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Phenol degradation by microorganisms adsorbed on activated carbon

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Summary. The phenol degradation by *Candida* sp. and *Pseudomonas* sp. immobilized on activated carbon was investigated. Thanks to its great adsorptive surface, activated carbon is suited as supporting material for microorganisms and also provides a high adsorption capacity for phenol.

The immobilization by adsorption avoids any unphysiological treatment of the microorganisms. One gram activated carbon adsorbed in 10 h about 4×10^9 *Pseudomonas* cells and 3×10^8 *Candida* cells. While the free cells did not tolerate more than 1.5 g/l phenol, the adsorbed microorganisms survived at temporary high phenol concentrations up to 15 g/l, and they degraded about 90% of the adsorbed phenol.

The activated carbon operated like a "depot": the adsorbed phenol diffused out of the carbon and could be metabolized by the microorganisms. The results give an explanation of the stimulating effect of activated carbon in the treatment of waste waters observed until now.

Introduction

Since several years activated carbon has been used to treat organic waste waters. Especially phenol and phenolic substances can be adsorbed on activated carbon filters (Klein and Jüntgen 1977; Jüntgen et al. 1981) which must be regenerated by physical, chemical or biological methods (v. Kienle and Bäder 1980).

Many bacteria and yeasts can utilize phenol as carbon source (Dagley 1971; Gaal and Neujahr 1979) and are active in its degradation in waste waters. Investigations concerning phenol degradation by immobilized cells of *Candida tropicalis* entrapped in different polymeric networks were reported by Klein et al. (1979). In their experiments problems of cell entrapment, e.g. impairment of metabolic activity by toxic substances and transport limitation, were especially obvious. Bettmann and Rehm (1984) found a clear increased phenol degradation rate with immobilized *Pseudomonas* cells entrapped in alginate and polyacrylamide-hydrazide.

Recent studies with undefined microorganisms have shown that activated carbon is suited as supporting material by its large adsorptive surface (Andrews and Tien 1981; Shimp and Pfaender 1982). The following investigations describe the adsorption of phenol as well as of *Pseudomonas* sp. and *Candida* sp. on activated carbon, and the phenol degradation by these immobilized microorganisms compared to that of free microorganisms.

Materials and methods

Strains of *Candida* sp. and *Pseudomonas* sp. were isolated from waste water. The following mineral salt medium was used consisting of (per liter): NH_4NO_3 1.0 g; $(NH_4)_2SO_4$ 0.5 g; NaCl 0.5 g; $MgSO_4 \times 7\,H_2O$ 0.5 g; K_2HPO_4 1.5 g; KH_2PO_4 0.5 g; $CaCl_2$ 0.01 g; $FeSO_4 \times 7\,H_2O$ 0.01 g; trace elements (Pfennig and Lippert 1966) 1 ml; final pH 6.9. After sterilization phenol was added.

The precultures were incubated at 21° C on a rotary shaker at 100 RPM in a 1 g/l phenol-containing medium. After complete consumption of phenol they were harvested for immobilization. This solution with a cell titer of approximately 7×10^7 cells/ml for *Candida* sp. and 1.5×10^9 cells/ml for *Pseudomonas* sp. was used for immobilization.

The investigations of phenol adsorption, immobilization and phenol degradation were made with a loop reactor with 200 ml working volume. In all experiments 10 g activated carbon and 200 ml medium were used according to Scheme 1.

The activated carbon of type AG 33/1-4 from the Bergbau-Forschung, Essen, had a diameter of 1–4 mm and an internal surface of 1,300 ± 50 m^2/g. The growth of free microorganisms was determined by measuring the optical density (OD) of the suspension at 546 nm. The quantity of immobilized microorgan-

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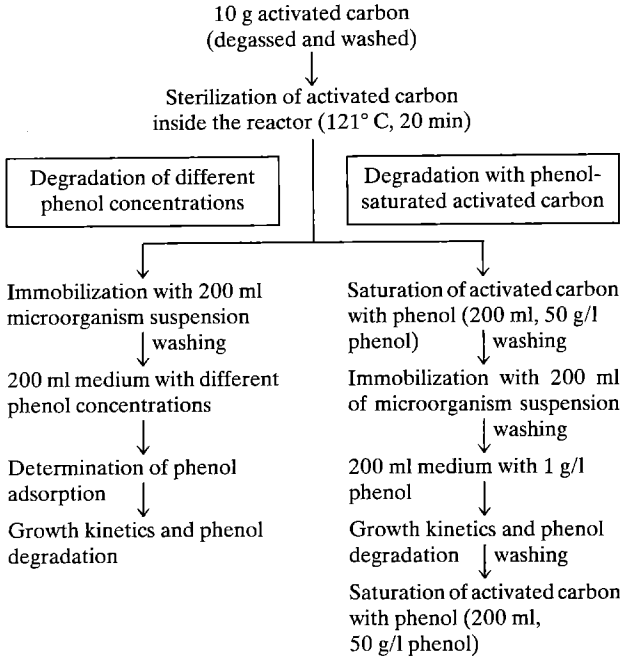
Scheme 1



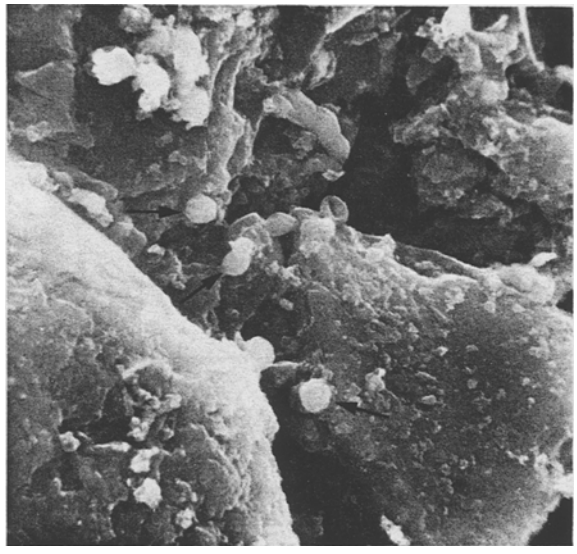


Fig. 2. *Candida* cells immobilized on the activated carbon surface (1500 ×) → *Candida* cells

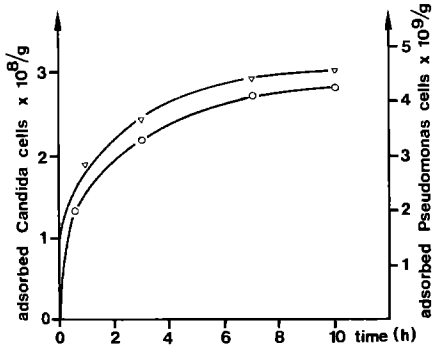


Fig. 1. Time-dependence of immobilization. ▽ adsorbed *Candida* cells/g activated carbon; ○ adsorbed *Pseudomonas* cells/g activated carbon

isms was calculated by the difference of the cell titer in the supension before and after the adsorption on activated carbon. The phenol concentration was quantitatively determined by a colorimetrical method described by Martin (1949), using 4-aminoantipyrine as colour reagent.

For electron microscopy (Stereoscan electron microscope) activated carbon particles with immobilized microorganisms were lyophilized. The scanning electron micrographs were made in the Bergbau-Forschung, Essen.

Results

Immobilization of microorganisms

The quantity of immobilized microorganism cells attached to the surface of the activated carbon is dependent on immobilization time. After about 8 h

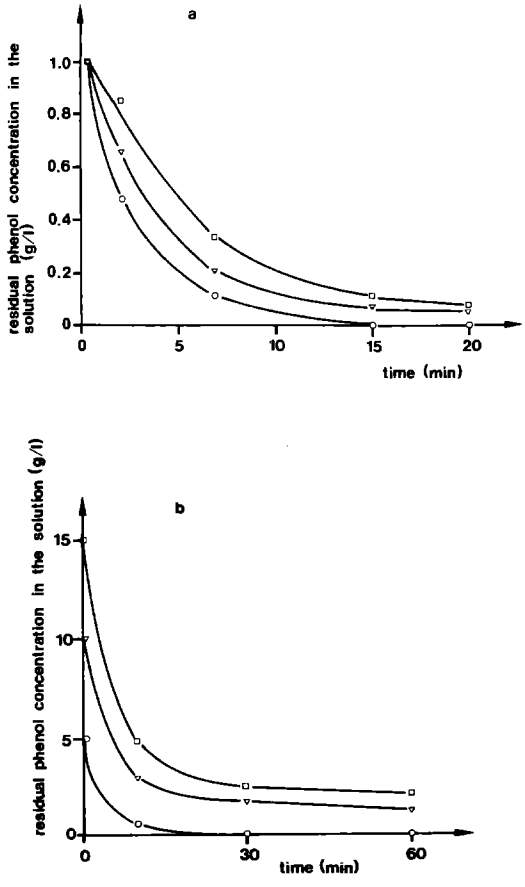


Fig. 3a, b. Kinetics of phenol adsorption on activated carbon. **a** Adsorption of 1 g/l phenol. Activated carbon: ○ without microorganisms; ▽ with immobilized *Candida;* □ with immobilized *Pseudomonas*. **b** Adsorption kinetics of different phenol concentrations. Adsorption of: ○ 5 g/l phenol; ▽ 10 g/l phenol; □ 15 g /l phenol

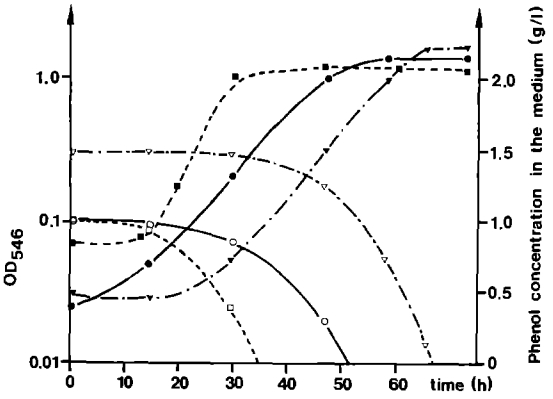


Fig. 4. Degradation of phenol by free microorganisms. Initial concentration of phenol 1 g/l: O———O phenol concentration and ●———● OD in *Candida* culture; □– – –□ phenol concentration and ■– – –■ OD in *Pseudomonas* culture. Initial concentration of phenol 1.5 g/l: ▽— · —▽ phenol concentration and ▼— · —▼ OD in *Candida* culture

an adsorption balance was reached between free and immobilized cells (Fig. 1). To get a high cell density on the carbon surface the immobilization time in further investigations was increased to 10 h. The immobilization of *Candida* cells could be demonstrated by scanning electron micrographs (Fig. 2).

Phenol adsorption

The activated carbon adsorbed phenol to about half of its own weight. Therefore it was difficult in the experiments to distinguish between phenol adsorption and phenol degradation by immobilized microorganisms. For this reason it was important to determine the kinetics of phenol adsorption on activated carbon. One gram per liter phenol was adsorbed in a few minutes. Immobilized cells delayed the phenol adsorption a few (Fig. 3a). The adsorption of 5 g/l, 10 g/l, and 15 g/l phenol required more time and was incomplete (Fig. 3b). At these concentrations no difference in adsorption between activated carbon without microorganisms and with adsorbed microorganisms could be observed.

Phenol degradation

Free cells of *Pseudomonas* and *Candida* degraded only low concentrations of phenol. *Pseudomonas* did not grow at 1.5 g/l phenol as sole carbon source (Fig. 4).

For the phenol degradation by immobilized cells, see Fig. 5a–d. After being added the phenol was rapidly adsorbed on the carbon (see Fig. 3a, b). The outgrowth of the immobilized microorganisms was

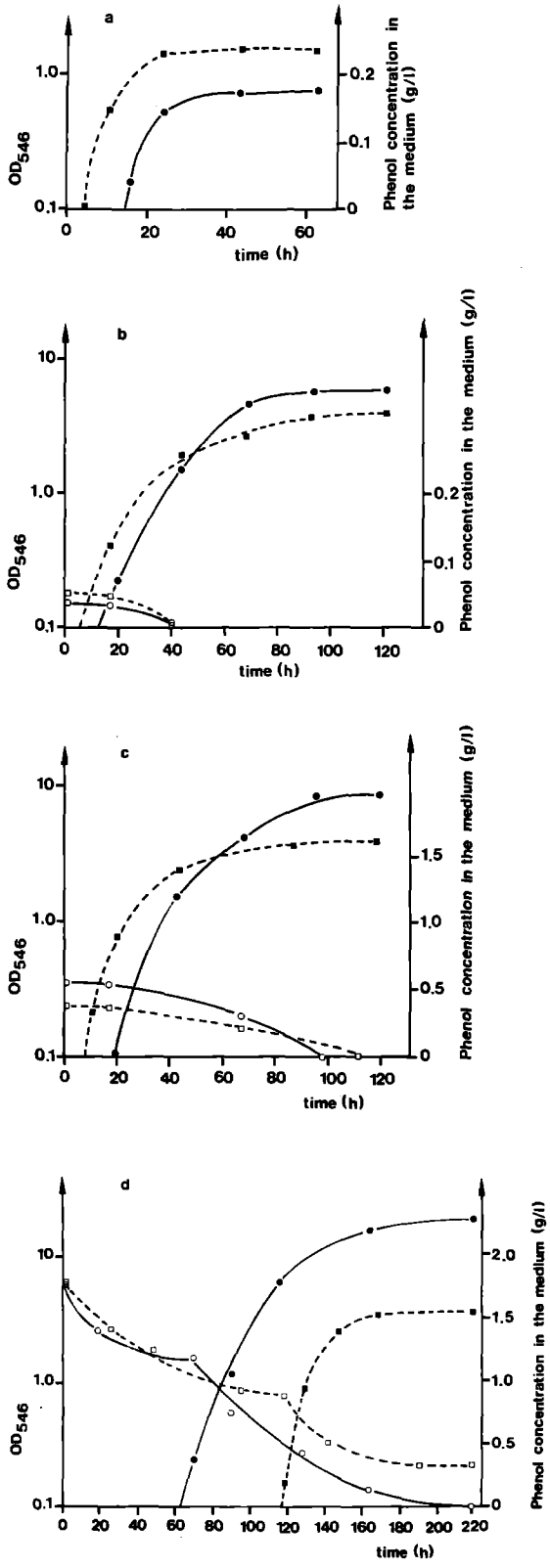


Fig. 5a–d. Growth kinetics and phenol degradation by *Candida* and *Pseudomonas* cells adsorbed on activated carbon (after addition and adsorption of **a** 1 g/l phenol, **b** 5 g/l phenol, **c** 10 g/l phenol, **d** 15 g/l phenol). O———O phenol concentration and ●———● OD in *Candida* culture; □– – –□ phenol concentration and ■– – –■ OD in *Pseudomonas* culture

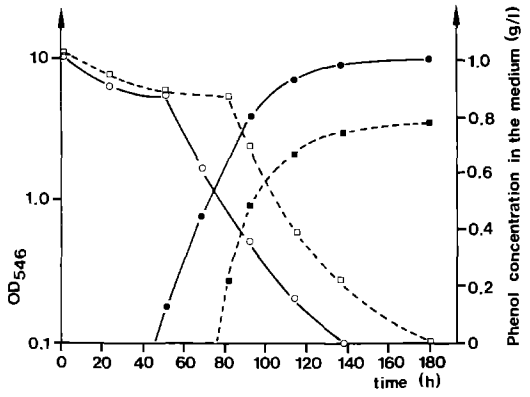


Fig. 6. Growth kinetics and phenol degradation of microorganisms immobilized on phenol saturated activated carbon (legend see Fig. 5)

determined by the optical density of free cells. The first free cells appeared in the medium, when the immobilized microorganisms already grew in the log-phase.

The immobilized cells remained active in spite of the addition of high phenol concentrations up to 15 g/l. The added phenol rapidly disappeared by adsorption and degradation. After a few hours no phenol was left in the medium but the cells continued to grow by degrading the phenol which had been adsorbed by the activated carbon (Fig. 5).

After addition of 15 g/l phenol about 2 g/l phenol could be observed after 2 h and decreased slowly under the amount of 1.5 g/l after several hours (see Fig. 3b). At this concentration the cell growth was delayed for 60 h (*Candida*) and 200 h (*Pseudomonas*). The bacteria were unable to degrade the total amount of phenol (Fig. 5d).

The activated carbon was saturated with phenol before immobilization to quantify the phenol degradation and phenol desorption from the activated carbon. After fermentation of about 120 h (Fig. 6) the activated carbon was saturated again. From the difference between the first and the second saturation a phenol degradation of approximately 90% was calculated.

The phenol concentration in the medium decreased clearly when the adsorbed microorganisms started growing. This growth made it possible to distinguish between phenol adsorption and phenol degradation (see also Figs. 5d, 6).

Discussion

Immobilization by adsorption on activated carbon offers the advantage of immobilization of microbial cells with very easy handling under favorable physiological conditions. The outer pores of the activated carbon have a diameter of $> 5 \times 10^{-8}$ m (Jüntgen et al. 1981) which is smaller than the diameter of a *Pseudomonas* cell of about 5×10^{-7} m. Therefore the cells will attach on the external surface of the carbon and can enter only some big macropores. The delay of the phenol adsorption by immobilized microorganisms might be caused by cells which narrowed or obstructed some pores and hindered the diffusion of phenol into the carbon.

The survival of the immobilized cells in spite of the addition of normally toxic phenol concentrations may result from the rapid phenol adsorption by the activated carbon. By this quick adsorption the immobilized cells are exposed only for a short time to the toxic phenol concentrations. The phenol concentration in the medium after adsorption is important for growth and degradation activity of the immobilized microorganisms (max. 1.5–2 g/l).

The immobilized microorganisms could utilize most of the adsorbed phenol. The cells still grew although no phenol was present in the medium. The adsorbed phenol diffused out of the activated carbon and was available to the microorganisms.

The activated carbon operated like a "buffer and depot": it protected the immobilized microorganisms by adsorbing toxic phenol concentrations and set low quantities of the adsorbed phenol free for biodegradation gradually. Therefore this combination of physical adsorption and biological degradation of phenol seems to be applicable for the treatment of waste waters containing temporarily high phenol concentrations which are toxic for biological treatment plants.

This also explains the stimulating effect of activated carbon in the treatment of waste waters observed until now.

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