Investigation of gene expression in multi layer biocatalytic coatings and biocatalytic membranes.

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This project involves the investigation of prokaryotic gene expression during starvation and desiccation, latex film coating technology, and characterization of the permeability of coatings that contain viable E. coli or Thermotoga maritima.

Initial work on development of biocatalytic coatings - high volume fraction, latex film entrapped viable bacteria permanently immobilized by a top or sealant polymer coat - used expression of ß-galactosidase activity to follow gene expression of very high cell density thin latex films of E. coli (Swope and Flickinger 1996a, 1996b) on polyester sheets. This early coating method has recently been significantly improved and refined into multi-layer patch coating (Lyngberg et al., 1998) and filament coating methods (Flickinger et al., 1998).

One concept in the design of a hyper stable biocatalyst is film entrapment of viable microorganisms in a dried coating that remain capable of new protein synthesis when induced without significant cell division. Gene expression studies originally carried out in E. coli latex coatings using a variety of stationary-phase inducible or starvation-inducible promoters such as Pssp and PmcB are being continued along with investigation of the dominant forms of RNAP present in non-growing, film entrapped cells. One of the most useful promoters for gene expression in non-growing film-entrapped cells is the mercury sensitive promoter the activity of which is followed using mer/lux fusions (Lyngberg et al., 1998). This has resulted in demonstration of the utility of biocatalytic patch coating as inexpensive, disposable, biosensors using detection of mercury and expression of Lux. The kinetics of induction can be quantitated in a scintillation counter (Lyngberg et al., 1998).

Cryogenic scanning electron microscopy (cryo-SEM) is being used to obtain images of the micro porosity of these biocatalytic coatings and the polymer particle-cell interactions during film formation, particle coalescence, drying, and film rehydration (Thiagarajan et al., 1996; 1998; Huang et al., 1998).

Recently, latex films of the hyperthermophile Thermotoga maritima have been made which are stable at 85° C. These high cell density T. maritima coatings will be used for investigation of the usefulness of this organism to carry out biotransformations at elevated temperatures.