the xylitol dehydrogenase defective mutants (XDH) from *Pichia stipitis* CBs 5776 and investigate the characteristics of xylitol fermentation by a xylitol dehydrogenase defective mutant PXM-4 in an effort to determine the optimum conditions for the high yield production of xylitol from xylose. The XDH defective mutants were screened by a xylose assimilation test. Among about several hundreds mutant screened, the best mutant PXM-4 was selected. And also gluconic acid was selected as a appropriate co-substrate for the xylitol fermentation. Since gluconic acid neither blocked xylose transport nor repressed xylose reductase expression. An increase in gluconic acid concentration reduced the rates of xylitol production and cell growth by decreasing medium pH. The optimal concentration of gluconic acid for xylitol production was determined at 20 g/l with approximately 100% xylitol conversion yield. A fed-batch cell culture resulted in 42.4 g/l xylitol concentration with 97% yield based on xylose consumed.

SL315

Quantitative Immunoassay for Polychlorinated Biphenyl Compounds in Electrical Insulating Oils

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The development and performance of a competitive indirect immunoassay for the quantitative measurement of polychlorinated biphenyl compounds in insulating oils is presented. Reagent preparation and the assay characterisation, optimisation and validation steps are described. The dynamic range of the assay for Aroclors 1254 and 1260 in methanol was 50-800 μg/ml with 50% signal inhibition values of 217 and 212 μg /ml respectively. Impending legislation in the UK is likely to decree that oils containing >50 μg/ml PCB be considered contaminated. Assay sensitivity increased with the degree of PCB chlorination. The assay of structurally related compounds of environmental concern yielded cross-reactivity values of under 0.6%. The immunoassay proved reliable for the analysis of transformer oils containing >70 μg/ml PCB, but over-estimated PCB levels in oils containing <20 μg/ml of the analyte with the oils requiring pre-treatment using either solid-phase extraction techniques or washing with KOH-ethanol/sulphuric acid to remove matrix interferents. The analytical performance of the assay was compared against a commercially available semi-quantitative immunoassay kit for PCBs in soil and water.

SL316

A Molecular Biotechnology for Removal of Toxic Heavy Metals

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The thiosulfate reductase gene (*phsABC*) from *Salmonella typhimurium* was expressed in *Escherichia coli* in order to produce sulfide from inorganic thiosulfate and precipitate metals as metal sulfide complexes. A 5.1 kb DNA fragment containing the native *phsABC* and a 3.7 kb DNA fragment, excluding putative promoter and regulatory regions were inserted into expression vectors pTrc99A and pJB866, respectively. Upon

expression of *phsABC*, *E. coli* DH5α harboring the *phsABC* constructs showed higher thiosulfate reductase activity and produced significantly more sulfide than the control strain (*E. coli* DH5α) under both aerobic and anaerobic conditions. Among the four constructs, *E. coli* DH5α harboring pSB74 produced the highest level of thiosulfate reductase and removed most of heavy metals from solution under anaerobic conditions. In a mixture of 100 μM each of cadmium, lead, and zinc, the strain could remove 99% of the total metals from solution within 10 hours. Cadmium was removed first, lead second, and zinc last. In contrast, a negative control did not produce any measurable sulfide and removed very little metals from solution. These results have important implications for removal of metals from wastewater contaminated with several metals.

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Carbon Tetrachloride Dechlorination and Metal Chelating Properties of Pyridine-2,6-bis (thiocarboxylic acid) Produced from *Pseudomonas stutzeri* Strain KC

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Pseudomonas stutzeri strain KC was originally characterized as having, under iron-limiting conditions, novel carbon tetrachloride (CCl_4) dehalogenation activity, specifically, a net conversion of CCl_4 to CO_2. The exact pathway and reaction mechanisms are unknown, but chloroform is not an intermediate and thiophosgene and phosgene have been identified as intermediates in trapping experiments. Previous work by others using cell-free preparations has shown that cell-free culture supernatants that have been passed through a low-molecular-weight cutoff membrane can confer rapid CCl_4 transformation ability upon cultures of bacteria which otherwise show little or no reactivity toward CCl_4. We used a cell-free assay system to monitor the complete purification of compounds showing CCl_4 degradation activity elaborated by iron-limited cultures of strain KC. Electerospray tandem mass spectroscopy, NMR spectroscopy, and comparisons with synthetic material have identified pyridine-2,6-bis (thiocarboxylate) (PDTC) as a metabolite of strain KC which has CCl_4 transformation activity in the presence of chemical reductants, e.g., titanium[III] citrate or dithiothreitol, or actively growing bacterial cultures. We have tested the ability of complexes of synthetic PDTC and several transition metal ions, as well as the uncomplexed ligand, to dechlorinate CCl_4 in assays, which include either bacterial cells, chemical reducing agents, or the complex alone with CCl_4. Of the Fe, Ni, Co, and Cu complexes, only the Cu:PDTC complex showed activity under all conditions tested. The reaction between Cu:PDTC and CCl_4 took place both aerobically and anaerobically. Identification of end products and intermediates of the reaction suggested a reaction pathway that includes trichloromethyl radical coupling to one of the sulfur atoms of PDTC, and hydrolysis of the resulting thioester.

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Developmental Pattern Formation Controlled by *patS* in a Cyanobacterium

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