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Influence of physicochemical bacterial surface properties on adsorption to inorganic porous supports

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Summary. The effect of the hydrophobicity and the electrostatic charge of bacterial cell surfaces on the initial phase of adsorption to inorganic porous supports with SiO_2 or Al_2O_3 as the main components was investigated. The physicochemical surface properties of various Gram-positive and Gram-negative bacteria were characterized by water contact angle and zeta-potential measurements. The influence of microbial charge on adsorption was investigated by varying the ionic strength of the suspending liquid. The amount of *Escherichia coli* cells adsorbed to Siran and Bi 86 supports increased with increasing electrolyte concentration. The effect of cell surface hydrophobicity on the extent of adsorption was demonstrated at high ionic strength (0.15 M NaCl) where charge effects were reduced. The supports applied in this study promoted the adsorption of hydrophilic bacteria.

Introduction

Immobilization of microorganisms by the natural phenomenon of adsorption to porous supports is often applied in biotechnological processes on a laboratory scale (Klein and Ziehr 1990). Examples are ethanol fermentation with *Zymomonas mobilis* (Weuster et al. 1988), the production of glycerol (Hecker et al. 1990) and L-leucine (Büchs 1988) and the degradation of phenol (Ehrhardt and Rehm 1989; Mörsen and Rehm 1990). The application of porous glass as a support matrix in large-scale reactors for anaerobic waste-water treatment was reported by Aivasidis (1989).

Two theoretical concepts are discussed that describe the influence of physicochemical interactions on microbial adsorption with respect to adhesion: the DLVO theory and the surface free energy approach. Long-range electrostatic interactions (separation distance > 1.5 nm) between charged surfaces are considered by the DLVO theory of colloid stability, developed by Derjaguin, Landau, Verwey and Overbeek (Shaw 1980). According to this theory the total interaction energy between two particles is the sum of the attractive van der Waals forces and the attractive or repulsive elctrostatic forces. The thermodynamic surface energy approach takes into account the surface tension with respect to the surface free energies of the three components involved in the process of adhesion: cell, support and suspending liquid. According to this theory adsorption is favoured when the change in the Gibbs free energy for the process of adhesion is negative. Further details on the energetics of bacterial adhesion are discussed by van Loosdrecht and Zehnder (1990).

Fundamental studies on the influence of physicochemical interactions on microbial adsorption have been published, for example Busscher et al. (1984, 1986), Mozes et al. (1987) and van Loosdrecht et al. (1987a, b). A general survey of various methods applied to characterize the physicochemical properties of microbial cell surfaces is given by Krekeler et al. (1989).

Plane surfaces such as microscope slides and various polymer plates were preferentially used as model supports. In addition, we studied the influence of bacterial surface properties on the adsorption to porous carrier materials applied as immobilization matrices in several laboratory-scale biotechnological processes. Two different kinds of supports were compared: porous glass and ceramic materials with SiO_2 or Al_2O_3 as the main components.

Materials and methods

Microorganisms and culture conditions. The bacterial strains, growth media and cultivation times are listed in Table 1. Variations in cell surface properties were obtained by investigating Gram-positive and Gram-negative organisms of different taxonomic groups according to Bergey (1986).

The composition of media, using Millipore water prepared by a Milli-Q system unless stated otherwise, were as follows. CY: casein-peptone, 3.0 g/l; yeast extract, 1.0 g/l; $CaCl_2 \cdot 2H_2O$, 1.0 g/l (pH 7.2). EBS: casein-peptone, 5.0 g/l; proteose peptone, 5.0 g/l;

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Table 1. Bacterial strains, growth media and cultivation times

No. and name of bacterial strains	Medium	Cultivation time (h)
1 *Sorangium cellulosum* So ce 12	CK112b	48
2 *Bacillus cereus* 28	TSB	12
3 *Staphylococcus aureus* Strain Oxford	EBS	12
4 *Arthrobacter simplex* ATCC 6946	CY	24
5 *Escherichia coli* 72	TSB	12
6 *Alcaligenes eutrophus* JMP 134	TSB	15
7 *Acetobacter xylinum* 284	HP6	24

Alcaligenes eutrophus JMP 134 was a gift from Prof. H.-J. Knackmuss, Institut für Mikrobiologie, Azenbergstrasse 18, W-7000 Stuttgart (FRG), all other bacteria were obtained from the culture collection of Prof. H. Reichenbach, Gesellschaft für Biotechnologische Forschung, Mascheroder Weg 1, W-3300 Braunschweig (FRG)

meat extract, 1.0 g/l; yeast extract, 1.0 g/l (pH 7.0). TSB: tryptic soy broth, 15.0 g/l (Difco, Detroit Mich., USA) (pH 7.0). HP6: $MgSO_4 \cdot 7H_2O$, 1.0 g/l; yeast extract, 1.0 g/l; vitamin B_{12}, 0.5 mg/l; sodium-L-glutamate$\cdot H_2O$, 10.0 g/l; glucose, 5.0 g/l (pH 7.2). CK112b: (a) 0.15% $MgSO_4 \cdot 7H_2O$, 0.2% Tris(hydroxymethyl)aminomethane (Sigma, St. Louis, Mo., USA) (pH 7.2); (b) 0.2% KNO_3, 0.0125% K_2HPO_4; (c) 0.1% $CaCl_2 \cdot 2H_2O$, 8.0 mg/l NaFe(III)-EDTA; (d) 0.5% fructose; (e) 0.1% casein-peptone; (f) 0.1% glycerol. After separate sterilization of components a–f, component b was added first to component a.

The microorganisms were cultivated in two steps. *Sorangium cellulosum* was grown in 250-ml erlenmeyer flasks with 100 ml medium (preculture and culture) in the light (15 W fluorescent tube) at 30° C on a rotary shaker (Lab-Shaker LSR-V, Adolf Kühner, Birsfelden, Switzerland) at 150 rpm. All other bacteria were cultivated at 27° C and 120 rpm on the same rotary shaker. Precultures were grown in 250-ml erlenmeyer flasks (50 ml medium) and cultures in 500-ml erlenmeyer flasks with two baffles (150 ml medium). Cultures were inoculated from precultures to an initial optical density (E_{623} for *S. cellulosum* and E_{546} for all other bacteria) of 0.045.

Cells were harvested at the early stationary phase (for cultivation time see Table 1) by centrifugation (15 min at 6° C and 7700 *g*, Suprafuge 22, Heraeus Sepatech, Osterode, FRG), washed three times with deionized water and resuspended in solutions and at cell concentrations depending on the experiment. *S. cellulosum* was harvested after an incubation time of 48 h (exponential phase) to prevent cell aggregation in older cultures. All experiments were carried out with freshly harvested microorganisms.

Supports. Inorganic porous carrier materials with SiO_2 or Al_2O_3 as the main components were applied. They are characterized in Table 2. The carriers were used after washing with deionized water and drying in a microwave oven (Micromat 112 Z, AEG, Frankfurt, FRG). No further treatment was applied. Particle densities of the carriers were measured with a pycnometer (volume 10 ml) by weight.

Zeta potential measurement. Microorganisms were suspended at a concentration of about 2×10^8 cells/ml in NaCl solutions of varying ionic strength (0.001–0.150 M). The pH was adjusted just before zeta potential measurement at 25° C (Peltier temperature control). The instrument used was a ZetaSizer 3 (Malvern Instruments, Malvern, Worcs., UK; software version 1.31, 1989) equipped with a quartz capillary (diameter 4 mm) as electrophoresis cell. A voltage of 120 V was applied for 20 s. To prevent sample heating at ionic strengths above 0.001 M the cell temperature was additionally controlled by an external thermostat and the voltage was switched off periodically. An "on time" of 25 and an "off time" of 225 (in terms of modular cycles) were chosen for each measurement cycle. Zeta potentials were calculated from the electrophoretic mobilities by the equation of Smoluchowski (Shaw 1980). Measurements were usually carried out in triplicate for two independant cultures. The standard deviation of the mean zeta-potential values was in the range of ± 2 mV.

Electrostatic interaction chromatography (ESIC). Measurements were carried out in duplicate as described by Mozes et al. (1987). Instead of using a hydrophobic interaction chromatographic support, positively charged DEAE-Sepharose CL-6B (Sigma) and negatively charged Carboxymethyl(CM)-Sepharose Cl-6B (Pharmacia, Uppsala, Sweden) were applied at pH 3.0 and 7.0 (no addition of salt).

Contact angle measurement. Sample preparation was performed as described by Mozes and Rouxhet (1987) and van der Mei et al. (1987). The filters with the bacterial layers were air-dried at room temperature overnight. Contact angles were determined at room temperature by the sessile drop technique, using water as the wetting agent. Measurements were performed with equipment enabling automatic determination of the contact angle with a video camera and a computer system (System G 30, Krüss, Hamburg, FRG, software version 6.1, 1986). For each bacterium the water contact angle of two independently prepared cultures was determined from about 30–40 measurements.

Table 2. Characterization of the porous supports

Support	Company	Mean particle diameter (mm)	Mean pore diameter (μm)	Composition		Density	Porosity
				SiO_2 (%)	Al_2O_3 (%)	(g/cm^3)	(%)
Siran	Schott[a]	1.0–2.0	60–300	≈75	—	2.4769	55–60
Bi 54	Otto Feuerfest[b]	1.0–2.5	20	41	54.3	2.7264	77
Bi 54 S		1.0–2.5	24	41	53.5	2.8782	78
Bi 54 T		1.0–2.5	—	—	—	2.6228	—
Bi 85		1.0–2.5	14	31	67.0	2.9047	72
Bi 86		1.0–2.5	22	35	62.0	3.2109	70
Stuttgarter Masse	Schumacher[c]	2	—	90	—	2.5643	40–60

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[c] Schumacher, Zur Flügelau 70,W-7180 Crailsheim, FRG

Adsorption experiments in shaking flasks. Microorganisms were suspended in solutions of "high" (0.9% NaCl, pH 7.0) or varying ion concentrations (0.001–0.150 M NaCl) at a concentration of 5×10^8 cells/ml. Carrier material (3 g) and 30 ml cell suspension were slowly agitated (70 rpm) in a 100-ml erlenmeyer flask on a rotary shaker (Lab-Shaker LSR-V) at 30° C. After incubation for 28 h, the amount of fixed cells was determined. The supernatant was decanted and the carriers were gently rinsed three times with about 50 ml deionized water to eliminate non-adsorbed cells. The ionic strength of the washing solution did not significantly influence the amount of cells adsorbed. The carriers were dried in a microwave oven (45 min, ca. 250 W, Micromat 112 Z) and ground for about 2 min (Fritsch pulverisette, Idar-Oberstein, Germany). The powdered material was then suspended in 10 ml of 0.5 M NaOH and incubated for 2 h at 60° C to disintegrate the cells and extract the protein. Then the carrier material was separated by centrifugation (5 min at 11000 rpm; centrifuge 5415 Eppendorf, Hamburg, Germany) and the protein of the supernatant was determined according to Lowry et al. (1951).

Protein content and cell number (determined on a Thoma counting chamber) were correlated with the corresponding values obtained from a suspension culture to calculate the number of cells adsorbed per volume of carrier from the amount of protein for each adsorption experiment.

Results and discussion

Microbial surface charge

Two different methods were applied to characterize bacterial surface charge: zeta potential measurement and ESIC. Figure 1 shows the relationship between zeta potential and pH at low ionic strength (0.001 M NaCl) for *Bacillus cereus* and *Alcaligenes eutrophus,* two of six microorganisms investigated in our laboratory. At physiological pH they both carry a net negative zeta potential, explicable by an excess of negatively charged acidic ionogenic groups at the cell surface. At low pH, when dissociation of acidic groups is suppressed, the zeta potential of *B. cereus* became positive due to the presence of ionized basic groups. In comparison, *A. eutrophus* and also *Arthrobacter simples* (data not shown) were almost uncharged at low pH values, which could be explained by the absence of detectable ionized basic groups on the outer cell surface.

This diverse behaviour, already described by Marshall (1976), was also observed with ESIC. Figure 2 shows the retention of *B. cereus* and *Alcaligenes eutrophus* in charged gels at different pH values. Independent of pH, both microorganisms were completely retained on DEAE-Sepharose (anion exchanger), i.e. the bacteria carried negatively charged groups on their surfaces. In comparison, retention of the bacteria on CM-Sepharose (cation exchanger) was different. *A. eutrophus* was eluted from the gel to a high degree at both pH values, i.e. this bacterium only showed a small amount of positively charged surface groups. In contrast to this, the retention of *B. cereus* was very high at pH 3.0, due to a high amount of positively charged groups. One should consider that different parameters are determined by the two methods applied. With ESIC the interaction between charged regions on the cell surface and the gel is measured while the zeta potential represents the net charge at the plane of shear.

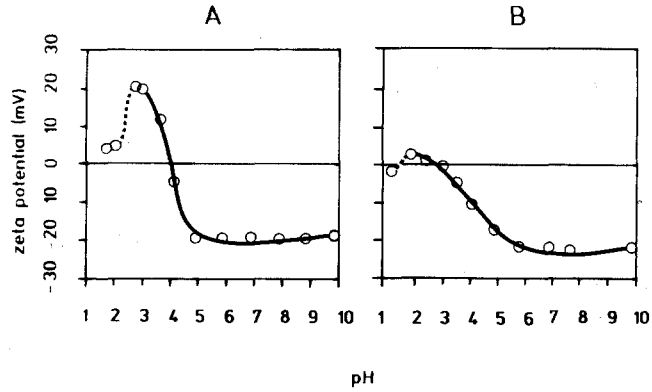

Fig. 1. Influence of pH on the zeta potential of *Bacillus cereus* (**A**) and *Alcaligenes eutrophus* (**B**): measurements in 1 mM NaCl. The decrease in zeta potential at low pH (*dashed line*) is not necessarily due to reduced charged groups on the cell surface but can also originate from an increase in the ionic strength

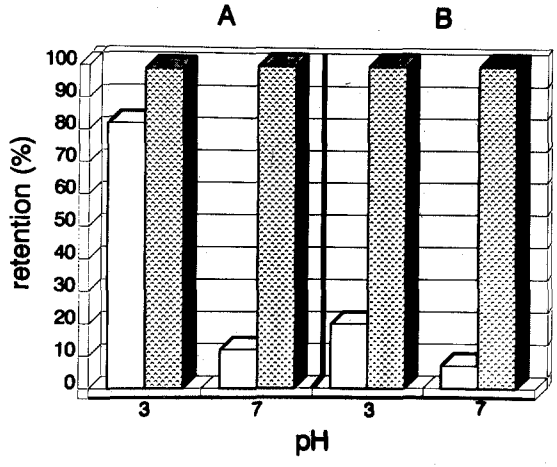

Fig. 2. Electrostatic interaction chromatography of *B. cereus* (**A**) and *A. eutrophus* (**B**): □, carboxymethyl(CM)-Sepharose; ⊠, DEAE-Sepharose

The electric potential at microbial cell surfaces is generated by ionized basic and acidic groups. Amory et al. (1988) and van Haecht et al. (1982) concluded from X-ray photoelectron spectroscopy data, that the negative charge of microorganisms is mainly due to deprotonated phosphate groups that are partly neutralized by protonated amino groups.

Microbial surface charge is characterized by various parameters: isoelectric point (Büchs et al. 1988; van Haecht et al. 1982), zeta potential and electrophoretic mobility at pH 4.0 (Amory et al. 1988) or at a more physiological pH (Büchs et al. 1988, Abbott et al. 1983, van Loosdrecht et al. 1987b, Champluvier et al. 1988). The corresponding parameters of the bacteria investigated were determined from the pH zeta-potential curves. They are listed in Table 3. No fundamental differences between Gram-positive and Gram-negative bacteria could be detected. In this study, cell surface charge is characterized by the zeta potential at pH 7.0 because adsorption experiments were also performed at

Table 3. Parameters characterizing bacterial surface charge

Microorganism	pI	Zeta potential			Gram reaction
		(1 mM NaCl)		(150 mM NaCl)	
	(pH)	pH 4.0 (mV)	pH 7.0 (mV)	pH 7.0 (mV)	
Alcaligenes eutrophus	2.9	− 8.33	−19.65	+ 0.76	−
Arthrobacter simplex	<1.5	−20.64	−18.12	− 8.06	+
Bacillus cereus	4.1	− 1.42	−23.79	− 9.68	+
Escherichia coli	2.3	−32.15	−31.30	−13.70	−
Sorangium cellulosum	3.6	−19.94	−33.31	−12.20	−
Staphylococcus aureus	3.3	−18.61	−29.27	− 2.08	+

Table 4. Characterization of bacterial cell surface hydrophobicity by water contact angle measurement

Classification	Microorganism	Contact angle[a] (°)	y_{CV} (mJ/m^2)	y_{CL} (mJ/m^2)
Very hydrophilic	*Alcaligenes eutrophus*	17.9±2.0	69.14	0.19
Hydrophilic	*Acetobacter xylinum*	20.4±2.0	68.21	0.30
	Escherichia coli	22.5±0.5	67.41	0.43
	Arthrobacter simplex	27.9±3.7	64.96	0.94
Hydrophobic	*Staphylococcus aureus*	34.5±2.4	61.60	1.90
	Bacillus cereus	45.1±1.1	55.87	4.61
	Sorangium cellulosum	52.3±2.5	51.79	7.16

y_{CV}, cell surface free energy; y_{CL}, cell-liquid interfacial free energy
[a] Mean values ±standard deviation

this pH. The variability among the bacteria could be easily detected and variations of pH in the range 6.0–10.0 did not influence the zeta potential significantly (Fig. 1).

Cell surface hydrophobicity

Various methods have been compared to determine bacterial hydrophobicity: hydrophobic interaction chromatography, bacterial adherence to hydrocarbons and contact angle measurement (Krekeler 1990). Among these, measurement of the water contact angle has been found to be the best method for characterizing cell surface hydrophobicity and detecting variations among different bacteria.

We have classified the bacteria in three categories of hydrophobicity (Table 4): very hydrophilic (contact angle <20°), hydrophilic (contact angle 20°–30°) and hydrophobic (contact angle >30°). With the exception of the myxobacterium *S. cellulosum* the Gram-negative organisms had in general lower contact angles than the Gram-positives. Burchard et al. (1990) also suggested hydrophobic surface properties for several other gliding bacteria. They seemed to be due partly to cell envelope proteins. Gram-positive organisms such as *B. cereus* might be rendered hydrophobic by cell wall lipoteichoic acids. In the case of *Corynebacterium glutamicum* there seems to be a strong influence of the phosphate concentration of the medium (Büchs et al. 1988). Hydrophilic cell surface components can be the *O* side chains of the lipolysaccharides of Gram-negative bacteria or carbohydrate capsules (Nikaido and Nakae 1979).

Two theoretical approaches are applied for the calculation of surface free energies by measuring contact angles: the equation of state (Neumann et al. 1974) and the geometric mean approach (Busscher et al. 1984). Van Loosdrecht et al. (1987a) reported almost identical results using both approaches for various bacteria. Table 4 shows the surface and interfacial free energies of the microorganisms studied. They were determined via the equation of state from tables published by Neumann et al. (1980a).

Due to the fact that microbial contact angles are correlated to the surface and interfacial free energies it is sufficient to characterize cell surface hydrophobicity by the contact angle measured. In addition to our conclusion the applicability of surface and interfacial free energy calculations is still considered controversial. Both approaches are based on a non-thermodynamic assumption, needed for the determination of solid/liquid interfacial tensions from the surface tensions of the interacting phases (solid/vapour and liquid/vapour) (van Loosdrecht et al. 1987a). Besides, there are experimental difficulties in qualifying the validity of such data (Mozes and Rouxhet 1987). The main problem is that the cellular films have to be dried before contact angle measurement. Therefore conformational changes or denaturation of the cell surface should also be taken into consideration.

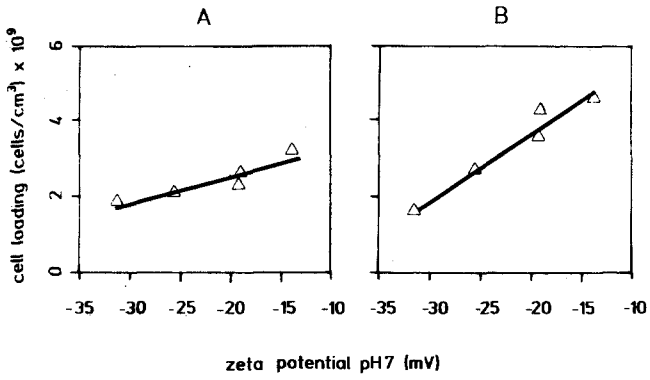

Fig. 3 A, B. Influence of the zeta potential of *Escherichia coli* on the adsorption to porous supports. **A** Siran. **B** Bi 86. The experiments were carried out in shake flasks; electrolyte concentration 0.001–0.150 M NaCl. The standard deviation in the adsorption experiment was in the range ±3%)

Microbial surface charge and adsorption

Electrostatic interactions are classified as long-range interactions, therefore their influence on microbial adsorption is significant by enabling a bacterium to approach a surface or not. To investigate the influence of microbial charge on the adsorption to porous supports, adsorption experiments were performed in shake flasks. The number of cells adsorbed was calculated from the amount of protein determined after an incubation time of 28 h. Adsorption kinetics in shake flasks displayed that after 28 h the quantity of cells adsorbed could not be further increased. Carrier fixed protein was determined directly instead of calculating it from the decrease in protein content of the supernatant. This method has the advantage of distinguishing between adsorbed cells and those included in the inter-particle or pore space.

A linear correlation between microbial zeta potential (*Escherichia coli* was selected as test organism) and cell loading on porous supports could be calculated ($r=0.92$ for Siran, $r=0.98$ for Bi 86). Adsorption increased with decreasing negative zeta potential (Fig. 3), which was achieved by varying the ionic strength of the suspending liquid from 0.001 to 0.150 M NaCl. By this method the electrostatic repulsive forces between the surfaces of the same charge (bacteria and carriers) were reduced and adsorption was enhanced.

It has been shown by polyelectrolyte titration, adsorption of the positively charged dye crystal violet and zeta-potential measurement of powdered carrier material, that the supports applied in this study were negatively charged above pH 3.0 (Krekeler 1990). However, only qualitative results could be obtained. Increasing the ion concentration always resulted in a less negative microbial zeta potential, as can be seen from Table 3. This can be attributed to the reduced thickness of the diffuse part of the electrical double layer. The determining factor is the ionic strength, i.e. the valency and the concentration of the electrolyte.

In biotechnological processes where adsorption of microorganisms to negatively charged supports is intended, the reduction of the repulsive electrostatic forces seems to be an important factor in promoting adsorption. Adsorption is favoured by increasing the ionic strength of the suspending liquid as pointed out in Fig. 3, e.g. by using media with high ion concentrations. The modification of the support or microbial surfaces is another possibility. Champluvier et al. (1988) increased the adhesion of *Kluyveromyces* cells to various supports (glass, polycarbonate, polystyrene) by treating the microorganisms or the supports with polycations (chitosan). Büchs et al. (1988) enhanced the adsorption of phosphate-saturated cells of *C. glutamicum* to Siran by treating the porous glass with DEAE-dextran and Triaminosilane.

At high ion concentration (0.9% NaCl) the linear correlations between the zeta potentials of the bacteria applied in this study and cell loading were not significant ($r \leq 0.64$). This can be interpreted theoretically to indicate that adsorption is also influenced by factors other than microbial charge.

Cell surface hydrophobicity and adsorption

In addition to bacterial surface charge the influence of cell surface hydrophobicity on the process of adsorption was investigated. At high electrolyte concentration (0.9% NaCl) a linear correlation between cell loading and microbial contact angle could be calculated for all carriers and bacteria tested, with correlation coefficients ranging from −0.89 (Stuttgarter Masse) to −0.98 (Bi 54 S). The lower the water contact angle, i.e. the

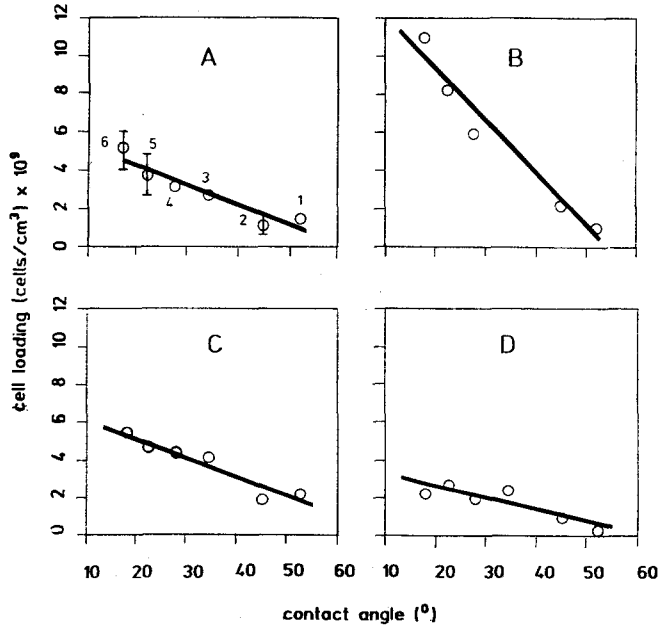

Fig. 4 A–D. Influence of bacterial contact angle on the adsorption to porous supports. **A** Siran. **B** Bi 54 S. **C** Bi 86. **D** Stuttgarter Masse. The experiments were carried out in shake flasks; electrolyte concentration 0.9% NaCl. For reference numbers see Table 1. The *bars* indicate the average standard deviation in the adsorption experiments

more hydrophilic the bacteria, the higher was the amount of cells adsorbed per volume unit of carrier (Fig. 4). The same trend was observed from adsorption kinetics performed in fixed bed columns with Siran, Bi 54 and Bi 86 as supports (Krekeler 1990). No influence of the changed experimental system (shake flasks or fixed-bed columns) on the correlation was found.

Differences between the various suppors were only observed concerning the number of cells adsorbed per volume unit of carrier material, but no correlation with the chemical composition of the supports was found. The larger pore diameters of Siran compared to the ceramic materials did not enhance the quantity of bacteria adsorbed.

The change in the Gibbs free energy for the process of adsorption (ΔG) was calculated from the interfacial free energies between the systems involved (microorganism, support, suspending liquid) in order to discuss hydrophobicity and cell loading on a thermodynamic basis. While the surface free energies of the bacteria could be determined (Table 4), it was technically not possible to measure the contact angles of the supports. Therefore hypothetical values for the surface free energies of the carriers were assumed to determine ΔG values according to Neumann et al. (1980b). Based on the surface free energy of water as the suspending liquid ($\gamma_{LV} = 72.5$ mJ/m^{-2}), increasing cell loading concomitant with decreasing microbial contact angle was found for supports with surface free energies higher than the suspending liquid ($\gamma_{SV} > \gamma_{LV}$). We interpret the correlations presented in Fig. 4 by the fact that the porous inorganic carriers applied in this study have a higher surface free energy than water. This is supported by Busscher et al. (1986) who reported a surface free energy of 109 mJ/m^2 for glass.

With high-energy supports ($\gamma_{SV} > \gamma_{LV}$) the change in the free energy for the process of adsorption was positive, i.e. microbial adsorption should not be energetically favourable under these conditions. Adsorption despite positive ΔG values was also reported by Busscher et al. (1986). They found that positive values were associated with reversible adsorption and negative values led to irreversible adsorption. High-energy surfaces seem to promote the adhesion of high-energy bacteria and vice versa, i.e. the organisms prefer to adsorb to supports with surface free energies most similar to their own.

It has been shown that microbial hydrophobicity is influenced by several parameters such as phosphate concentration of the medium (Büchs et al. 1988), carbon source (Neufeld et al. 1980), growth rate (van Loosdrecht et al. 1987b), and growth temperature (Hazen et al. 1986). In this study the change in cell surface hydrophobicity through the growth cycle of *S. cellulosum* was investigated in shake cultures. This bacterium is of biotechnological interest due to the formation of the secondary metabolite Sorangicin A, a macrolide-polyether antibiotic (Jansen et al. 1985). Figure 5 shows that, at the beginning of cultivation, cell surface hydrophobicity increased with culture time. The changes in cell surface composition or structure of *S.*

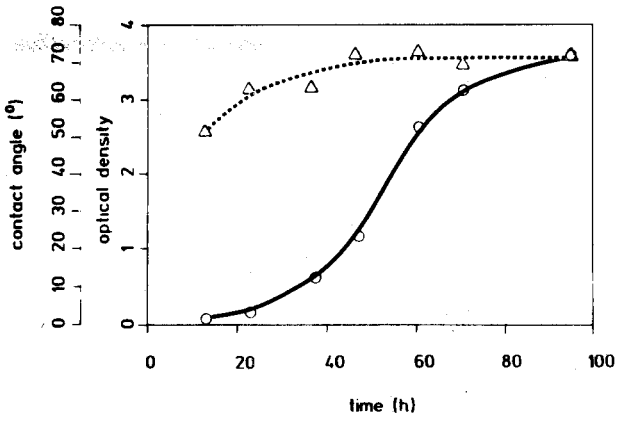

Fig. 5. Contact angle of *Sorangium cellulosum* as a function of cultivation time: O–O, optical density (E_{623}); Δ– –Δ., water contact angle. Bacteria were cultivated in shake flasks

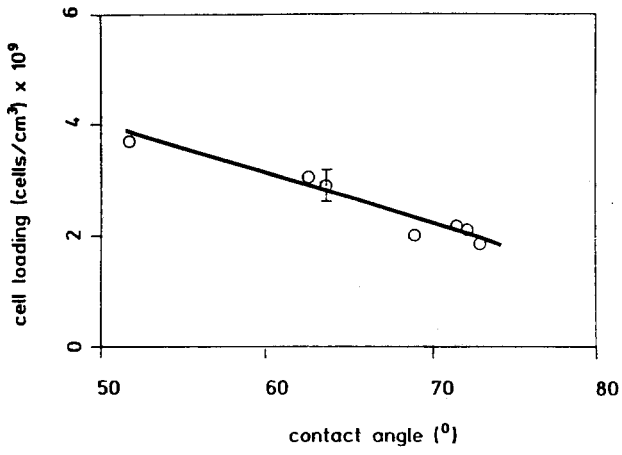

Fig. 6. Adsorption of *S. cellulosum* on Bi 86 as a function of bacterial contact angle. The experiments were carried out in shake flasks; electrolyte concentration 0.9% NaCl. The *bar* indicates the average standard deviation in the adsorption experiments

cellulosum resulting in an altered hydrophobicity have not yet been studied. An increased hydrophobicity with increasing dilution rate, i.e. increasing growth rate in chemostat cultures of several bacteria was reported by van Loosdrecht et al. (1987b).

The effect of the growth-phase-induced change in cell surface hydrophobicity on the extent of adsorption of *S. cellulosum* to the support Bi 86 was studied; the results are shown in Fig. 6. Cell loading increased with decreasing water contact angle, i.e. the trend reported above was confirmed. We suppose that the adsorption of the hydrophobic bacterium *S. cellulosum* could be enhanced by supports with lower surface free energies than those tested. The fact that cell surface hydrophobicity varied with culture time could probably be used to control and modify the adsorption behaviour of *S. cellulosum* by changing the dilution rate of the chemostat culture.

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