Influence of physico-chemical properties of porous microcarriers on the adhesion of an anaerobic consortium

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The ability for biomass colonization of four porous mineral microcarriers (sepiolite, clay, pozzolana and foam glass-Poraver™), was studied and related to their surface properties. The surface hydrophobicity of the mineral carriers was a more important factor influencing colonization by the anaerobic consortium than was surface charge. It was possible to correlate linearly the degree of hydrophobicity with the biomass retention capacity. Although the thermodynamic theory did not explain adhesion, an increase in cell attachment was directly related to the decrease of the positive values of the free energy of adhesion. Surface roughness, porosity and the amount of surface Mg^{2+}, were also determinant factors in bacterial immobilization. However a great biomass accumulation can originate a decrease in biological activity due to mass transfer limitations. *Journal of Industrial Microbiology & Biotechnology* (2000) **24**, 181–186.

Introduction

In the last two decades anaerobic digestion technology significantly improved due, in large part, to better biomass immobilization technology. Therefore, knowledge of the basic processes of biomass immobilization is important.

Microorganisms present in biofilms or microbial aggregates are more protected from adverse environmental conditions [9,16]. Although biomass immobilization in the popular Upflow Anaerobic Sludge Blanket (UASB) reactor is based on self-granulation, anaerobic filters (AF) and fluidized bed (FB) digesters are examples of true biofilm reactors. In FB digesters, sludge retention is primarily governed by adhesion and growth on a moving surface and the performance of this kind of digester was reported to be highly dependent on the characteristics of the support material used for biomass colonization [12].

The colonization of solid surfaces is ruled by the surface properties of both interacting bodies (surface charge, surface tension, wettability, composition, porosity and roughness). Surface charge and surface tension are responsible for short and long range interactions. Long range interactions have been described by DLVO (after Derjaguin, Landau, Verwey and Overbeek) theory which postulates that the net force of interaction between cells and the adhesion substratum arises from the balance between van der Waals forces of attraction and electrostatic forces of repulsion [19]. Short-range interactions take place when the cells are close to the surface (<2 nm) and include Born repulsion forces, hydration forces and hydrophobic and steric interactions [10]. When a bacterium and a surface enter into direct contact, the water film present between the interacting entities has to be removed. This is in accordance with the thermodynamic theory of adhesion and is expressed by the Dupré equation [1] which states that the Gibbs free energy of interaction can be calculated assuming that the interfaces between bacteria/liquid medium and solid/liquid medium are replaced by a bacteria/solid interface. Hydrophobicity has been considered the most important short-range interaction force in bacterial attachment [8,30]. A number of authors reported that the hydrophobicity of the adhesion surface plays a determinant role in bacterial adhesion, being even more important than the surface charge [3,11,23,24,26]. It should be stressed that surface charge is usually assessed in terms of zeta potential. As they are directly related, both are used interchangeably throughout the literature to express qualitatively the electrical surface properties of colloidal particles.

Apart from these properties, surface morphology is also important. Adhesion is enhanced on porous surfaces on account of a higher surface area for bacteria attachment. However, pore size is an important feature for surface colonization [17]. Accordingly, the best pore size for attachment should range between the smallest cell dimension and five times its largest dimension. Shimp and Pfaender [22] also reported that microbe-size crevices favor surface colonization.

Some authors claim that surface roughness is more important for colonization than internal surface area [20]. Scanning electron microscopy revealed that the initial colonization starts from surface irregularities such as cracks, grooves or abrasion defects. Moreover, on a rough surface, microorganisms are more protected against shear forces [21].

Many authors reported an enhancement of adhesion in the presence of specific surface groups, such as Ca^{2+} and Mg^{2+} [5,26,28] due to cation bridges between the negatively charged bacterial surface and the substratum [19].

The aim of this work was to study the surface properties of four porous carriers (clay, foam glass, pozzolana and sepiolite) and relate these properties with the biomass retention capacity, taking into account methanogenic activity.

Table 1 Characteristics of the microcarriers

Material	Diameter (mm)	Dry grain density (g cm^{-3})	Wet grain density (g cm^{-3})	Internal porous[a] volume (cm^3 cm$_{particle}^{-3}$)
Clay	3.35–4.75	0.714 ± 0.017	1.094 ± 0.001	0.381 ± 0.019
Foam glass	3.35–4.75	0.307 ± 0.012	1.101 ± 0.013	0.796 ± 0.016
Pozzolana	3.35–4.75	1.532 ± 0.068	2.039 ± 0.049	0.508 ± 0.100
Sepiolite	3.35–4.75	0.991 ± 0.066	1.627 ± 0.049	0.638 ± 0.114

[a]Water accessible.

Materials and methods

Microcarriers and biomass retention capacity

Four porous microcarriers were tested: sepiolite, foam glass (Poraver™, Dennert, Poraver GmbH, Germany), clay and pozzolana. Table 1 summarizes some of their relevant characteristics. The dry grain density was measured by mercury picnometry. The wet grain density was determined by water picnometry, after forcing the penetration of water inside the pores of each material (autoclaving). water accessible porosity was considered to be a more suitable parameter than total porosity to characterize the susceptibility of each material for internal microbial colonization.

The biomass retention capacity, expressed as volatile solids (VS) per volume of particle, of each microcarrier was determined in a previous work [2]. Each material (heated at 550°C and autoclaved) was placed in a continuous reactor, under similar environmental conditions. At the end of the adhesion experiment the specific methanogenic activity of the adhered biomass was determined by measuring the initial rate of degradation of a stock solution of volatile fatty acids (VFA) in batch assays, as detailed elsewhere [2].

Surface tension

The surface tension of the anaerobic consortium was calculated according to the approach of van Oss *et al* [32], using the values of the contact angles formed by water, formamide and di-iodomethane on bacterial lawns [6]. The surface tension of the carriers, due to their shape, was assessed by means of the thin layer wicking technique as described by Teixeira *et al* [27].

Zeta potential

The zeta potential of the mineral carriers was determined (after grinding) by measuring the electrophoretic mobility in a Zeta-Meter 3.0+ (Zeta-Meter Inc, USA) at 100 V. Determinations were performed in the culture medium adjusted to pH values in the range between 6.7 and 7.7 with NaOH and HCl. The same procedure was followed to obtain the zeta potential of the cells. However, in this case anaerobic conditions were maintained inside the electrophoretic cell by saturating the culture medium with nitrogen.

Surface morphology and chemical composition

The surface morphology of each material was evaluated by scanning electron microscopy (Leica, Cambridge S360) [2]. Prior to microscopic examination, samples were sputter-coated with gold. The chemical composition of each

Table 2 Attached biomass (± 95% confidence interval)

Material	Attached biomass (g VS L$_{particle}^{-1}$)[a]
Sepiolite	24.5 ± 1.5
Foam glass	15.4 ± 1.2
Clay	14.9 ± 0.5
Pozzolana	13.3 ± 0.4

[a]Volatile solids per volume of particle. Determined by weight loss after heating at 550°C.

material was determined by EDS (Energy Dispersion Spectroscopy) (Leica, Cambridge S360).

Results and discussion

Biomass colonization

The four assayed carriers exhibited different susceptibilities to bacterial colonization. Sepiolite was the most colonized material followed by foam glass, clay and pozzolana (Table 2).

In a previous work describing the adhesion experiment, the amount of volatile solids (VS) attached was expressed per internal porous volume [2]. This representation allowed differentiation and qualification of the internal porosity relative to the biomass retention capacity, which was related to surface morphology, particularly to pore size. Table 3 summarizes these results.

The rough surface of sepiolite with cell-size crevices favored adhesion. On the other hand the relative smooth surface and large pores of foam glass accumulated less biomass.

The conclusion that sepiolite is an efficient microcarrier for biomass immobilization is in accordance with earlier works. Huysman *et al* [14] observed that sepiolite was the

Table 3 Characteristics of the microcarriers and attached biomass, expressed per internal porous volume (± 95% confidence interval)

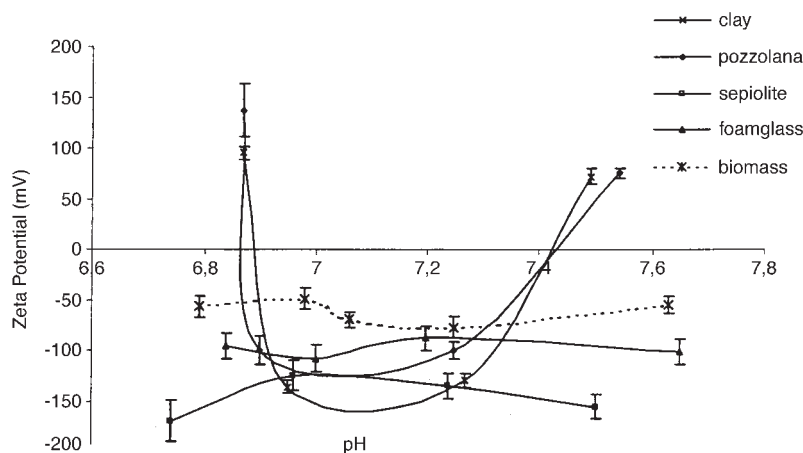| Material	Roughness	Surface appearance/pore size	Attached biomass g VS L$_{internal\ porous\ volume}^{-1}$
Sepiolite	++++	cell size crevices	38.4 ± 2.4
Clay	+++	10–100 μm	35.1 ± 1.0
Pozzolana	++	10–300 μm	29.3 ± 1.3
Foam glass	+	20–1000 μm	19.3 ± 1.4

Figure 1 Zeta-potential values of the anaerobic consortium and microcarriers in the operating pH range.

most efficient material for microbial colonization, when compared with glass beads, argex, activated carbon and a zeolite. The amount of macro and micropores and the surface roughness of sepiolite were suggested to be important factors for its ability to achieve a good and fast microbial colonization [18,22].

In the present work an attempt to explain the different degrees of colonization was made by investigating some surface properties such as surface tension, hydrophobicity and chemical composition of the four materials.

Surface charge

The surface charge of the interacting entities (cells and carriers) was evaluated by means of zeta potential (Figure 1). Both pozzolana and clay behaved similarly showing an oscillatory pattern of zeta potential with pH: negative for pH between 6.9 and 7.5 and positive outside this pH range. In the pH range 6.9–7.5, where all the materials show a negative zeta potential, foam glass is less negatively charged. Bacteria also present a negative zeta potential for pH values between 6.9 and 7.5. Therefore, in this pH range a high electrostatic repulsion would prevent bacteria from adhering to the carriers. Moreover, it was not possible to find any direct correlation between a less negative surface charge and a higher tendency for adhesion. Thus, in the present situation, this parameter has a minor influence on bacterial adhesion.

Surface tension

According to van Oss *et al* [32], the surface tension of a substance comprises a component arising from Lifshitz–van der Waals interactions (γ^{LW}) and a component related to polar interactions (γ^{AB}) of the electron acceptor-electron donor type, γ^+ and γ^-, respectively ($\gamma^{AB} = 2 \ [\gamma^+ \ \gamma^-]^{1/2}$).

From the data presented in Table 4 it is clear that all the surfaces are predominantly electron donor ($\gamma^- \gg \gamma^+$). The electron acceptor parameter of all the surfaces studied is very small; therefore the apolar component has a greater contribution for the overall surface tension than the polar component. Knowing surface tension values it was possible to calculate the free energy of adhesion [1].

Thermodynamically, adhesion is favored when it leads to a decrease of the free energy (ΔG) at the interface bacteria

Table 4 Surface tension and surface tension components of the mineral carriers and the bacterial cells (values in mJ m^{-2} at 20°C)

Surface	γ^{LW}	γ^+	γ^-	γ^{AB}	γ^{tot}
Cells	29.6	3.5	55.9	27.9	57.5
Clay	50.7	0.8	52.0	12.9	63.6
Sepiolite	42.1	0.7	37.0	10.2	52.3
Pozzolana	49.3	3.4	62.6	29.2	78.5
Foam glass	52.9	0.1	80.4	5.7	58.6

γ^{LW} = apolar component of surface tension; γ^+ = electron acceptor parameter of the polar component of surface tension; γ^- = electron donor parameter of the polar component of surface tension; γ^{AB} = polar component of surface tension; γ^{tot} = total surface tension.
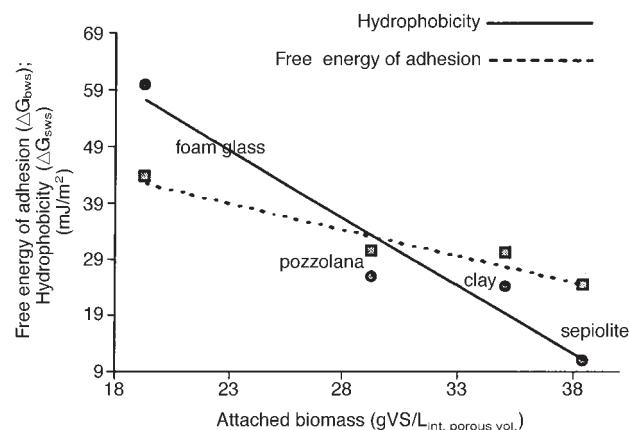

Figure 2 Interfacial free energy of adhesion (ΔG_{bws}) and hydrophobicity (ΔG_{sws}) of the carriers *vs* attached biomass expressed as volatile solids per litre of internal porous volume.

(b)/water (w)/substratum (s), meaning a negative value of ΔG_{bws}. In this case, theoretically, the anaerobic biomass would not adhere to the assayed carriers because the free energy of adhesion is always positive (Figure 2). This anomaly reveals limitations in thermodynamic theory, which has also been reported by other authors [7]. Nevertheless a decrease in the interfacial free energy seems to correlate
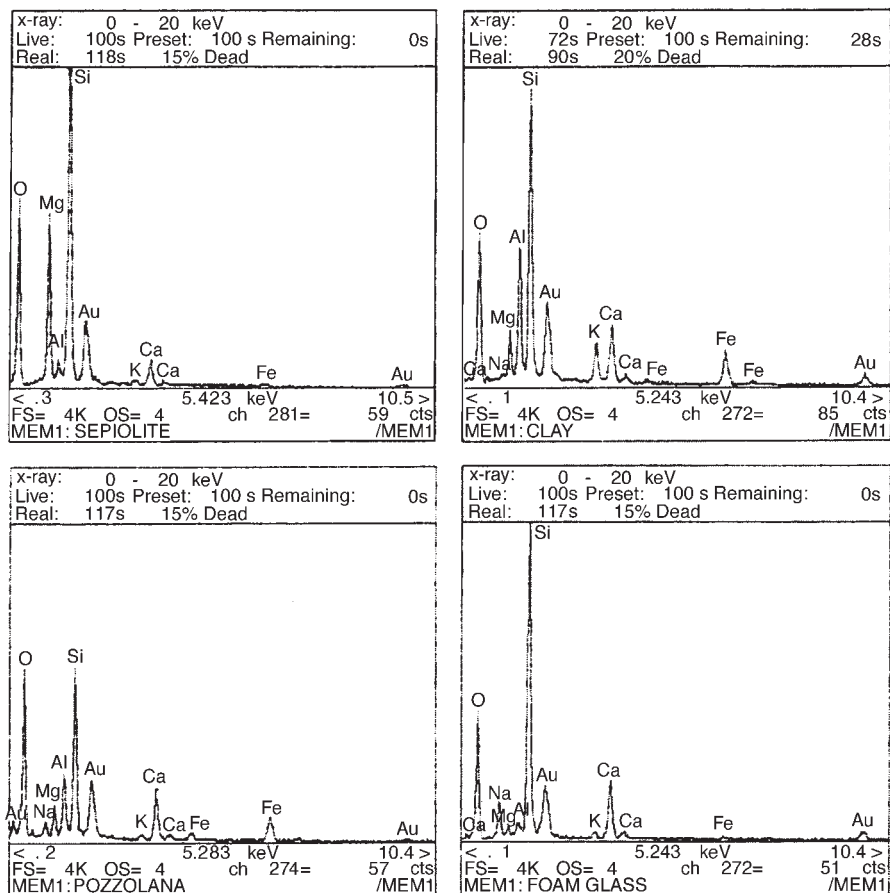
Figure 3 Elemental surface composition of foam glass, sepiolite, pozzolana, and clay obtained by energy dispersion spectroscopy (EDS) analysis.

with the biomass retention capacity of each material (Figure 2). In fact, the material that had the highest biomass retention capacity, sepiolite, showed a less positive free energy of interaction with the cells, followed by clay, pozzolana and foam glass. Other authors reported a similar behavior for different types of microorganisms and surfaces [7,25]. This means that for each bacterial strain a decrease in the free energy of adhesion is indicative of an increase in the number of adhering cells.

Surface hydrophobicity

According to the definition of hydrophobicity proposed by van Oss and Giese [31], a solid is considered hydrophobic when the free energy of interaction between its surface molecules in the presence of water (ΔG_{sws}) is negative; otherwise it will be hydrophilic. It should be pointed out that based on this concept the absolute degree of hydrophobicity or hydrophilicity can be precisely expressed in applicable SI units, which also makes possible the use of mathematical correlations.

With the data presented in Table 4 it was possible to calculate ΔG_{sws} [4,31]. As all the materials have a $\Delta G_{sws} > 0$, it can be said that they are all hydrophilic.

Foam glass was the most hydrophilic support assayed (Figure 2), mainly due to its greater electron donor ability (Table 4). The other carriers were less hydrophilic than foam glass, having a lower electron donancy. As cells are

also very hydrophilic ($\Delta G_{sws} = 29.8$ mJ m^{-2}), the interactions took place between two hydrophilic entities. Some authors [8,29] claim that adhesion is favored between hydrophobic surfaces because a closer contact is attained when the water layer between the two interacting entities is removed, which is also facilitated if at least one of the surfaces is hydrophobic. Although all the surfaces assayed were hydrophilic it was found that a decrease in surface hydrophilicity leads to an increase in cell attachment (Figure 2).

Surface composition

The presence of divalent cations has also been considered important in the adhesion process [26]. Divalent cations such as Ca^{2+} and Mg^{2+} may provide the existence of punctual positive charges on the material surface that can promote the establishment of ionic bridges, resulting in an attractive interaction. From the EDS analysis (Figure 3), it can be seen that a significant amount of Mg^{2+} was found in sepiolite, the microcarrier that exhibited the highest biomass retention capacity.

Methanogenic activity

A good carrier for biomass colonization should not only promote high cell retention but also high biomass activity. Clay and pozzolana supported the highest specific biological activity and had an intermediate level of colonization,

Table 5 Specific methanogenic activity (SMA) of the attached biomass and volumetric methanogenic activity (VMA) of the colonised microcarriers (± 95% confidence interval)

Biomass attached to	SMA[a] (g VFA-COD g $VS_{attached}^{-1}$ day^{-1})[b]	VMA (g VFA-COD $L_{colonized particle}^{-1}$ day^{-1})[c]
Clay	0.329 ± 0.003	4.4 ± 0.4
Foam glass	0.289 ± 0.010	4.4 ± 1.2
Pozzolana	0.340 ± 0.038	5.1 ± 0.5
Sepiolite	0.173 ± 0.007	4.2 ± 1.5
Non-attached	0.348 ± 0.022	–

[a]The methanogenic activity of the attached biomass was determined by measuring the initial rate of volatile fatty acids (VFA) degradation in strict anaerobic batch assays. A volume of 0.26 ml of a VFA solution of acetate, propionate and butyrate with a chemical oxygen demand (COD) ratio 24:34:41 was added to the biomass to make up a working volume of 50 ml with an initial COD of 2.65 g L^{-1} in each vial.
[b]VFA consumption expressed as COD loss per gram of volatile solids attached to each carrier per day.
[c]VFA consumption expressed as chemical COD per litre of colonized particle per day.

whereas the great amount of biomass that colonized sepiolite had a low specific methanogenic activity (50% of the non-attached biomass, Table 5). Differences in the methanogenic activity of the colonised microcarriers were only marginal (Table 5, last column).

The balanced effect observed between the specific methanogenic activity (SMA) and the attached biomass suggests a loss of activity of the biomass immobilized in the highest colonized carriers, probably due to diffusion limitations [2]. On the other hand, the accumulation of inert organic matter, ie extracellular polymers, may indirectly affect the specific activity, overestimating the biomass measured as volatile solids. A similar result was obtained by Garcia-Calderón *et al* [13] who found a similar overall performance for three fluidized bed reactors operating with different microcarriers, in spite of the differences in the attached biomass. Kuba *et al* [15] also reported the existence of an estimated active biomass concentration smaller than the measured attached volatile solids due to accumulation of inert organic matter.

For practical purposes, the importance of the microcarrier characteristics seems to be more relevant for the startup process, which is greatly influenced by the adhesion kinetics. A surface, such as sepiolite will assure a fast adhesion and consequently the start-up time will be reduced. During steady-state operation, manipulation of operating conditions can control unwanted excessive biofilm growth.

Conclusions

The results of this study show that the surface hydrophobicity of the mineral carriers is more important for colonization by the anaerobic consortium than the surface charge. In fact, the biomass retention capacity correlates linearly with the degree of surface hydrophobicity. The thermodynamic theory was not suitable to explain the attachment of the biomass. However, a direct relation was found between a decrease in positive free energy of interaction and an increase in cell retention capacity. Adhesion occurred preferentially in rough surfaces. Porosity was also

a very important surface characteristic, adhesion being enhanced in the carriers with smaller pore size.

From the colonization point of view the best carrier was sepiolite which presented the lowest value of free energy of adhesion, the greatest hydrophobicity, the highest roughness, and the presence of a higher amount of Mg^{2+} at the surface. However, it is also important to take into account the effect of mass transfer limitations due to a great biomass accumulation, which can decrease the biological activity of a well-colonized carrier. In practice this problem can be overcome by manipulating operating conditions such as upflow velocity.

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