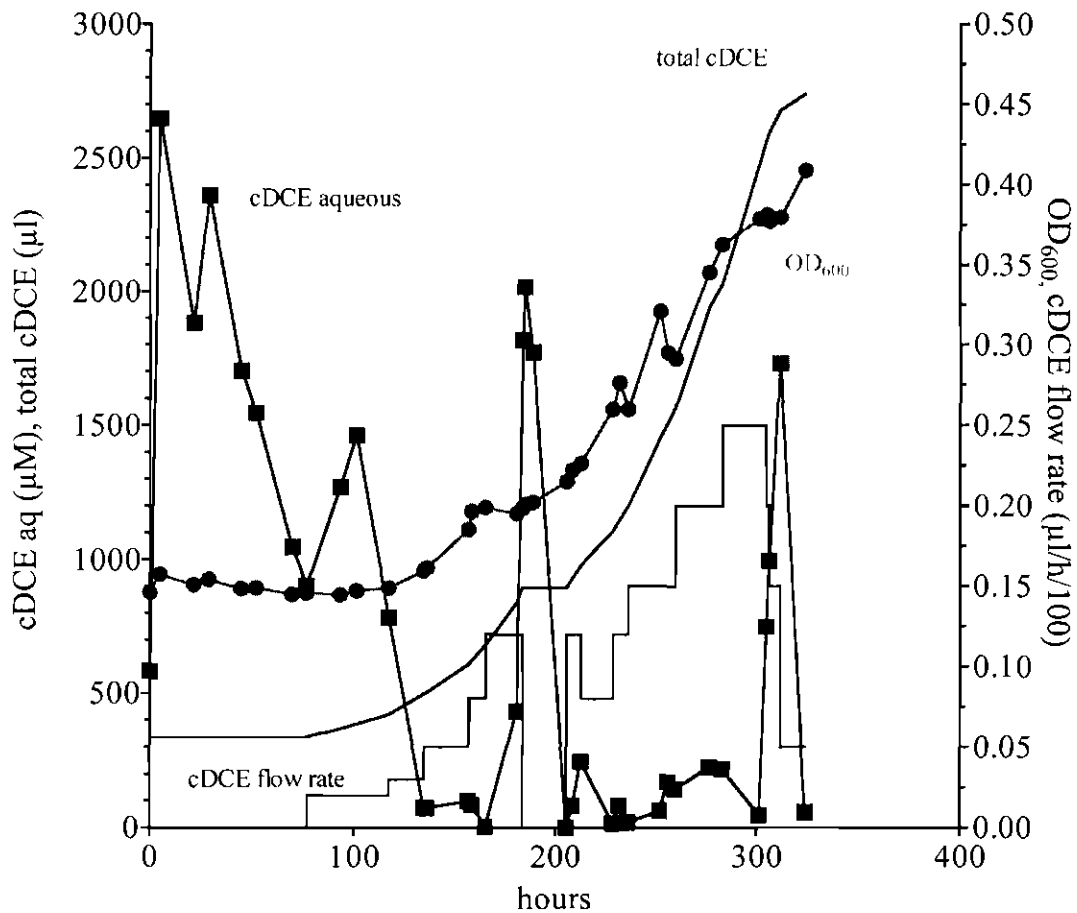


### Subtask 1

In the last report we described experiments in which we attempted to remove chloride from serum bottle cultures using Amberlite IRN-78. A preliminary experiment suggested that the resin added to serum bottles at 10 g/L enabled JS666 cultures to consume more cDCE than cultures without the resin. In a subsequent experiment a range of resin amounts (10 to 40 g/L) were added to duplicate bottles. More cDCE was consumed in bottles with 10 or 20 g/L resin than in control bottles without resin. Much less cDCE was consumed in bottles with 40 g/L resin than in the control bottles. Bottles with 40 g/L resin also developed a white precipitate, presumably due to disruption of the balance of ionic species in the medium by the action of the anionic exchange resin.

g/L Resin	0	10	20	40
Bottle 1 $\mu$ l cDCE consumed	47.0	61.4	89.9	12.0
Bottle 2 $\mu$ l cDCE consumed	47.6	63.0	80.8	14.1

JS666 was grown in a 1-L bioreactor as described before, using modified 1/2-MSB as the growth medium, with 10 mM rather than 40 mM phosphate buffer. A small column filled with approximately 5 g of Amberlite IRN-78 resin was substituted for the base addition bottle. When the pH of the culture medium dropped below the pH 7.2 setpoint, culture medium was pumped from the reactor vessel, through the column and back into the reactor. The column was exchanged for a fresh column when the resin was no longer able to restore the pH to the setpoint. All other operating conditions were as described for previous bioreactors. Before the run was terminated by a break in the recycle line (and loss of the culture), OD<sub>600</sub> tripled, and approximately 2800  $\mu$ l of cDCE was consumed, and growth had not slowed. The flattening of growth around 300 h was due to the inability of air addition to maintain 30% O<sub>2</sub> saturation in the reactor. Automatic continuous aeration stripped cDCE from the reactor and growth stopped. When O<sub>2</sub> was substituted for air, there was a 10 h lag period before growth resumed. Growth in previous bioreactors slowed or stopped after the addition of approximately 1300  $\mu$ l of cDCE and one doubling. The results indicate that the removal of excess chloride rather than the addition of base to neutralize chloride yields better growth of JS666. The reactor will be run under the same conditions again to determine the maximum amount of growth that can be achieved before a medium exchange is required.



Growth of JS666 on cDCE in a bioreactor with chloride removal by anionic exchange resin.

### Subtask 8

We have not detected transfer of the genes for cDCE degradation from JS666 to *Polaromonas naphthalenivorans* CJ2 (rifampicin resistant) when mating mixtures of the two cultures were incubated on the surface of rich medium (1/4 Tryptic Soy Agar plates) in the absence of cDCE. It is possible that the presence of cDCE is required for the optimal transfer of the cDCE genes. Therefore, we tested for the transfer of the cDCE degradation genes from JS666 to strain CJ2 (rifampicin resistant) in the presence of cDCE. Mating mixtures of the two cultures were resuspended in liquid or on the surface of solid minimal media (1/2 MSB) with cDCE added to the headspace. After 1 week, mating mixtures were collected, then spread onto the surface of 1/2 MSB + rifampicin plates, and finally the plates were incubated under a headspace of cDCE. No growth has yet occurred, although it has been less than one week. Theoretically, only strain CJ2 that has acquired the cDCE degradation genes will grow under these conditions.