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Polymeric supports for the adhesion of a consortium of autotrophic nitrifying bacteria

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The nitrifying performance of the biofilm formed onto polymeric supports (high density polystyrene, polyethylene, polypropylene, polyvinylchloride and polymethyl-methacrylate) was correlated with the hydrophobicity and surface charge of both bacteria and support media. Polypropylene , the most hydrophobic material, had the best properties for biofilm formation. The adhesion of nitrifying bacteria was mainly governed by hydrophobic interactions though electrostatic interactions were a determinant when the supports had identical hydrophobicity.

Introduction

Biological removal of nitrogen uses nitrifying bacteria which in aerobic conditions oxidise ammonium to nitrate in two stages: $NH_3 \rightarrow NO_2^- + H^+$ and $NO_2^- \rightarrow NO_3^-$ (Metcalf and Eddy, 1991). As nitrifying bacteria grow very slowly, bacteria washout from the bioreactors can be prevented by their immobilisation in fixed film or biofilm reactors.

The early stages of bacteria adhesion can be described by *van der Waals* forces of attraction and electrostatic forces of repulsion as formulated by DLVO theory, formerly proposed by Derjaguin, Landau, Verwey and Overbeek to explain the stability of lyophobic colloids (Oliveira, 1992). According to the thermodynamic model, adhesion is favoured when the surface energy of the associated solids is lower than that of the liquid medium (Mozes *et al.*, 1992). The surface energy of a solid can be estimated from measurements of contact angles and from thermodynamic considerations (van Oss *et al.*, 1995). In spite of existing various theoretical approaches for its computation, none has yet been universally accepted, thus, quite often the surface hydrophobicity is evaluated from the measurement of contact angle alone (Mozes *et al.*, 1992). The main goal of this work was to study the ability of some polymeric materials to be used as supports for a consortium of autotrophic nitrifying bacteria especially in laboratory rotating reactors and floating bed reactors. It was also intended to correlate that ability with surface properties, due to their especial role on the process of adhesion and consequently on biofilm development. According to this, glass was also used in order to test a well known hydrophilic surface.

Materials and methods

Culture conditions

The inoculum used was obtained from an activated sludge unit incorporated in a sewage treatment plant and was maintained in an inorganic medium (0.85 g $(NH_4)_2SO_4$, 0.25 g $Na_2HPO_4.12H_2O$, 0.25 g KH_2PO_4, 2.15 g $NaHCO_3$, 0.015 g $CaCl_2$, 0.15 g $MgSO_4.7H_2O$ and 0.005 g $FeSO_4.7H_2O$ in 1 litre of distilled water) in order to promote an enrichment in nitrifying microorganisms. To isolate ammonia oxidising bacteria the culture medium was inoculated with the mixed population and when the ammonia concentration was ~10 mg/l the cells were filtered, washed and introduced into fresh medium. The isolation of nitrite oxidising bacteria was obtained by inoculating the above mentioned media in which $(NH_4)_2SO_4$ was replaced by 1.2 g $NaNO_2$ /l and 1.5 g of $NaHCO_3$/l was added.

Batch experiments

The batch experiments were performed in a laboratory-scale reactor (6 L) using 5 L of culture medium and carried out at 26°C. In each experiment three slides of each type of support were suspended inside the fermenter. After 7 days, the slides were removed and washed thoroughly with distilled water in order to eliminate all non adhering cells. One slide of each material was dried in an oven at 50°C for 3 hours and then observed in a scanning electron microscope. The other two slides were utilised as inoculum in new experiments.

Analytical methods

The samples collected for the determination of nitrite and nitrate ions were filtered through a 0.2 μm filter.

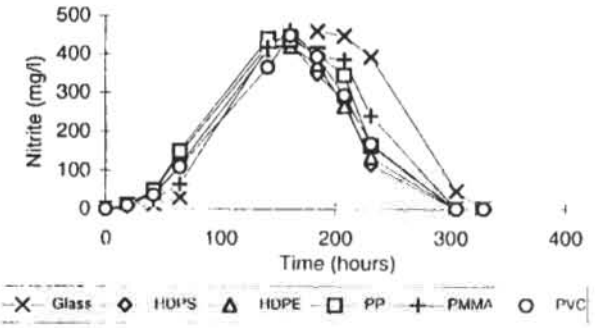

Figure 1 Variation of nitrite concentration in culture medium as a function of time, using as inoculum the biomass adhered to the different types of supports: glass, high density polystyrene (HDPS), high density polyethylene (HDPE), polypropylene (PP), polymethyl-methacrylate (PMMA) and polyvinylchloride (PVC).

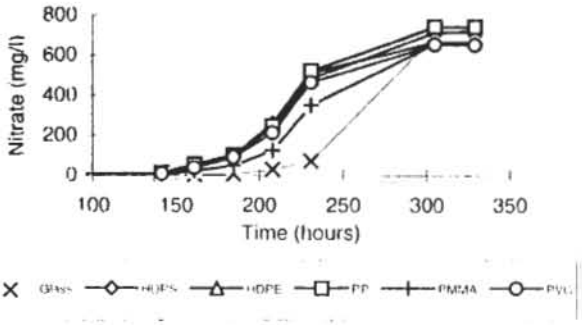

Figure 2 Variation of nitrate concentration in culture medium as a function of time, using as inoculum the biomass adhered to the different types of supports: glass, high density polystyrene (HDPS), high density polyethylene (HDPE), polypropylene (PP), polymethyl-methacrylate (PMMA) and polyvinylchloride (PVC).

Nitrite was detected by a colorimetric assay using N-(1-naphthyl)-ethylene-diamine. Nitrate was detected by measurement of UV absorption at 220 nm (APHA, 1989).

Support materials

Two millimetres thick slides (2 cm × 2 cm) of the polymeric materials: high density polystyrene (HDPS), high density polyethylene (HDPE), polypropylene (PP), polyvinylchloride (PVC), polymethyl-methacrylate (PMMA) and glass were used as substrata for biofilm development.

Characterisation of supports and cells

Surface hydrophobicity was determined by the measurement of contact angles of water in a standard contact angle apparatus (Kruss-GmH, Hamburg). The surface charge was measured as electrophoretic mobility in a Zeta-Meter System 3.0+ (Zeta-meter Inc., New York) operating at 100 V.

For the measurement of surface hydrophobicity of bacteria, the cells were harvested during exponential growth and washed once with ultra-pure water, and then with increasing concentrations of ethanol (10%, 25%, 50%, 70%, 90%) and finally resuspended in pure ethanol to a final concentration of 10^9 cells/ml. This cell suspension was immersed in a sonication bath during 5 min and 250 µl of this suspension was spread over a agar plate (20g/l of agar and 10% glycerol) with $1.5 \times 1.5 cm^2$ of size. After drying the first layer of cells, two more layers were added, completely covering the agar plate.

Results and discussion

The results obtained for nitrification using the slides with attached biofilm as inoculum are presented in Figures 1 and 2. PP is the support that exhibits the higher rate of ammonia oxidation, followed by the other polymeric supports. This tendency is evidenced by the rate of nitrate production (Fig. 2). Glass was the support where nitrite production and nitrate production occurred for a longer period of time, which might be explained by a smaller quantity of biomass adhered. PMMA revealed an intermediate behaviour between glass and the remaining polymeric materials (Fig. 1 and Fig. 2). This hypothesis is in agreement with the observation of the supports under scanning electron microscopy, which enabled to distinguish different covered areas in the various types of supports. However, it was not possible to make reasonable cell counts, due to the great tendency of the cells to form aggregates and to appear enmeshed in exopolymers.

After isolation of ammonia oxidising bacteria and nitrite oxidising bacteria the electrophoretic mobility was measured to obtain information on the net charge of their surfaces (Fig. 3). Both ammonia oxidising bacteria and nitrite oxidising bacteria exhibited a negative charge during their growth period.

The evaluation of the net surface charge of the supports was also made by measurement of electrophoretic mobility. All the supports were found to have a negative surface charge at pH 7.5 to 9 (Fig. 4) and presented approximately the same surface charge. However, PMMA was the most negative and glass showed a rather negative surface charge in comparison with the polymeric materials. Since both bacteria and supports have a negative surface charge, the electrostatic interactions are repulsive. If electrostatic interactions were solely involved, adhesion should not take place. However, as all the supports were colonised by the autotrophic bacteria (Fig. 1 and Fig.2) it can be concluded that the adhesion was controlled by other interactions.

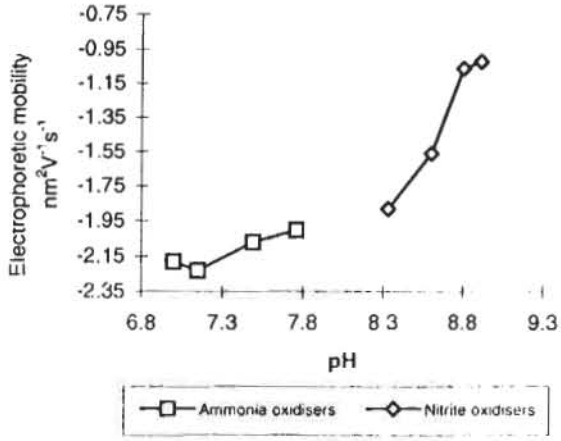

Figure 3 Bacterial electrophoretic mobility measured at 100 V during their growth period in culture medium as a function of pH.

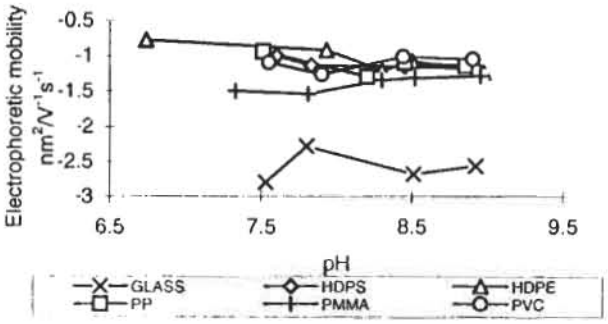

Figure 4 Electrophoretic mobility of the various supports measured at 100 V in culture medium as a function of pH.

Loosdrecht *et al.*(1990), showed that surface hydrophobicity plays a dominant role in bacteria adhesion because the *van der Waals* forces of attraction increases with the surface hydrophobicity. The hydrophobicity of both cells and supports are shown in Tables 1 and 2. Glass is highly hydrophilic, PP, HDPS and PVC are the more hydrophobic supports whereas PMMA and HDPE presented an intermediate behaviour. Comparing the ability of the support media to bacteria colonisation (Fig. 1 and Fig. 2) with their surface hydrophobicity, adhesion seems to be favoured on solids of lower surface energy.

Surface properties of ammonia oxidiser and nitrate oxidisers are different, ammonia oxidisers have the smaller hydrophobicity and the smaller charge whereas nitrite oxidiser are more hydrophobic and less negative. This fact can be responsible for the increase in the dissimilar behaviour of the supports concerning nitrite oxidation.

The hidrophobicity of PMMA and HDPE are approximate the same (Table 2) whereas their performance is not (Fig. 1 and Fig. 2). PMMA is the most negative polymeric material and exhibits a smaller nitrifying activity. These facts may lead to the conclusion that in the adhesion process of these microorganisms, not only interfacial energy should be considered but also electrostatic interactions, moreover, electrostatic interactions becomes more important in less hydrophobic surfaces.

The results obtained show that adhesion to hydrophilic supports also occurred, in spite of being in a lesser extent. This is in agreement with the results of several authors (Loosdrecht *et al.*, 1990; Oliveira *et al.*, 1994; Dufrene and Rouxhet, 1996). The initial attachment of bacteria to high energy hydrophilic surfaces may be due to two factors: first hydrophilic surfaces can be preconditioned by the adsorption of more hydrophilic macromolecules, which can be metabolites excreted by the microorganisms, secondly a initial small population of attached microorganisms may grow to develop a thick microbial slime layer.

According to the results obtained, the rotating discs of nitrifying RBC reactors should be built in a high hydrophobic material, like polypropylene. Adhesion would also be favoured if the surface of the discs is rubbed and carved with concentric lines, in order to increase the roughness. For circulating floating bed reactors, besides hydrophobicity, the particles have to be less dense than water and preferentially with some degree of porosity to increase the surface area for biomass attachment. So, the type of material to be used is depen-

Table 1 Contact angles of water on the ammonia oxidising and nitrite oxidising bacteria

bacteria	*Contact angle of water (°) ±2°*
Ammonia oxidising	15
nitrite oxidising	18

Table 2 Contact angles of water on the various supports and cells

Support	*Contact angle of water (°) ±1°*
PP	79
HDPS	76
PVC	72
PMMA	67
HDPE	65
Glass	16

dent on the applicability of the new techniques of polymer production to obtain porous granules. For instance, polystyrene fits all these requisites.

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