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Sorption isotherms and kinetics in the primary biodegradation of anionic surfactants by immobilized bacteria: II. *Comamonas terrigena* N3H

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Abstract

Comamonas terrigena N3H was immobilized by covalent linking on silanized inorganic supports and by physical entrapment of cells within calcium alginate beads and reticulated polyurethane foam. Both entrapped cells were efficient for the primary biodegradation of the anionic surfactants dihexyl sulphosuccinate (DHSS) and dioctyl sulphosuccinate (DOSS), furthermore, exhibiting, in the case of polyurethane immobilized cells, a positive fractionating effect of the substrate by adsorption onto the polymer matrix. The overall kinetics for the surfactant removal from water were well-fitted to a biphasic process, a rapid passive sorption step of the surfactant onto the cell-loaded support and the intrinsic primary biodegradation slower step, both acting synergically.

Keywords: Anionic surfactant; Dioctyl sulphosuccinate; Dihexyl sulphosuccinate; Sodium dodecyl sulphate; Dodecylbenzene sulphonic acid; Immobilization; *Comamonas terrigena* N3H; Polyurethane; Alginate; Sorption isotherm; Primary biodegradation; Kinetics

1. Introduction

Predominant among the surfactants are those possessing strongly anionic hydrophilic groups such as sulphonate ($-C-SO_3$), ester sulphate ($-C-O-SO_3^-$) or dialkyl sulphosuccinate ($-O-CO-CHSO_3^--CH_2-CO-O-$). The group of anionic surfactants represents 65% of global production of synthetic surfactants which counted for 26–30 million tons in 1993. However, future prognoses do not predict significant changes in these figures; extensive usage of surfactants leads to their substantial increase in surface and waste waters with undesirable effects on microflora and also on the processes associated with waste-water treatment. For this reason, there is an urgent need to search for new possibilities of enhanced degradation of surfactants. Bacterial biodegradation of pollutants has proved to be very efficient especially in combination with immobilization methods [1]. Immobilization of bacterial cells offers several advantages such as the prevention of cell losses in

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continual processes, and allows working with high cell densities. Furthermore, immobilized microorganisms are more resistant to adverse effects occurring during the degradation processes like changes of physico-chemical parameters such as pH, temperature, fluctuations of substrate concentration, presence of toxic substances, etc. [2–4].

One of the major environmental factors influencing microbial physiology and cell growth is nutrient availability. Most natural environments are characterized by low bioavailability of nutrients [5]. In general, microorganisms do not respond to depletion of nutrients by simply arresting all metabolic activity and stopping growth. Instead, they carry out starvation-induced programs that allow them to exit from the cell cycle, maintain viability during starvation and resume growth when nutrients become available [6]. There are many papers on the effect of starvation on morphology [7,8], alterations of the cell surface structures [9,10], proteosynthesis and genetic regulation [11–16], formation of cross-protection against heavy metals such as Cd^{2+} [17–19], but only very few articles deal with problems of influence of starvation stress on the degradation of xenobiotics [20].

Alginate is most widely used as a polysaccharide matrix for the immobilization of viable cells [21,22]. Entrapment of cells within spheres of Ca^{2+}-alginate gels ensures a very mild immobilization procedure and, moreover, this system secures the minimization of viability and activity losses [23]. Immobilization by entrapment methods also allows working with high cell densities in comparison with attachment methods [24,25].

The aim of the present study was to investigate the performance of immobilized *Comamonas terrigena* N3H cells for surfactant primary biodegradation and to find some stress factor (starvation) which can accelerate the biotransformation capability of the used strain. The kinetics of the biochemical process was assessed for sulpho-succinate surfactants and different immobilized *C. terrigena* N3H.

2. Materials and methods

Details on materials, such as substrates (anionic surfactants sodium dodecyl sulphate (SDS), dioctyl sulphosuccinate (DOSS), dihexyl sulphosuccinate (DHSS) and dodecylbenzene sulphonic acid (DBS)); supports of the immobilized cells (inorganic supports, polyurethane reticulated foam), bioreactors (packed bed); instruments such as the Zeiss Digital Scanning Microscope 949; methods for measuring the moisture and biomass contents; kinetic assays for measuring the surfactant biodegradative activities of free and immobilized and for discriminating between sorption and biodegradation of the surfactants; the methods of immobilization of cells (covalent linking to silanized inorganic supports, biofilm coating, physical entrapment in reticulated polyurethane) and curve fitting of the data can be checked in the previous paper [26].

2.1. Microorganisms

The organism chosen for the present study was *C. terrigena* N3H (NCIMB 11753) (Gram negative rod bacteria), originally isolated from a site with elevated levels of surfactant pollution and after the screening process towards surfactant biodegradation. This versatile microorganism is able to utilize dialkyl sulphosuccinate surfactants as sole sources of carbon and energy and degrading them. These cells were batch-grown for 48 h at 28°C (150 rpm) in YEP medium containing 2.0 g Peptone, 0.2 g yeast extract, 0.2 g NH_4NO_3 in 1 l of distilled water (pH 7.4). When the growth curve had reached steady-state, the bacteria were collected by centrifugation (3800 rpm for 20 min, 4°C) and were washed with a mineral medium consisting of 5.4 g NH_4Cl, 0.4 g KH_2PO_4, 0.4 g $MgSO_4$, 50 mM Tris/HCl (pH 7.4). Following this treatment, they were stored in slant agar (meat–peptone agar) for 1 month, 4°C or in glycerine for 1 yr at −20°C.

Starvation experiments were performed in a 50-ml TP medium containing (g l^{-1}) in distilled water: NH_4Cl 5.4, KH_2PO_4 0.4, $MgSO_4$ 0.2, Tris 6. The pH was adjusted to 7.4. Equal aliquots of the cells (each variant contain cells harvested from 50 ml of YEP medium with $OD_{575} = 0.35$) were resuspended in 50 ml of TP medium. The surfactant (100 mg) was added to the system after different chosen intervals of starvation and than assayed by the MBAS method.

2.2. Supports of immobilized cells

Powdered sodium alginate (Protanal LF 20/60, Drammen, Norway) and powdered sodium pectate was a gift from the Institute of Chemistry of the Slovak Academy of Sciences (Bratislava).

Immobasil R grade 2 Raschig rings (Ashby Scientific Coalville, LE67 3FB, UK) (4 mm × 4 mm, 1.5 mm internal diameter) are made from silicone rubber, with a sponge-like structure and randomly interconnecting pores and are non-toxic. The pores are largely uniform in size (100 μm) and give a void volume of greater than 40%, creating a usable surface area of 50 m^2 l^{-1}. The specific gravity is 1.065 g ml^{-1}, making it suitable for use in stirred tank reactors. Such properties of this new commercially available support makes it a good candidate for the physical immobilization of cells.

2.3. Immobilization in alginate beads

Alginate, the major structural polysaccharide of marine brown algae, forms a viscous solution in the presence of monovalent cations even at low concentrations. In contrast, in the presence of divalent cations, especially calcium, gelation occurs. Increasing the concentration of the alginate solution, and to a certain extent that of the $CaCl_2$ solution, will result in more tightly 'cross-linked' gels. The calcium ions may, if necessary, be replaced by other divalent cations such as barium. It is also important that chelating agents, such as phosphate and citrate, be avoided as these disrupt the gel structure by binding calcium.

Aqueous samples of cells were mixed with a water solution of sodium alginate to make a final concentration of 3% alginate (v/v). This suspension was injected through a syringe needle with air flow into a precipitation bath containing a 2% solution of $CaCl_2$. The diameter of the resulting beads depends on the rate of air flow. Prior to use they were kept in $CaCl_2$ for 1 h.

The alginate beads, prepared as mentioned were hardened by the following procedure: three volumes of 2% (v/v) polyethyleneimine (PEI) in 0.05 M $CaCl_2$ were added to one volume of alginate beads and kept under agitation for 12 h. After removal of excess PEI by washing (3 ×) with distilled water, beads were thoroughly mixed with two volumes of 1% (v/v) glutaraldehyde for 1 min and finally washed 6 × with distilled water [27].

3. Results and discussion

With a view to obtaining an optimum biodegradative activity towards anionic surfactants [dioctyl sulphosuccinate (DOSS), dihexyl sulphosuccinate (DHSS)] by immobilized *C. terrigena* N3H cells, different inorganic and organic supports (sepiolite, ceramic beds, reticulated polyurethane foam, silicone rubber rings, calcium alginate beads) and cell immobilization methods (silanization, biofilm growth, physical entrapment, correticulation) were screened, characterized and evaluated for stability and suitability for large scale use.

3.1. Enhancement of biodegradative activity by starvation of cells

This strain exhibits a very interesting attribute especially from the practical point of view. The strain is able to retain the biodegradation ability after depletion of surfactant for

weeks. The concentration changes of xenobiotics in waste-water treatment plants is a common problem especially during the weekends or vacations when the waste-water production is lowered. For this reason it is necessary to apply such microorganisms which are able to overcome this period of time.

Scientific reports dealing with the possible effect of starvation on biotransformation or biodegradation are very scarce. Truex et al. [20] studied the effect of starvation on quinoline degradation by *Pseudomonas cepacia* finding that long-time starvation (60–80 days) leads to more efficient conversion of quinoline to degradation products with quinoline concentrations of 39 and 155 mM. However, the long-time starved cells required 3–5 × longer time for induction of quinoline degradation in comparison with short-time starved cells (2 days).

Based on our results, it was confirmed that starvation influenced the metabolic activity of bacterial cells. It is evident that starvation positively affected the biotransformation potential of *C. terrigena* cells towards DHSS. Following the experimental protocol for the starvation of microorganisms (see above), it was found that the protein content in cells remained stable in each variant during both the starvation period and the time period for complete biotransformation of DHSS. The average protein concentration value was 37 mg l^{-1}. From this data, the specific transformation rate for each starvation variant was calculated (Fig. 1). All variants exhibited improved biotransformation ability in comparison with nonstarved cells. The highest biotransformation rate was observed in the case of 16 h of starvation, representing 144% of the nonstarved culture. Starvation for more than 16 h leads to gradual decrease of specific biotransformation rate.

The study of starvation effect is important for potential biodegraders because the depletion of nutrients is a common stage occurring with bacteria in natural environment and in the small waste-water treatment plants. Only strains which are able to overcome the depletion of nutrients

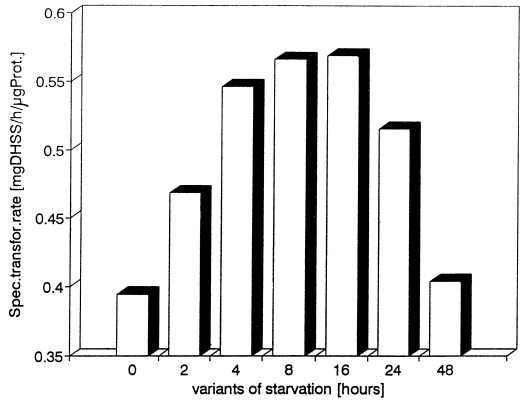

Fig. 1. Effect of starvation time on the specific transformation rate of DHSS.

with no injury or, in the better case, with an improved biodegradation ability can be effective and suitable for introduction in waste-water treatment processes.

3.2. C. terrigena N3H cells immobilized on inorganic supports: immobilization performance

Taking into account the effective immobilization performance for *Pseudomonas* C12B by the silanization method (without reduction of the Schiff base) on sepiolite and the good activities of such immobilized cells for removing SDS and other anionic surfactants [26], one preliminary test was carried out following identical covalent immobilization of *C. terrigena* N3H cells on sepiolite, but without finding any DHSS degrading activity (data not shown). Following Proksova et al. [28], the viability of the cells is not a prerequisite for DHSS primary biodegradation.

3.3. C. terrigena N3H cells biofilms

Preliminary assays with *C. terrigena* N3H bound to commercial polyurethane foam and silicone rubber Immobasil rings by passive immobilization showed significant cell biofilms but the cell loading was not very high and some washout of cells had occurred. However, a significant degree of DHSS removal was observed

(data not shown). This simple method of immobilization was discarded in favour of others (cell entrapment in polyurethane foam, correticulation in calcium alginate gel) due to the higher degree of cell loading and reproducibility of immobilization resulting from this procedure.

3.4. C. terrigena N3H cells entrapped in calcium alginate

3.4.1. Diffusional limitations

An important factor to be considered is the diffusion restriction for substrate (products) and/or oxygen through the matrix employed as a carrier for immobilization of viable cells. In the case of growing cells, the intrinsic growth rate of immobilized cells should be the same as for free cells where diffusional limitation is absent [29]. Diffusion throughout the gel depends on different factors such as the cell density and particle size [2–4]. Moreover, the alginate concentration hinders oxygen diffusion as well. With higher gel concentration, the porosity of the gel is lowered and, hence, the diffusivity and uptake of oxygen is reduced [30]. The diffusion barrier depresses growth more in immobilized microorganisms with high respiratory activity and correspondingly high oxygen demand than in those with low reaction velocity [31].

In our previous paper [26], it was shown that for *Pseudomonas* C12B cells entrapped in polyurethane reticulated foam, internal and external diffusional limitations to substrate and/or products during surfactant conversion were found to occur as a result of a cell (enzyme) within a support particle being exposed to conditions that differ greatly from those of the bulk solution. It was assumed that this diffusion limitation for substrate, products or oxygen through the alginate gel matrix, carrier for immobilization of viable *C. terrigena* N3H cells, should also occur. To estimate the diffusion limitation, experiments with the cells immobilized in alginate beads of diameters ranging from 0.8 mm

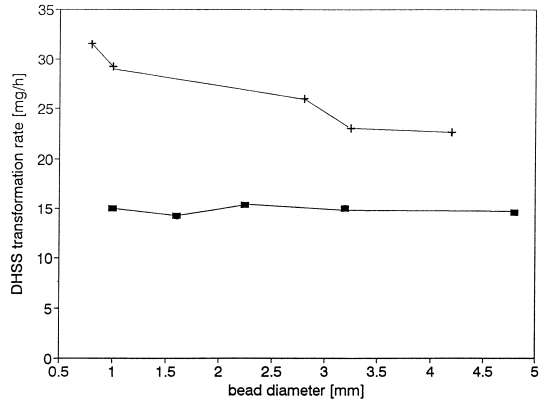

Fig. 2. Effect of bead diameter on the DHSS transformation at two biomass loads (600 mg wet weight (+), 60 mg wet weight (■)).

up to 4.8 mm were carried out. Furthermore, the effect of cell density (60 mg and 600 mg wet weight) on the diffusion was investigated (Fig. 2). It is evident that, in the case of low biomass, there is no difference in DHSS biodegradation rate between smaller and larger particles. On the other hand, when 10 × higher biomass loading was employed, the biodegradation rate curve showed a decreasing course depending on the increase of bead diameter. It can be predicted that by using even smaller beads the increase of biodegradation rate should be sharper. Considering that the oxygen penetration depth lay between 50–150 μm [32,33], the ideal particle size can be obtained by multiplying these values by 2. However, preparation of such smaller beads requires special equipment and technology [34].

3.4.2. Sorption of DHSS and biodegradative activity of alginate entrapped cells

Due to amphiphilic properties (hydrophobic and hydrophilic moieties in one molecule) of surfactants, in general, they tend to bind to surfaces or to interfaces. The amount of adsorpted surfactant depends on the physicochemical character of the surface, number of charged groups and pH [35]. As expected, the adsorption of DHSS onto alginate beads loaded with heat-killed cells and onto empty beads was

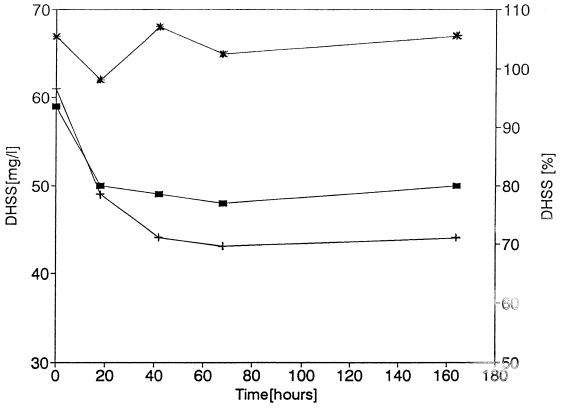

Fig. 3. Estimation of passive adsorption of DHSS onto alginate beads (control variant (∗), empty beads (■), heat-killed immobilized cells (+)). See text for explanation.

different (Fig. 3). Adding 10 ml of alginate beads into 50 ml of buffer led to the 20% dilution of the solution. The same removal of surfactant was observed in the case of cell-free alginate beads. Thus the alginate did not affect the absorption of surfactant molecules. From this viewpoint the alginate gel is appropriate for the study of surfactant biodegradation in comparison with polyurethane foam where the support exhibits a very high affinity towards DHSS (see below). The immobilized dead cells show roughly 10% higher amounts of removed surfactant in comparison to empty beads, which cannot be explained by a simple dilution effect. Thus, on the surfaces of cells there are sites where the surfactant can be bound. It is questionable how undesirable this phenomenon is when we assume that the first step of biodegradation is the adsorption of chemicals on the cells surfaces with a subsequent transport into the cell where they can be attacked by intracellular enzymatic systems.

3.4.3. Effect of biomass loading

The biomass loading clearly affects the biodegradation rate (Fig. 4). With higher concentration of immobilized biomass higher biodegradation rate of DHSS is obtained, reaching a value of 37 mg h^{-1}. However, the difference when 964 mg vs. 482 mg wet weight of cells was immobilized is negligible. As there are some additional effects involved, e.g., the negative influence on gel strength, the optimum of biomass loading towards DHSS degradation was around 482 mg (wet weight) cells.

3.4.4. Effect of hardening procedure on the stability of alginate gels

One of the main disadvantages of alginate gels is its low physical stability in the presence of phosphate or citrate ions due to the liberation of cross-linked Ca^{2+} ions from the gel structure leading to destabilization of the beads [25]. In our experiments we have not observed particular differences between alginate and pectate gels; the operating stability of the support beads in semicontinuous process was 6 days for alginate and 8 days for pectate. Afterwards, the beads become eroded releasing live and active cells to the bioreactor void volume. We observed much improved stability of alginate gel after treating the beads with polyethyleneimine (PEI) and subsequently by glutaraldehyde. The hardened variant showed a very good physical stability for at least 2 months. However, the biodegradation rate at the start of the experiment in the variant with hardened beads was lower than in the nonhardened variant, probably due to the toxic effect of glutaraldehyde during hardening.

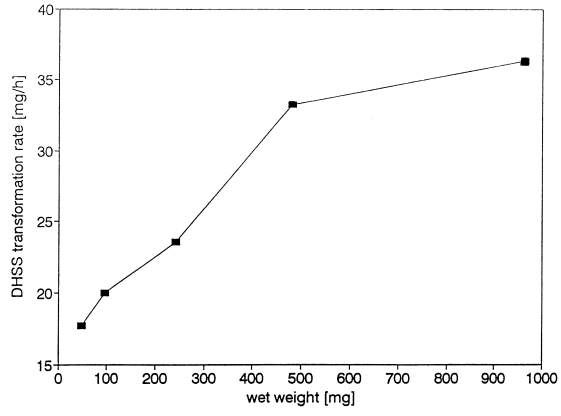

Fig. 4. Effect of biomass loading on the DHSS transformation.

The immobilized cells can recover and speed up the biodegradation rate within a few cycles of adding 100 mg l^{-1} DHSS to reach the biodegradation rate close to that of the nonhardened variant. Our strain exhibits a very interesting attribute especially from the practical point of view; it is able to retain the biodegradation ability after depletion of surfactant for weeks. The concentration changes of xenobiotics in waste-water treatment plants is a common problem, especially during the week-ends or vacations when the waste-water production is lowered. For this reason it is necessary to apply such microorganisms which are able to overcome this period of time.

Compared with freely suspended cells, the immobilized cells exhibit a slower biodegradation rate which can be explained by diffusional restriction of substrate, products or oxygen through the gel. There are a couple of methods for solving this problem. One strategy is the oxygenation of the bulk medium. The other method is the use of oxygen carriers and in situ oxygen generation within the matrix (for a review see Ref. [34]). Another way for minimizing this effect is making the beads as small as possible. This can be done by using special equipment—sonic vibration transducer [36], emulsion techniques [37] or fine aerosol techniques [38].

The other advantages of hardening procedure is linked with starvation experiments; overnight treatment of beads by polyethyleneimine is analogous to starvation conditions and therefore leads to the stimulation of biodegradation activity.

However, the application of alginate gels, or other kinds of natural polymers, in open systems such as waste-water treatment plants is not suitable because of their low stability and the high abundance of alginate degrading bacteria. Anyway, alginate represents the best laboratory model for testing the behaviour of immobilized cells and the results obtained with alginate are in close correlation with many other synthetic hydrogels such as polyvinylalcohol [39].

3.5. C. terrigena N3H cells entrapped on reticulated polyurethane foam

3.5.1. Operational stability of the bioreactor removing DOSS and DHSS

Long kinetic runs were carried out for several days, testing the remaining surfactant (DOSS, DHSS) concentration in water vs. time of operation of the recirculation bioreactor packed with polyurethane reticulated foam particles with and without (controls) *C. terrigena* N3H entrapped cells (flow rate 28 ml min^{-1}, pH 7.1, known weight of support, initial DOSS concentration 0.24 g l^{-1}). After each day of operation, the two bioreactors were challenged with a new cycle with the same initial surfactant concentration and after four cycles of 1 day of operation, when the support particles became saturated with the surfactant, the bioreactors were incubated in a MBS–Mg solution for 24 h (for the surfactant being desorbed from the support) and then challenged again with successive cycles of surfactant solutions. The biodegradation kinetics could be obtained by subtracting the sorption kinetics of controls (Fig. 5, empty points) from the sorption plus biodegradation kinetics (Fig. 5, full points) for bioreactors challenged with consecutive cycles of the surfactant.

As in the case of immobilized *Pseudomonas* C12B for the removal of SDS, DHSS and DBS [26], it may be concluded that during 5–6 cycles (days) of continuous operation, the DOSS- and DHSS-removing activity of immobilized *C. terrigena* N3H is maintained, mainly due to the physical sorption of DOSS during the first 2 cycles (days) while afterwards primary biodegradation of the surfactant becomes increasingly important. The bioreactors recover their surfactant removing activity after each operational cycle of 3 or 4 days by desorbing the substrate and products from the support particles by recirculating a high-ionic strength MBS-Mg solution through them for 24 h.

The overall DOSS and DHSS removals from water for polyurethane foam without entrapped *Comamonas* cells was well fitted to first-order

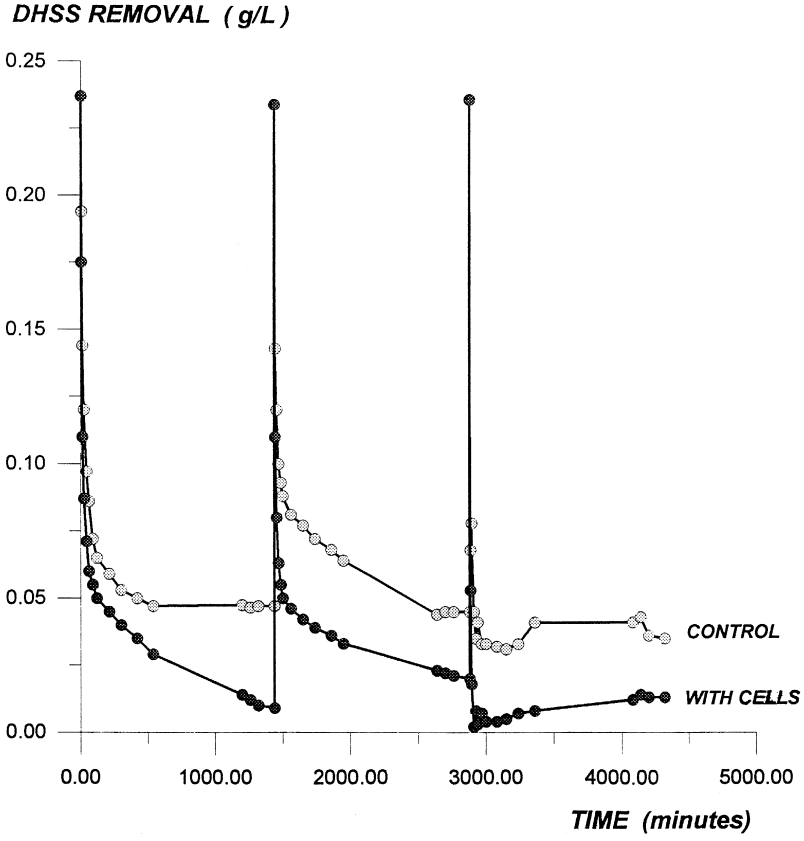

Fig. 5. Operational stability of DHSS removal by *C. terrigena* N3H immobilized on polyurethane foam (empty support (○), cell loaded support (●)). For other experimental conditions see text.

kinetics (remaining substrate concentration vs. time: [Surfactant] $= Ae^{-k_1 t} + C$ ($k_1 = 0.22$ min^{-1} for DOSS, 0.16 min^{-1} for DHSS; $(t_{1/2})_1 = 3.2$ min and 4.3 min, respectively). For the cells entrapped in polyurethane foam, the overall kinetics were well fitted to a biphasic process ([Surfactant] $= Ae^{-k_1 t} + Ae^{-k_2 t} + C$). First, there was a rapid sorption step of the surfactant on the cell-loaded support ($k_1 = 0.092$ min^{-1} for DOSS, 0.082 min^{-1} for DHSS; $(t_{1/2})_1 = 7.5$ min and 8.5 min, respectively) followed by the slower intrinsic primary biodegradation step ($k_2 = 4.9 \times 10^{-4}$ min^{-1} for DOSS, 1.8×10^{-3} min^{-1} (for DHSS); $(t_{1/2})_2 = 23.6$ h and 6.4 h, respectively). Depending on time, the remaining surfactant concentration is mainly due to sorption or primary biodegradation or both synergic processes. The k_1 for adsorption of surfactant when the support is empty of cells (control) (0.22, 0.16 min^{-1}) is greater than k_1 when the support is cell-loaded (0.092, 0.082 min^{-1}), meaning that the cells on the support particles are interfering the (specific) surfactant-support interaction by occupying some of the ligand binding sites of the particle.

3.5.2. Sorption isotherms of surfactants on silicone rubber support

The sorption isotherms for SDS, DHSS, DOSS and DBS on reticulated polyurethane foam have been established as type II of the Brunauer, Deming, Deming and Teller (BDDT) classification [26]. In this work, sorption isotherms for SDS, DBS and DOSS on silicone rubber Raschig rings (Immobasil) have also been established as type II of BDDT classification (Fig. 6). The surfactant after being sorpted as a

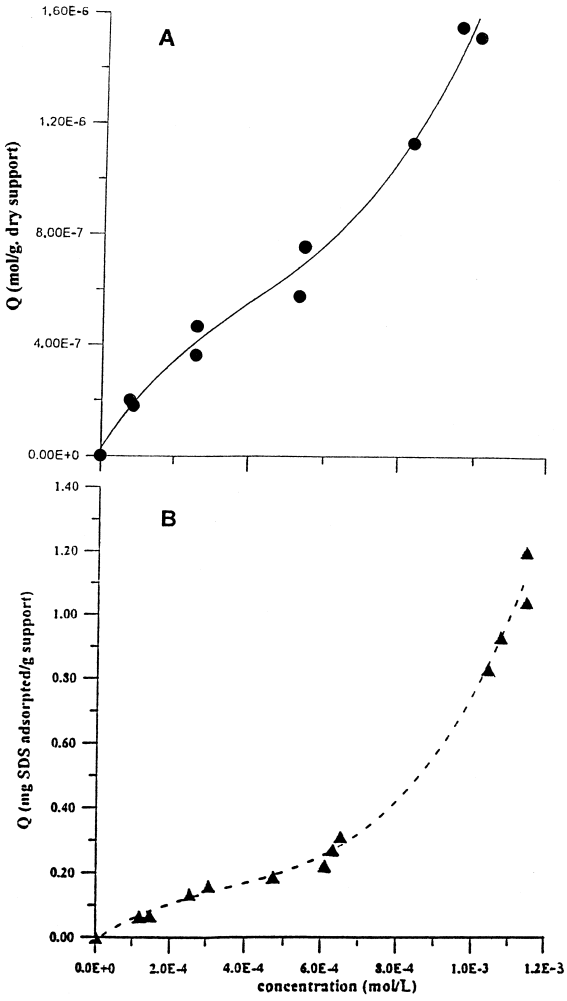

Fig. 6. Sorption isotherms for DOSS (A) and SDS (B) on silicone rubber Immobasil rings. For experimental conditions see text.

monolayer continues its sorption as multilayer on the support particle. The data, moles of sorpted surfactant per gram dried support (Q) vs. the surfactant molar concentration at equilibrium (C), are well fitted by an empirical third order polynomial whose parameters (a, b, c) are shown in Table 1.

$$Q = aC + bC^2 + cC^3$$

As may be seen from Fig. 6 and Table 1, the shapes of the adsorption isotherms are similar and, thanks to the highest value of the parameter a, DBS is adsorbed more efficiently (for $C = 1 \times 10^{-4}$ M, $Q_{DBS} = 3.56 \times 10^{-7}$, $Q_{SDS} = 2.62 \times 10^{-7}$, $Q_{DOSS} = 1.75 \times 10^{-7}$). The adsorption of these surfactants onto silicone rubber Immobasil rings could be due to a specific polar-type reversible hydrophilic interaction that becomes patent at low surfactant concentrations (up to 4×10^{-4} M) (on the first stretch of the adsorption isotherms a Langmuir type monolayer adsorption is seen, Fig. 6), together with a nonspecific interaction, which is also reversible, that becomes apparent at higher surfactant concentrations ($> 4 \times 10^{-4}$ M) (on the second stretch of the adsorption isotherms physical adsorption in multilayers is seen). These interactions are physical in nature; that is, adsorption is reversible because in the presence of high ionic strength the spontaneous desorption of these surfactants occurs (data not shown).

4. Conclusions

Both alginate and polyurethane immobilized *C. terrigena* N3H cells have been shown to be appropriate for the effective biodegradation of the anionic surfactants dihexyl sulphosuccinate (DHSS) and dioctyl sulphosuccinate (DOSS), and in view of the low cost and ease of synthesizing the supports, these systems seem to have

Table 1
Sorption isotherms of anionic surfactants on silicone rubber Immobasil rings fitted to an empirical polynomial (see text). Parameters and correlation coefficient

Surfactant	a (mol sorpted l/mol g support)	b (mol sorpted l^2/mol^2 g support)	c (mol sorpted l^3/mol^3 g support)	r^2
SDS	0.0032	−6.39	5928.6	0.957
DOSS	0.0020	−2.65	2203.4	0.995
DBS	0.0038	−2.42	501.5	0.988

more advantages than others when attempting to remove these representative anionic surfactants.

As in the case of polyurethane-entrapped *Pseudomonas* C12B [26], a simultaneous and synergic action of both sorption and primary biodegradation of the anionic surfactants (DHSS, DOSS), providing enhanced kinetics for the overall removal of the surfactant, was also found for polyurethane-entrapped *Comamonas*. The kinetics of the surfactant removal from water were well fitted to a biphasic process ([Surfactant] $= A_1 e^{-k_1 t} + A_2 e^{-k_2 t} + C$), a rapid passive sorption step of the surfactant onto the cell-loaded support ($k_1 = 0.082,\ 0.092\ \text{min}^{-1}$) and the intrinsic primary biodegradation slower step (Michaelis–Menten (Monod) kinetics under low substrate concentration regime) ($k_2 = 1.8 \times 10^{-3},\ 4.9 \times 10^{-4}\ \text{min}^{-1}$), both acting synergically.

Starvation positively affected the biodegradation potential of cells towards DHSS: cells exposed for 16 h in carbonless media showed the highest biotransformation rate, representing 144% of the nonstarved culture. Alginate gel did not affect the disappearance of DHSS due to adsorption. Biodegradation rate of DHSS increased with the higher amount of immobilized biomass. The diameter of alginate beads affected biodegradation rate in case of immobilization of high biomass (about 600 mg wet weight). With larger diameter of beads, the biodegradation rate was reduced but with low biomass (60 mg wet weight) no effect was observed. Treatment of alginate beads with polyethyleneimine and subsequently with glutaraldehyde improved the physical stability of beads, retaining their stability for at least 2 months in a semicontinuous system.

Sorption isotherms for the surfactants on silicone rubber Raschig rings (Immobasil) have been established as type II of the Brunauer, Deming, Deming and Teller (BDDT) classification. The adsorption could be due to a specific polar-type reversible hydrophilic interaction that becomes patent at low surfactant concentrations (Langmuir type monolayer), together with a nonspecific interaction, which is also reversible, that becomes apparent at higher surfactant concentrations (physical adsorption in multilayers).

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