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The importance of surface properties in the selection of supports for nitrification in airlift bioreactors

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Abstract The selection of the supports for biomass immobilisation is of great importance to obtain a stable biofilm leading to a high nitrification efficiency. In this work five type of supports were assayed in external loop air-lift reactors: sand, limestone, basalt, pumice and poraver. The efficiencies of the supports were compared and discussed in terms of their surface-properties: surface free energy of interaction, surface charge, porosity and shape. The results showed that materials leading to low surface free energies of interaction and with less negative surface charges favour bacterial adhesion and the formation of stable biofilms. High porosities are also relevant for bacterial colonisation. However, irregular shaped particles may promote the erosion of attached biomass.

1 Introduction

Biofilms are a collection of cells entrapped within a gelatinous matrix comprising mostly insoluble extracellular polymers that the cells secrete [1]. Any surface in contact with a biological fluid is a potential target for microbial cell adhesion. Once cells are attached, subsequent growth and replication of surface-attached cells, cell exopolymer production, further recruitment of planktonic cells from the fluid phase, and various biofilm detachment processes constitute what is known as biofilm formation and persistence. Although useful in many situations, biofilm processes are used widely in wastewater treatment, namely, in nitrogen compounds removal.

The key requirement for nitrification in wastewater systems is that the sludge age in the reactor is larger than the specific growth rate of nitrifying bacteria [2]. In the activated sludge processes this is accomplished in relatively large bioreactors. Airlift reactors offer several advantages over the traditional systems: ease of construction, ease of operation, fewer chances of media contamination, lower energy consumption, low shear rate and low cost. However, the main advantage of this bioreactor is the possibility to maintain high concentrations of biomass in the reactor. This is achieved by growing biofilms on small suspended carriers. The selection of the supports for biomass immobilisation is of great importance to obtain a stable biofilm leading to a high nitrification efficiency. In this way, the support must allow microbial adhesion, must be hard, must have a high fluidity (to reduce the energy required for fluidization), a low cost and a great availability. Parameters like porosity, roughness and particles diameter, are still of great importance.

Rougher carrier particles may help attachment of cells. This may be due to the increase of the surface area available to adhesion. Moreover, microroughness acts as a protection to cells from shear forces. However, in rougher particles, the biofilm detachment is great because in particle-particle collisions, the energy transfer during collision will be higher then in smooth carriers [3]. Messing et al. [4] shows that the maximum accumulation of stable and viable biomass occurs when the pore diameter is between one and five times the major dimension of the microorganisms. The surface properties of cells and support, like hydrophobicity, surface charge and surface energy, play an important role in the adhesion process. Normally, an organism tends to adsorb irreversibly to minimise the free energy of the system [5]. However, charge repulsion or steric exclusion may prevent close approach. Hydrophilic surfaces may be stabilised by ordering of surface-associated water molecules into the bulk phase, increasing their free energy. Hydrophobic surfaces should attract strongly because water molecules moving away from the surface into the bulk will decrease their free energy as their level of hydrogen bonding will increase [6]. Most bacteria are hydrophilic [5] so an approach to a hydrophilic surface would be thermodynamically unfavourable. Alternatively, the approach of a hydrophilic cell to a hydrophobic surface would yield a stronger interaction. Microbial cell surfaces possess an electric charge, which is usually negative [7]. Thus, it may be expected that adhesion will be favourable to supports with positive charge. Kida et al. [8] showed this when evaluating eight different kinds of supports. However, ideal supports for microbial adhesion are not always available since solid materials usually have negative surface charge, creating an electrostatic repulsion with cells which, near neutral pH, are negatively charged too. Surface energy considerations enables the computation of the free energy of adhesion

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between two surfaces (the thermodynamic model), by regarding the transformation as a replacement of two solid-liquid interfaces by one solid-solid interface. According to this model, bacterial adhesion will be favoured if the process itself causes the free energy to decrease. Thus adhesion is favoured when the surface free energy of both solids is lower than that of the liquid medium [9].

So, the best support will be the one that allows the greatest substrate removal for the same organic load, under the same operating conditions.

2
Materials and methods

2.1
Culture conditions

The supports were previously inserted in 1000 ml flasks with culture medium inoculated with a nitrifying consortium obtained from an activated sludge unit incorporated in a sewage treatment plant, at 26 °C with shaking at 130 rpm. They were kept during one month. Every 7 days the medium was decanted, the supports were washed and introduced into fresh medium. The composition of the culture medium used was: 0.85 g $(NH_4)_2SO_4$, 0.25 g $Na_2HPO_4 \cdot 12H_2O$, 0.25 g KH_2PO_4, 2.15 g $NaHCO_3$, 0.015 g $CaCl_2$, 0.15 g $MgSO_4 \cdot 7H_2O$ and 0.005 g $FeSO_4 \cdot 7H_2O$ in 1 litre of distilled water.

2.2
Supports

Five different types of materials were compared: sand, limestone, pumice, basalt and poraver (expanded glass). The particles of the supports had a diameter in the range of 0.4–0.5 mm. The porosity was determined by nitrogen adsorption and expressed in %. The surface free energy of supports was determined by the "Thin-Layer Wicking" technique, proposed by van Oss et al. [10] and adapted by Chibowsky and Holysz [11]. The experiments were carried out using glass plates (25 × 100 mm) on which a suspension of the powdered materials was deposited. The suspension was prepared with 5 g of powdered material in 100 ml of double-distilled water and 5 ml portions were pipetted onto each plate placed horizontally to get a uniform layer. After water evaporation (overnight) the plates were dried at 150 °C for 2 h and kept in a desiccator. Some plates were allowed to equilibrate in a closed vessel with saturated vapour of the test liquid for 20–24 h. To test the penetration of the liquids, the plates were placed in a teflon chamber in a horizontal position. The chamber was 11 cm long, 4 cm wide and 0.6 cm deep. The top wall of the chamber was a glass plate, so the moving boundary was well visible. On the edges of the chamber, 1 cm sections were marked, which allowed the recording of the penetration time for particular distances (9 cm were marked). The test liquid was placed at the end of the plate with a pipette and at that very moment the penetration time counting was started. The test liquids were: water, decane and formamide. In each experiment the temperature of the liquid was measured. The viscosity of the liquid was taken from literature. All experiments were carried out at room temperature, 20–22 °C. For each liquid six plates were used: three bare-plates and three precontacted plates. The surface charge was measured as electrophoretic mobility in a Zeta-Meter 3.0+ (Zeta-meter Inc., New York) operating at 100 V. The supports were reduced to particles smaller than 38 μm. The electrophoretic mobility was determined in culture medium adjusted to different pH values between 6.0 and 9.0 with NaOH or HCl, as necessary. The measurements were performed after a soaking period of 24 hours in the culture medium to establish an equilibrium between the particles and the liquid.

2.3
Cell surface energy

2.3.1
Preparation of the bacteria substrata

Bacterial cells were harvested by centrifugation, washed firstly with deionized water, secondly with increasing concentrations of ethanol (10%, 25%, 50%, 70%, 90%) and finally resuspended in pure ethanol to a concentration of 10^8 cells/ml. This procedure with increasing ethanol concentrations, was used to avoid changes in cell wall structure due to dehydration. This cell suspension was immersed in a sonication bath for 5 minutes in order to obtain better homogenisation. Meanwhile, a solution of 20 g/l of agar and 10% glycerol, was cast in glass Petri dishes and cooled at room temperature. Finally, the agar plates were cut into small squares of 1.5 × 1.5 cm^2. A small volume of the cell suspension (250 μl) was spread over the agar square. After drying the first layer of cells, two more layers were added, to completely cover the agar square.

2.3.2
Contact angles measurements

Contact angles were measured on the agar plates covered with cells. The measurements were carried out in a standard contact angle apparatus (Kruss-GmH, Hamburg). The determinations of the contact angles were performed automatically, with the aid of an image analysis system (Kruss-GmH, Hamburg 1993). The images were received by a video camera connected to a 486 DX4 100 MHz personal computer, with an automatic measuring system (G2/G40) installed. All the measurements were made at room temperature and three different liquids were used: water, diiodomethane and glycerol.

2.4
Analytical methods

The rate of formation of nitrite was used to determine the activity of the ammonium oxidizing bacteria. The activity of the nitrite oxidizing bacteria was assessed based on the rate of nitrate production. Samples were taken every day. Ammonium was determined using a selective electrode (Orion, 720A). The samples collected for the determination of nitrite and nitrate ions were filtered through a 0.2 μm filter in order to remove interference from suspended particles. Nitrate was determined by UV absorbance at 220 nm and nitrite by a colorimetric method using *N*-(1-naphthyl)-ethylene-diamine, according to the Standard Methods of Analysis [12]. These determinations were made promptly after sampling.

2.5 Experimental conditions

The experiments were performed in three air-lift reactors with external loop. The bioreactors had a working volume of 0.7 l, 0.032 m of inner diameter of riser and 0.016 m of inner diameter of downcomer. The ratio between the area of the riser and the area of the downcomer, (A_r/A_d), was 4 and the ratio height/diameter (H/D) was 13. Air was sparged into the bioreactors through a perforated plate having pores of 100 to 160 μm of diameter and the aeration rate was measured with a calibrated rotameter. The air velocity was 2 cm/s. The experiments took place at room temperature and the pH was controlled between 7 and 9 with the automatic supply of hydrochloric acid, when necessary. The hydraulic retention time was 8 hours and the feed flow was 2.1 l/s. The carrier concentration was 20 g/l. The nitrogen organic load was 0.25 kg/m^{3}d, with an effluent ammonium concentration of 107 mg/l. To start-up the reactors, the supports were transferred to the reactors with the respective medium and the feed was turned on in order to complete the working volume.

3 Results

The results obtained for the porosity of the particulate materials are presented in Table 1. Contact angles with the various liquids employed and surface tensions components of the nitrifying bacteria studied are summarised in Table 2 and Table 3, respectively. It can be seen that both bacteria are hydrophilic. *Nitrobacter* is intrinsically the most hydrophilic.

Table 4 shows the data of the interfacial free energy of adhesion between nitrifying bacteria and support materials, immersed in water. The lowest values were obtained for limestone-*Nitrosomonas* and limestone-*Nitrobacter*, followed by the values for the interaction of the nitrifiers with basalt.

Table 1. Porosity of support materials

Support	Porosity (%)
Sand	29
Limestone	11
Basalt	15
Pumice	48
Poraver	79

Table 2. Contact angles (degrees) with various liquids on nitrifying bacteria

Bacterial strain	Water	Glycerol	Diiodomethane
Nitrosomonas	15.6	29.4	68.0
Nitrobacter	16.5	44.6	68.0

Table 3. Surface tension components of nitrifying bacteria (mJ/m^2)

Bacterial strain	γ_S^{LW}	γ^+	γ^-	γ_S^{TOT}
Nitrosomonas	24.00	4.80	55.23	56.56
Nitrobacter	23.99	1.67	68.90	45.45

Table 4. Interfacial free energy of adhesion (ΔG_{123}) between nitrifying bacteria (1) and support materials (2), immersed in water (3), in mJ/m^2, at 20 °C

Bacterial strain	Support	ΔG_{123}
Nitrosomonas	Sand	23.20
Nitrosomonas	Limestone	9.74
Nitrosomonas	Basalt	12.76
Nitrosomonas	Pumice	31.41
Nitrosomonas	Poraver	43.53
Nitrobacter	Sand	31.33
Nitrobacter	Limestone	11.67
Nitrobacter	Basalt	18.15
Nitrobacter	Pumice	43.05
Nitrobacter	Poraver	58.73

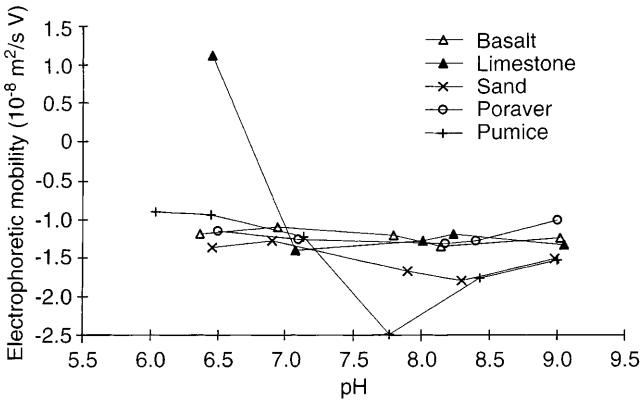

Fig. 1. pH dependence of electrophoretic mobility of supports

The surface charge of the supports was measured as electrophoretic mobility. Figure 1 shows the average values of the determinations. In the pH range 6–9 all the materials are negatively charged, except limestone that shows a positive charge for pH values under 6.7. Basalt and poraver have similar surface charges in the pH range studied. Pumice is the most electronegative followed by sand.

All over the experiments, due to the medium buffering capacity, the value of pH was in the range of 7.2–7.8, which is well within the optimal range for the nitrifiers. The results of nitrification in the airlift reactors are shown in Fig. 2. Poraver and limestone are the supports that showed greater efficiency. In the case of poraver it took 4 days for all the ammonia to be oxidised to nitrite, and all nitrite was oxidised to nitrate after 10 days. With limestone, both oxidations occurred in 2 and 12 days, respectively. Sand and pumice are the materials in which nitrification is slower. Basalt shows an intermediate behaviour.

4 Discussion

The adhesion of the nitrifying consortium to the different types of carriers seems to be strongly affected by the electrical charges of the interacting surfaces. Both types of bacteria present negative surface charges and they adhere

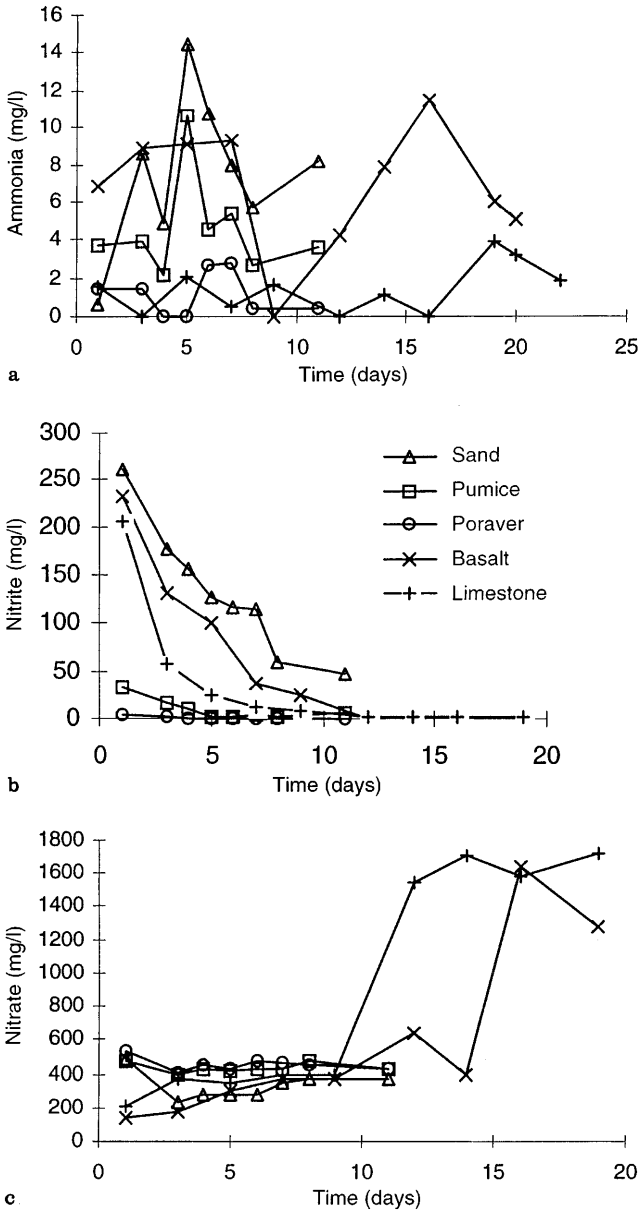

Fig. 2a–c. Time variation of nitrogen content in wastewater samples in air-lift reactors. **a** Ammonia; **b** Nitrite; and **c** Nitrate

preferentially to the supports that show higher surface charges. This explains the lower efficiencies obtained with sand and pumice, which are the most negatively charged particles.

The thermodynamic approach also helps to explain the observed behaviour of the different types of particles, as nitrification efficiency is concerned. Limestone and basalt, which show high efficiencies, have the most negative surface free energies of interaction with the bacterial cells, meaning that adhesion is more favourable. The small porosities of limestone and basalt also reinforce this reasoning, since they have less available surface area for bacterial attachment. Probably, the interaction established with the positively charged calcium ions present in the composition of these materials, mainly in limestone, may also contribute for a strong adhesion. It is well known the bridging effect of Ca^{2+} with negatively charged biomolecules.

Moreover, in the case of limestone, carbonate ions can affect the pH, since the protons released during the first step of nitrification can be neutralised by them, inhibiting a pronounced decrease in pH.

Besides, it is also possible that in the presence of limestone a higher concentration of inorganic carbon might be available for the metabolism of the autotrophic consortium. The good efficiency displayed by poraver, although not in good agreement with the thermodynamic approach, can be explained by some calcium bridging but mainly by its high surface area on account of its high porosity. The mechanical resistance of poraver is relatively low and this may lead to some disintegration of the particles with a consequent increase in surface area. However, if disintegration occurs to a large extent this may lead to the reactor wash-out.

Pumice has also a very high porosity, but when grounded to small particles do not acquire a round shape. By microscopic observation it is perfectly seen that they are very irregular and sharp. This type of shape increases the erosion phenomena and promotes a higher detachment of cells.

5
Conclusions

The surface properties of the materials have a great influence on the adhesion phenomenon. Materials having low surface free energies of interaction with bacteria favour the formation of more stable biofilms. The surface charge is also determinant for the bacterial attachment. As a consequence the basalt and limestone showed very high efficiencies. The porosity is also relevant for a good colonisation of the carriers as was proved by poraver.

Sand showed low efficiency and a great difficulty in fluidization.

Irregular particle shapes, like in the case of pumice, promote a strong abrasion and erosion of the biofilm.

Although poraver had a good performance, its low mechanical resistance can be detrimental when working with a great concentration of particles and high fluidising velocities.

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