# *Adsorption Isotherms of Azotobacter vinelandii on Cellex E®*

## INTRODUCTION

As recently reviewed by Jack and Zajic,[1] immobilization methods for whole microbial cells include entrapment, covalent coupling, and adsorption. We present here some observations of the adsorption equilibria of viable *Azotobacter vinelandii* on Cellex E®, an anionic exchange cellulose (ECTEOLA-C). Previous investigations of electrosorption of bacteria to anionic-exchange resins include the screening studies of Zvyaginstev[2] and Daniels and Kempe,[3] and the study by Johnson and Ciegler[4] of the invertase activity of spores of *Aspergillus* and *Penicillium* adsorbed onto ion-exchange celluloses. Hattori and coworkers[5–7] reported changes in physiological state, reproduction, and metabolism of *Escherichia coli* on Dowex 1® ion-exchange resin. Seyhan and Kirwan[8] immobilized *A. vinelandii* onto Cellex E® and maintained the nitrogenase activity of the immobilized organism for four days in a continuous-flow reactor.

## MATERIALS AND METHODS

For these experiments UW 590, a mutant strain of *A. vinelandii* obtained from the laboratory of Winston Brill,[9] was utilized. This strain is derepressed for nitrogenase synthesis and also does not produce the heavy slime layer characteristic of most *Azotobacters*. Adsorption was studied on Cellex E®, a cellulose-based anion-exchange resin (epichlorohydrin triethanolamine cellulose), obtained from Bio Rad Laboratories. The hydroxide form of the resin was employed which had a rated exchange capacity of 0.4 mequiv/g dry resin. The resin particles were cylindrically shaped bundles approximately 20 $\mu$m in diam by 80 $\mu$m in length.

The bacteria were grown in 100 ml batch cultures in modified Burk's media with 10 g/liter sucrose and no fixed nitrogen.[10] Cells were generally harvested in the logarithmic growth phase at a culture density of about $10^8$ cells/ml. Adsorption-desorption experiments were conducted at pH 7 either in Burk's media with 1 g/liter sucrose or Burk's media containing 1 g/liter sucrose and made 0.1*M* in NaCl. Seyhan[11] had previously established that adsorption occurs rapidly on Cellex E® in the pH range of 5.5 to 8.5. The harvested cells were centrifuged, the supernatant was decanted, and the cells resuspended to the desired cell concentrations in the above solutions.

Adsorption-desorption experiments were conducted at 23°C in the following manner: Ten ml solution of desired cell concentration ($10^7$ to $10^9$ cells/ml) were agitated with a known weight of resin. Preliminary experiments with contact times of 15 to 60 min established that adsorption was substantially complete in less than 15 min. The suspension was centrifuged and a sample of known volume of supernatant withdrawn through a 8 $\mu$m Millipore filter that allowed free passage of the cells but retained the resin. The supernatant cell count was determined by plating on Burk's media with agar. The cells adsorbed onto the resin were then simply calculated by a cell number balance. To conduct a desorption experiment the resin and solution remaining were diluted with a large volume (1000 ml) of fresh medium, agitated for 30 min, and the supernatant resampled and replated. Somewhat longer agitation times did not show significant increases in free cell population but time was limited because cell reproduction begins to affect the results. We recalculated the concentration of

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cells on the resin from a cell balance accounting for the cells lost in the earlier sampling. Since the entire adsorption-desorption experiment took place in less than 1.5 hr and the minimum doubling time for UW 590 is about 2.8 hr, cell reproduction did not significantly affect the results.

Some experiments were also conducted to determine at what solution ionic-strength cells would be completely desorbed from the resin but would not be lysed or their viability impaired. In these experiments known amounts of adsorbed cells, prepared as described above, were contacted with Burk's buffer with 1 g/liter sucrose and NaCl concentrations ranging from 0.1 to 1.0*M*. In one experiment adsorbed cells were contacted with distilled water. It was established that at a NaCl concentration of 0.25*M* all cells originally on the resin were free in solution and viable. At higher NaCl concentrations, cell viability was considerably reduced; while at lower NaCl concentrations, only partial desorption occurred. Distilled water desorbed very few cells. These results support the view that the adsorption mechanism is primarily an electrostatic effect as well as indicating methods for removal and counting of a sample of adsorbed cells.

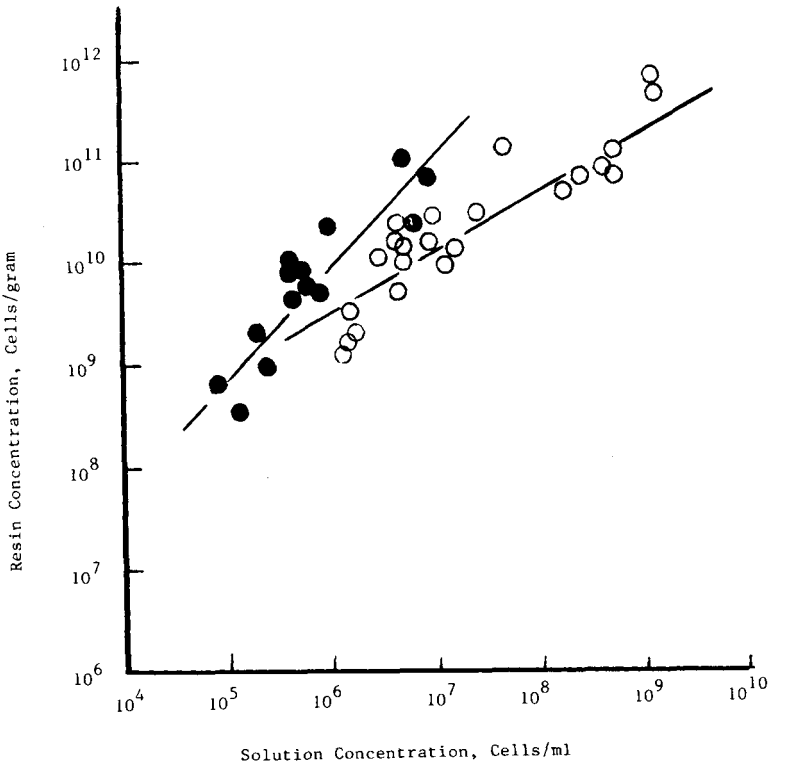


Fig. 1. Adsorption equilibria of *A. vinelandii* on Cellex E® in Burk's buffer at pH 7 and 23°C. (○) Adsorption experiments; (●) desorption experiments.

## RESULTS AND DISCUSSION

The adsorption and desorption isotherms for *A. vinelandii* on Cellex E® are presented in Figure 1 for Burk's buffer solution containing 1 g/liter sucrose. The corresponding results when the solution was also 0.1*M* in NaCl are shown in Figure 2. The data do not conform to a Langmuir isotherm form but, as evidenced by the lines on the plots, could be described by the Freudlich isotherm. There was no evidence for multilayer adsorption either from the isotherm data or from visible light microscopy of the resin. The resin did not appear to be saturated at the highest cell concentrations employed.

Both sets of data show a hysteresis effect in that the desorption curves lie to the left of the adsorption curves indicating that a true equilibrium is not established. This result is similar to that found in the adsorption of polymeric materials,[12,13] in that desorption is slow when the solution is replaced by a more dilute one, yet the solute is not irreversibly bound. The origin of this hysteresis effect in multisite attachment of solutes is that all binding sites between cell and resin must be nearly simultaneously broken for desorption to take place. Comparison of Figures 1 and 2

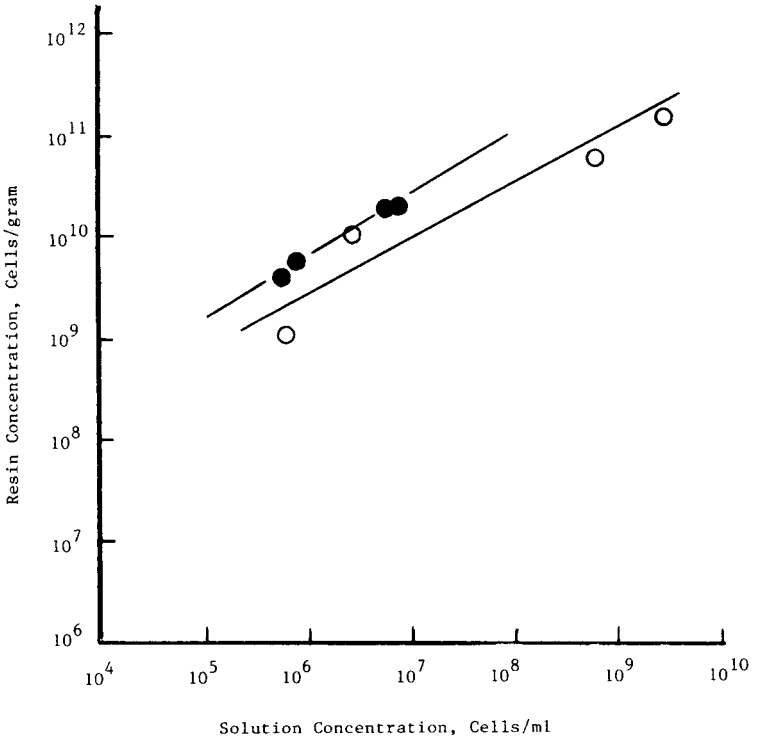


Fig. 2. Adsorption equilibria of *A. vinelandii* on Cellex E® in Burk's buffer with 0.1*M* NaCl at pH 7 and 23°C. (○) Adsorption experiments; (●) desorption experiments.

indicates that as the ionic strength of the solution increases, the kinetics of desorption increase resulting in a reduction of the hysteresis effect. As has been previously noted viable cells can be completely desorbed with 0.25*M* NaCl.

Of particular importance in these results is the demonstration that viable cell loadings as high as $10^{12}$ cells/g resin can be achieved. Since it is quite feasible to make slurries containing 10 g resin/100 ml, an effective cell concentration of $10^{11}$ cells/ml solution can be achieved. This provides effective biocatalyst loadings two orders-of-magnitude greater than that of cells free in solution. Work is in progress to fully assess the specific activities of adsorbed cells in comparison to free cells, but it is already clear that the activities can approach those of free cells.

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