Medium-Induced Inhibition of Microbial Adsorption to Nickel and Activated Charcoal

R. G. Protheroe,* R. H. Cumming, and A. Matchett
North East Biotechnology Centre, Department of Chemical Engineering, Teesside Polytechnic, Borough Road, Middlesbrough, Cleveland, TS1 3BA, United Kingdom

Accepted for publication September 14, 1988

Equilibrium adsorption studies on *Escherichia coli* and *Saccharomyces* sp. revealed the capacity and affinity of these organisms for the surfaces of powdered charcoal and nickel. In simple salt solutions both organisms readily adsorbed to each solid with an affinity and maximum loading capacity individual to each cell–solid combination. In the presence of common growth media (lab-lemco, nutrient broth, peptone, and yeast extract, individually at a concentration of 1.3%), each medium substantially inhibited adsorption. Each medium contained a proteinaceous constituent as determined by ultraviolet (UV) analysis. The degree of inhibition was relative to medium concentration present during assay. Cell wall extracts from whole-yeast cells also effectively inhibited adsorption. Cells adsorbed in the presence of sodium chloride solutions were susceptible to subsequent desorption by nutrient broth.

INTRODUCTION

In the biotechnological industry, cell separation from fermenter broth is usually the first step toward product recovery. In downstream processing, the small size and low density of microorganisms, in comparison with their aqueous environment, represents an inherent problem in separation. To effect suitable clarification, considerable investment in separation techniques such as centrifugation, membrane filtration, or flocculant-induced cellular aggregation is essential.[1]

Financial rewards are associated with the development of simple and potentially cost-effective clarifying techniques, which incorporate low running and capital costs. Research has therefore been initiated into new methods, including electrokinetic, ultrasonic, magnetic, and foam separations, together with liquid–liquid extraction.[2] One other technique of separation is the use of a solid settling promoter. Such a promoter would be a dense material with a high affinity for microbial adsorption. In theory, following mixing to permit contact, the cell–solid complex would settle quickly, thereby effecting clarification.

In an earlier study,[3] an assay procedure was used to identify solids with properties consistent with the requirements of settling agents. The high-cell-loading capacities of charcoal and nickel identified them as solids with the greatest potential, when compared with calcium carbonate, glass, PTFE, and sand. Preliminary adsorption assays were, however, performed in the presence of simple salt solutions, an environment not representative of industrial situations, where fermenter broth would be present. To investigate the usefulness of charcoal and nickel under environmental conditions where they would be employed industrially, adsorption assays were performed in the presence of four growth media.

MATERIALS AND METHODS

Organisms and Growth Conditions

Organisms were *Escherichia coli* (gram negative) and a *Saccharomyces* sp. (baker's yeast). *Escherichia coli* was grown in nutrient broth (Oxoid) aerobically in shake flasks at 36°C for 16 h. Cultures were harvested following the exponential phase by centrifugation using an MSE Hi-Spin 21 (23,600*g* for 10 min), and pellets were washed twice with 0.15*M* sodium chloride (British Drug Houses, AnalaR), pH 7.5, before use in adsorption assays. Baker's yeast was reconstituted with saline and harvested by centrifugation using an ICA Centra-7A centrifuge (14,000*g* for 5 min) and then washed twice with saline before use in adsorption assays.

Solids

Solid powders used in adsorption assays were activated charcoal (British Drug Houses, general-purpose reagent) and nickel powder (British Drug Houses, general-purpose reagent). Solids were wet sieved using deionized water to remove particles below 53 μm, dried in an oven at 40°C, and stored in a dry place before use in adsorption assays.

Adsorption Assay

The capacity of solids to adsorb cells was studied over a range of initial cell concentrations at a constant mass of solid. A typical assay involved the mixing of a 4-mL cell

* To whom all correspondence should be addressed at Department of Chemical Engineering, University of Technology, Loughborough, Leicestershire, LE11 3TU, United Kingdom.

Biotechnology and Bioengineering, Vol. 34, Pp. 896–901 (1989)

CCC 0006-3592/89/070896-06$04.00

suspension (cells were suspended by saline of 0.15*M* sodium chloride, pH 7.5) with solid (0.1 g charcoal or 0.2 g nickel) in a 20-mL universal bottle. Initial optical density (540 nm) was determined using a Pye Unicam PU 8600 spectrophotometer. Universals were shaken for 45 min by an orbital shaker (200 rpm) at room temperature (22 ± 2°C). Following mixing, universals were centrifuged at 3500*g* for 1 s to accelerate solid settling. Following settling, supernatant was sampled to determine optical density. Optical density loss following mixing was assumed to be a direct consequence of cell-to-solid adsorption. Control experiments were represented by solid with cell-free saline and cell-free medium and saline–cell and medium–cell suspensions in the absence of solid. Whether suspended by saline or medium, cells did not reproduce during assay. No appreciable adhesion occurred to universal walls during assay.

Optical density measurements were related directly to cell numbers per milliliter, by multiplication with a conversion factor individual to the particular organism under investigation. Conversion factors were determined by relating direct counts (using a counting chamber) and Coulter counter measurements (Coulter Counter ZM) with optical density.

Inhibition of adsorption resulting from the introduction of additives of known concentration into the assay was assessed by comparison to assays performed with 0.15*M* sodium chloride alone. Additives were lab-lemco (Oxoid), nutrient broth (Oxoid), peptone (Oxoid), and yeast extract (Oxoid). Additives did not alter pH. Equivalent albumin protein content of each medium (as determined by UV analysis) is given in Table I.

Following certain adsorption assays, known concentrations of nutrient broth were added to the system and mixed for a further 15 min. Following this period, supernatant optical density was again measured to determine whether desorption had occurred.

Cell and Charcoal Surface Precoating

For certain adsorption assays, *Saccharomyces* sp. and charcoal were precoated with nutrient broth before being challenged with either uncoated charcoal or uncoated cells. Cellular coating was achieved by mixing cells with 1.3% nutrient broth for 45 min. Following mixing, cells were harvested by centrifugation and washed 3 times with saline before use in adsorption assay. Controls were represented by cells treated in the same manner except nutrient broth was absent. Charcoal was coated by mixing solid with 1.3% nutrient broth for 45 min. Following mixing, solid was recovered by centrifugation, washed twice with saline and once with distilled water, and then dried in an oven at 40°C before use in adsorption assay.

The capacity of solids to adsorb components of growth media from solution was determined by mixing solid with nutrient broth and analyzing for the loss of UV adsorbance.

Table I. Equivalent protein content of growth media (using albumin standard).

	Equivalent g protein/g powder
Lab-lemco	0.56
Nutrient broth	0.82
Peptone	0.7
Yeast extract	1.16

Adsorption Isotherms

Adsorption isotherms are plotted as adherent cells per gram solid against supernatant cell density per milliliter at equilibrium. Numbers of adherent cells per gram solid (q) were calculated by

$$q = (C_i - C_e)(1/m) \times (\text{assay volume}) \qquad (1)$$

where C_i is initial cell numbers per milliliter, C_e is supernatant cell numbers at equilibrium per milliliter, and m is solid weight.

Outer Cell Wall Extraction from Yeast Cells

Outer cell wall components (including mannans and phospho-mannans) readily leach from yeast cell walls in the presence of warm mild alkali.[4] Cell wall components were extracted by mixing a 75-mL cell suspension in 0.01*M* KOH (~2.55×10^8 cells/mL) at 60°C for 10 min. Following cell removal by centrifugation (23,600*g* for 10 min) and supernatant filtration (0.8-μm pore), the pH of the resulting solution was adjusted to 7.5 before use in adsorption assay. The final concentration of cell wall extract in adsorption assay was 1.3% (albumin equivalent).

RESULTS

Adsorption Isotherms

The capacity of charcoal and nickel to adsorb *E. coli* and *Saccharomyces* sp. when suspended by simple salt solutions are represented by Figures 1–3. Solid cell loading is a function of cell concentration. The number q increases with C_e until the solid saturates. Saturation points were individual to the cell–solid combination. *Escherichia coli* effected saturation at an approximate C_e concentration of 8×10^9 cells/mL for charcoal (9×10^{10} cells/g). Charcoal and nickel were saturated by *Saccharomyces* sp. at C_e levels of 0.25×10^7 cells/mL (4.5×10^9 cells/g) and 1.75×10^7 cells/mL (7×10^8 cells/g), respectively. These values indicate *E. coli* to be the organism more capable of loading solid on a cells-per-gram basis and that the capacity of charcoal to adsorb cells was greater than nickel.

In the presence of four common growth media, adsorption was markedly reduced (Fig. 1–6). Nutrient broth and its constituents lab-lemco, peptone, and yeast extract (at a concentration in adsorption assay of 1.3%) prohibited cel-

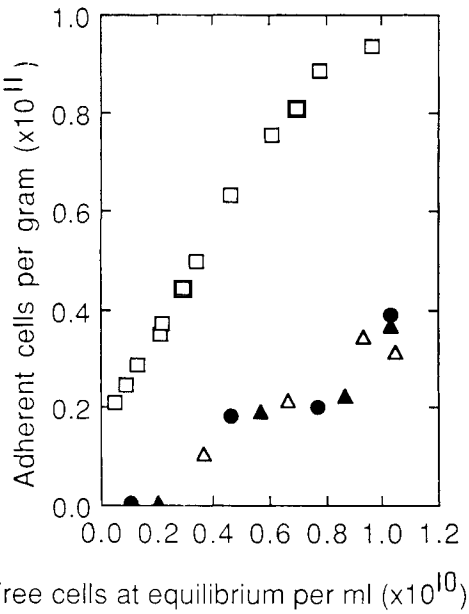

Figure 1. Effect of media on adsorption of *E. coli* to charcoal: (□) no added medium; (△) 1.3% lab-lemco; (▲) 1.3% peptone; (●) 1.3% yeast extract.

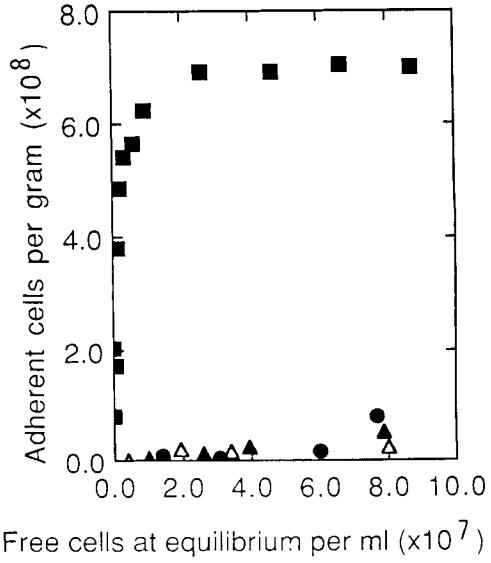

Figure 3. Effect of media on adsorption of *Saccharomyces* sp. to nickel: (■) no added medium; (△) 1.3% lab-lemco; (▲) 1.3% peptone; (●) 1.3% yeast extract.

lular attachment to nickel and caused an approximate 25% reduction of *E. coli* and a 90% reduction of *Saccharomyces* sp. adsorption to charcoal.

Microbial adsorption processes are accurately modeled by the Langmuir equation[3]:

$$1/q = [(1/A)(1/C_e) + 1]1/Q_0 \qquad (2)$$

where Q_0 is the maximum theoretical solid loading by cells and A is a constant, which is a measure of the observed affinity between interacting heterologous surfaces, calculated from experimental results. In the presence of adsorption inhibitors, the adsorption process is more accurately described by an adaptation of the Langmuir equation:

$$\frac{1}{q} = \frac{(1/A)(1/C_e) + 1]^1}{Q_0} + \frac{1}{A_I}\frac{1}{Q_0} \times \frac{1}{C_e} \qquad (3)$$

or

$$\frac{1}{q} = \frac{[(1/A + I/A_I)1/C_e + 1]^1}{Q_0} \qquad (4)$$

where I is inhibitor concentration and A_I is the apparent inhibition constant. Table II contains observed affinity and inhibition constants calculated using equations (2) and (3) for *E. coli* and yeast adsorption to charcoal and nickel as a function of nutrient broth concentration. It is evident that as growth medium concentration increases, intersurface affinity is reduced (Figs. 4–6). It is also evident that the calculated affinity of yeast for charcoal is similar to that for nickel.

Nickel was the solid most sensitive to nutrient broth presence.

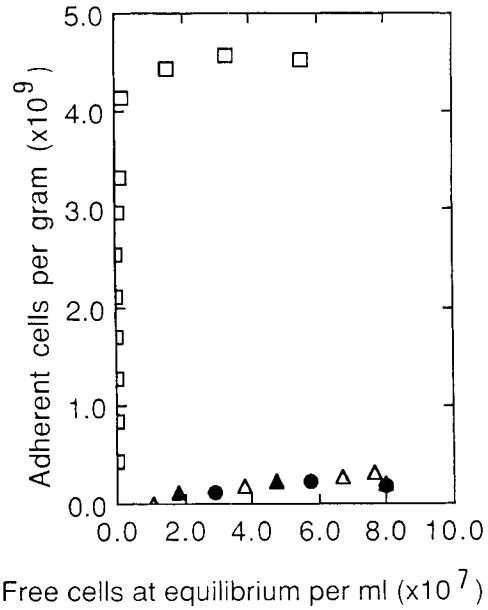

Figure 2. Effect of media on adsorption of *Saccharomyces* sp. to charcoal: (□) no added medium; (△) 1.3% lab-lemco; (▲) 1.3% peptone; (●) 1.3% yeast extract.

Cell and Solid Surface Precoating

To investigate the site of medium-induced inhibition of adsorption, certain assays were performed following yeast and charcoal preexposure to nutrient broth (Fig. 7). Cell coating did not affect adsorption, although solid preexposure markedly reduced the susceptibility of the solid surface to colonization.

Medium-Induced Desorption

The introduction of nutrient broth into adsorption assays following formation of a stable yeast–solid equilibrium

Table II. Observed affinity and inhibition constants for *E. coli* and *Saccharomyces* sp. adsorption to charcoal and nickel as function of nutrient broth concentration.[a]

Organism	Solid	Nutrient broth concentration, I (%)	Affinity constant, A (mL/cell)	Inhibition constant, A_I (mL/cell)
E coli	charcoal	0	6.99×10^{-10}	0
		0.013	6.99×10^{-10}	1.01×10^{-11}
		0.13	[b]	
		1.3	6.99×10^{-10}	1.66×10^{-10}
Saccharomyces sp.	charcoal	0	1.21×10^{-5}	0
		0.0026	[b]	
		0.13	1.21×10^{-5}	2.15×10^{-9}
		1.3	1.21×10^{-5}	2.89×10^{-8}
Saccharomyces sp.	nickel	0	1.13×10^{-5}	0
		0.0013	1.13×10^{-5}	2.54×10^{-8}
		0.013	[b]	
		0.13	1.13×10^{-5}	8.86×10^{-8}
		1.3	1.13×10^{-5}	1.26×10^{-7}

[a] Constants calculated using equations (2) and (3).
[b] Isotherm not accurately described by equation.

caused spontaneous desorption from charcoal and nickel (Figs. 8 and 9). The extent of desorption was dependent on concentration and the solid carrier. Charcoal was considerably more insensitive than nickel to medium presence, with 1.3% nutrient broth effecting a 6% desorption from charcoal (a statistically significant result at the 0.02 level) and a 97% desorption from nickel.

DISCUSSION

Adsorption in Presence and Absence of Media

Saturation isotherms were evident when charcoal and nickel were challenged with *E. coli* and *Saccharomyces* sp. when suspended by a solution of sodium chloride (Figs. 1–3). The presence of lab-lemco, nutrient broth, peptone, and yeast extract at a concentration of 1.3% inhibited adsorption to similar extents. Similarity in the extent of inhibition reflects similarity of composition. Ultraviolet analysis indicated each medium to possess equivalent proteinaceous concentrations (Table I).

In colloid science, polymeric (proteinaceous) interference of heterologous surface interaction is an established process. Proteins spontaneously adsorb at the solid–liquid interface,[5] resulting in the formation of a conditioning layer[6] or pellicle.[7] Depending on the properties of the adsorbed polymer, nonspecific cell–solid surface attachments are either promoted or inhibited. For certain polymers at discrete concentration, adsorption is promoted by polymer bridging.[8] Conversely, for adsorbed polymers soluble in an aqueous

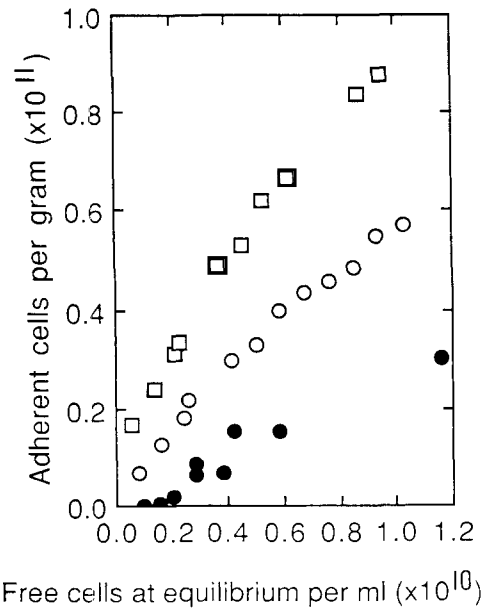

Figure 4. *Escherichia coli* adsorption to charcoal as function of nutrient broth concentration: (□) 0.013%; (○) 0.13%; (●) 1.3%.

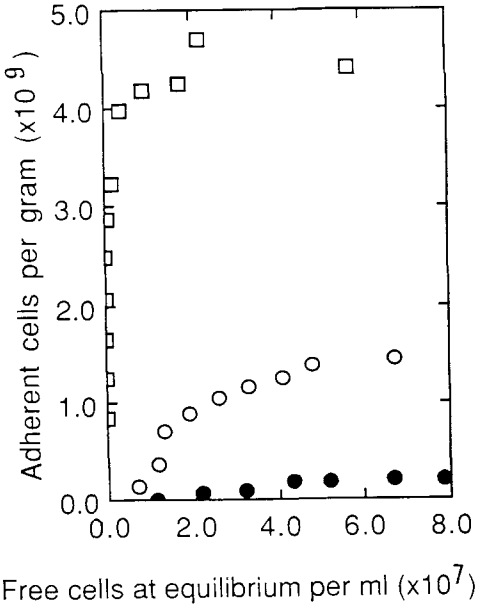

Figure 5. *Saccharomyces* sp. adsorption to charcoal as function of nutrient broth concentration: (□) 0.0026%; (○) 0.13%; (●) 1.3%.

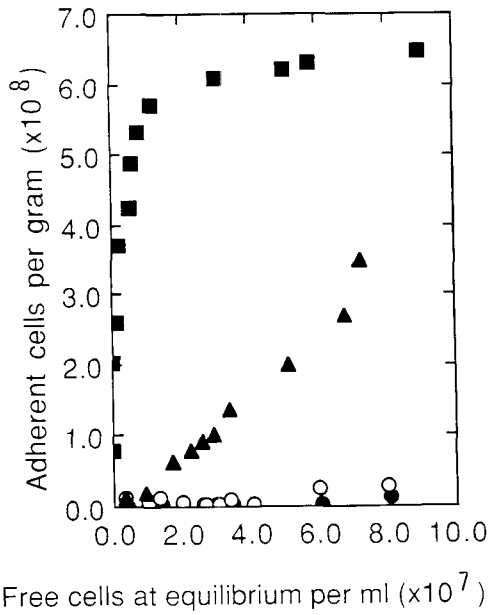

Figure 6. *Saccharomyces* sp. adsorption to nickel as function of nutrient broth concentration: (■) 0.0013%; (▲) 0.013%; (○) 0.13%; (●) 1.3%.

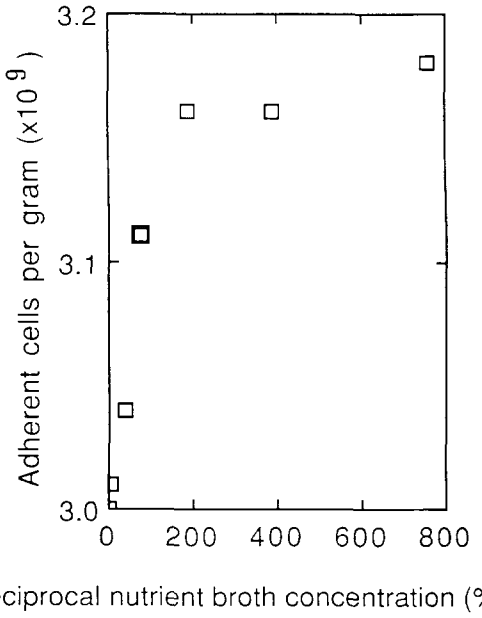

Figure 8. *Saccharomyces* sp. desorption from charcoal due to competition by nutrient broth.

medium, the intermingling of adsorbed polymer layers cause repulsive elastic and osmotic effects. These repulsive forces contribute to the steric effects, which are involved in the stabilization of suspended species.[9,10] In addition to surface effects, the presence of proteinaceous material in solution necessitates consideration of a hydrodynamic process. Proteins in solution increase viscosity, an effect lowering particle collision rate.[8] In this study, viscosity was, however, unlikely to influence adsorption due to the low solute concentrations used.

Inhibition of adsorption was therefore most probably associated with steric repulsions resulting from the surface conditioning of solids and cells.

Adsorption following Cell and Charcoal Preexposure to Nutrient Broth

Results of adsorption assays using coated *Saccharomyces* sp. and charcoal reveal that solid coating was more effective at reducing adsorption than cell coating. While it may be concluded that nutrient broth components were bound more tenaciously to charcoal (since washing failed to remove the coating which caused inhibition) than to *Saccharomyces* sp., this may be misleading. During culturing, cells would invariably be exposed to growth medium and therefore be "coated" from the outset. If already saturated, they would be insensitive to subsequent nutrient broth exposure.

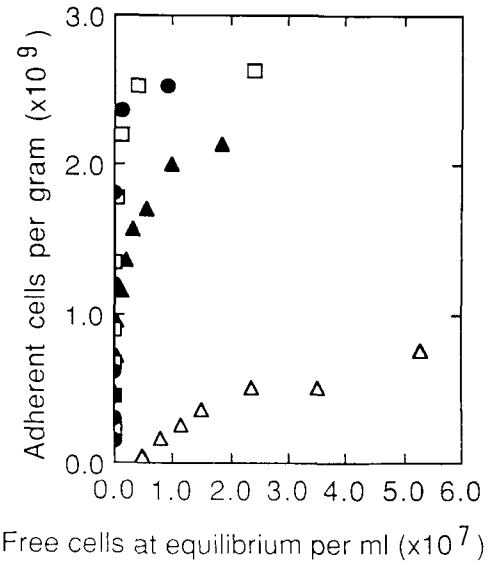

Figure 7. *Saccharomyces* sp. adsorption to charcoal following cell and solid precoating: (□) control yeast coating; (●) medium-coated yeast; (▲) control solid coating; (△) medium-coated solid.

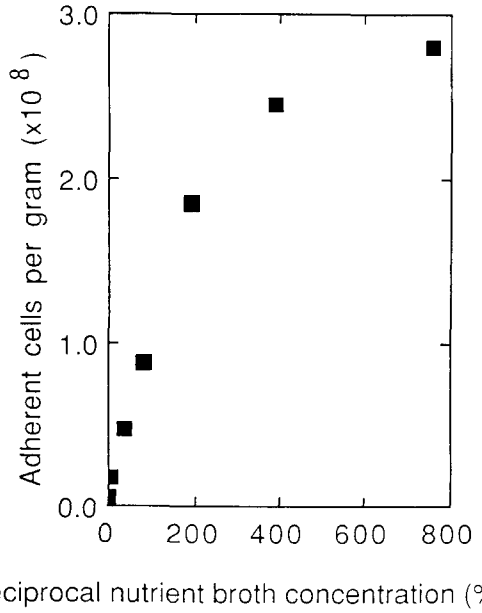

Figure 9. *Saccharomyces* sp. desorption from nickel due to competition by nutrient broth.

Table III. Percentage protein extraction from solution resulting from nutrient broth mixing with charcoal and nickel.

	Weight solid (g)	Volume (mL)	Concentration	Reduction (%)
Charcoal	0.1	4.0	1.3%	98.13
Nickel	0.2	4.0	1.3%	1.88

The results of Table III detail the proteinaceous adsorbing properties of nickel and charcoal. It is evident that the capacity of charcoal to adsorb proteinaceous material is 52-fold greater than for nickel. This effect results from the greater surface area for adsorption available on the porous activated charcoal, the pore size of which is below the resolution of scanning electron micrographs and therefore considerably smaller than cell diameters. Inhibition of adsorption caused by the presence of media reflects this situation. The readily saturated nickel surface is rendered incapable of cell adsorption at nutrient broth dilutions of 1 : 10. At this dilution, the charcoal surface is undersaturated by medium, and binding sites remain available on the outermost particle surface for cell attachment.

Cell Wall Extract Inhibition of Yeast Adsorption

Outer cell wall polymers, including mannans and phosphomannans, mediate the adsorption of yeast to solid surfaces. Coating solid surfaces with cell wall extracts would therefore be expected to block the adhesive interactions which hold cells in stable association with solids. Such a phenomenon was indeed observed, as illustrated in Figure 10, since the presence of soluble cell wall extract greatly reduced solid loading capacity by yeast.

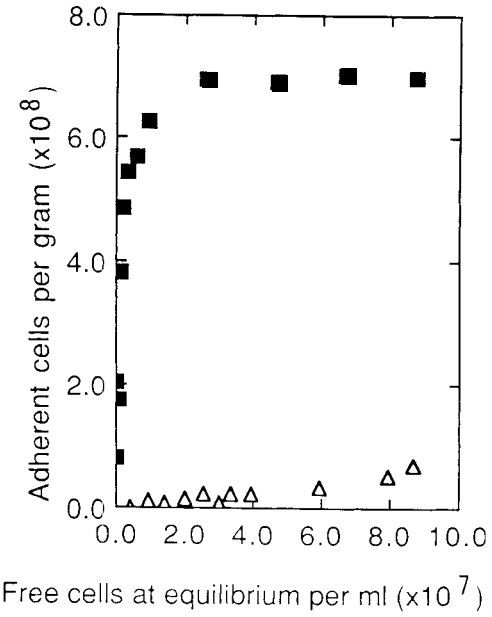

Figure 10. *Saccharomyces* sp. adsorption to nickel in presence and absence of cell wall extract: (■) control; (△) added cell wall extract.

Growth-Medium-induced Desorption

The ability of nutrient broth to effect desorption reflects the dynamic nature of the adsorption process. Cells spontaneously desorb from surfaces due to thermal motion and fluid shear. In the presence of simple salts, subsequent readsorption proceeds unhindered. In the presence of polymers (such as proteinaceous medium components), the vacated site becomes rapidly and tenaciously occupied, thereby blocking subsequent readsorption. As observed in Table II, the frequency of the adsorption–desorption process is similar for yeast with activated charcoal and nickel (when considering similarity in A). The greater sensitivity of nickel to desorption is thus a reflection of the greater affinity of blocking polymers for nickel in comparison with that for charcoal.

CONCLUSION

In the presence of growth media, steric interactions stabilize mixtures of either *E. coli* or *Saccharomyces* sp. with charcoal and nickel. Steric stabilization is manifested as cellular inhibition of adsorption to solids. Adsorption is, however, an essential element in the use of solid powders as settling agents. Consequently, unless a method for suppression of inhibition is developed, solid powders have no industrial use in primary cell separation from fermenter broths.

The authors wish to express their thanks to Dr. A. Abbott for constructive discussion which occurred throughout this investigation. We are also indebted to the National Advisory Body for funding this project.

References

1. D. I. C. Wang, in *Separations for Biotechnology,* M. S. Verall and M. J. Hudson, Eds. (Ellis Horwood, 1987), pp. 30–48.
2. C. P. Bowden, G. Leaver, J. Melling, M. G. Norton, and P. N. Whittington, in *Separations for Biotechnology,* M. S. Verall and M. J. Hudson, Eds. (Ellis Horwood, 1987).
3. R. G. Protheroe, R. H. Cumming, and A. Matchett, *Biotechnol. Bioeng.* (submitted for publication).
4. A. H. Rose, in *Chemical Microbiology* (Butterworth, London, 1976).
5. M. Fletcher and K. C. Marshall, *Appl. Environ. Microbiol.*, **44,** 184 (1982).
6. R. E. Baier, in *Adsorption of Microorganisms to Surfaces,* G. Bitton and K. C. Marshall, Eds. (Wiley Interscience, New York, 1980), pp. 59–104.
7. W. B. Clark, L. L. Bammann, and R. J. Gibbons, *Inf. Immun.*, **19,** 846 (1978).
8. P. R. Rutter and B. Vincent, in *Microbial Adhesion to Surfaces,* R. C. W. Berkeley, J. M. Lynch, P. R. Rutter, and B. Vincent, Eds. (Ellis Horwood, 1980), pp. 79–91.
9. D. J. Shaw, *Introduction to Colloid and Surface Chemistry* (Butterworth, London, 1980).
10. B. Vincent, *Adv. Coll. Interf. Sci.*, **4,** 193 (1974).