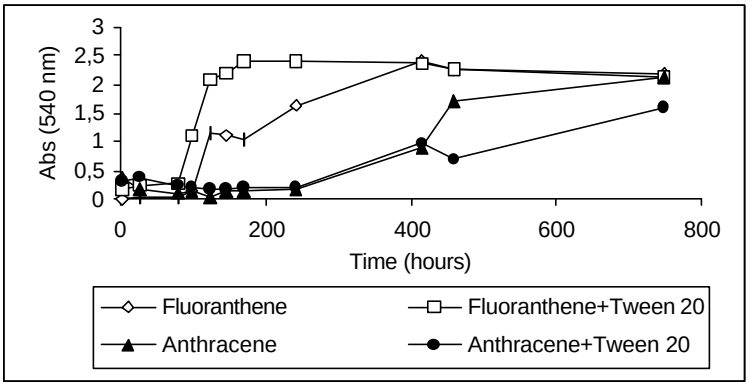

Figure 3 – Cell growth on fluoranthene and anthracene with and without Tween 20 (100 mg/L).

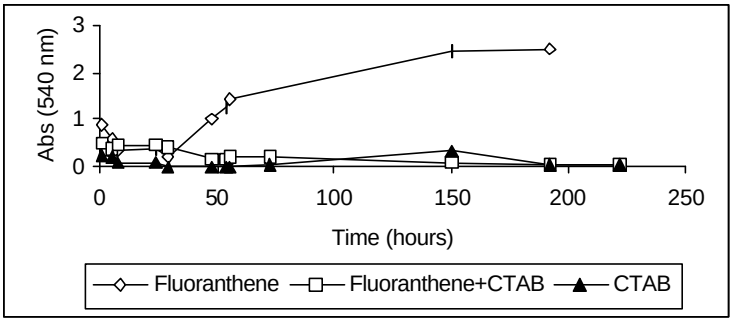

Figure 4 – Cell growth on fluoranthene with and without CTAB (100 mg/L).

It was observed that *P. putida* grows slower on anthracene than on fluoranthene. This result was confirmed by the higher cell activity obtained when fluoranthene was used as sole carbon source (Figures 1 and 2). The lower water solubitity of anthracene was certainly the cause for such result. In the case of fluoranthene

(Figure 3) the bacteria grew faster, reaching the stationary growth phase more rapidly, when Tween 20 was added. As the stationary growth phase was reached, the cell density was the same with and without added surfactant. Nevertheless, the cell activity obtained when Tween 20 was added was lower than the one observed without surfactant (Figure 1). On the other hand, using anthracene as sole carbon source (Figure 4), the cell density obtained was higher than in the presence of Tween 20. Yet, the activity of the cells was lower (Figure 2). The reason for this discordance is that the bioactivity was monitored during the first 3,5 hours of growth, while the batch experiments were performed within 30 days.
The batch experiments performed with CTAB confirmed the results obtained in the respirometric assays. The fluoranthene oxidation rate decreased to almost zero when CTAB was added (Figure 4).

CONCLUSIONS

The findings of this study show that the effect of surfactants on the biodegradation of hydrophobic compounds like PAHs depends on the nature and concentration of the surfactant and on the PAH itself. In the case of anthracene, the cell activity improved significantly in the presence of Tween 20 (100 mg/L). However, higher cell densities were reached using this PAH as sole carbon source. On the other hand, Tween 20 did not affect significantly fluoranthene biodegradation. Moreover, the bioactivity was reduced when the surfactant was added, indicating a potential negative effect of the surfactant. Tween 20 concentrations of 500 and 1000 mg/L might be toxic to bacteria. The cationic surfactant CTAB inhibited fluoranthene oxidation.

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