Model Micro-Channels for the Study of Aerobic, Nano-Porous Biocatalytic Latex Coatings

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Abstract:

Embedding bacteria in a nano-porous, self-assembled polymer coating would create highly reactive biocatalysts useful in micro-channel reactors. Our model system is an ~ 65 μm thick nano-porous acrylate/vinyl acetate latex coating containing the bacterium *Gluconobacter oxydans*. *G. oxydans* is a rod shaped obligate aerobe, which can carry out many oxidations, such as D-sorbitol to L-sorbose, using membrane bound dehydrogenases. This oxidation is non-growth associated, oxygen dependant and can be measured using HPLC.

Bioconversion of D-sorbitol to L-sorbose was initially studied using 2.5 cm^2 latex coatings in a non-growth media. A high reaction rate per surface area of coating was observed. Model micro-reactor channels (~ 500 μm to 1000 μm deep) where designed. Microscopic images of a nano-porous coating in ~ 450 μm wide channels where obtained.

A macro-channel, 10 mm wide, in which coated strips of *G. oxydans* can be tested was developed in order to measure the reaction rates accurately with HPLC. The reaction rates obtained in this larger channel, with a three-phase bubbly slug flow, will help us predict biocatalytic activity of *G. oxydans* in < 500 μm micro-channels, and aid us in the engineering of nano-porous biocatalytic coatings for micro-channel bioreactors.

Introduction:

The use of living, catalytically active microorganisms as industrial biocatalyst highly depends on the use of a suitable matrix for immobilization. An appropriate immobilization matrix must enhance catalytic activity, stability and protect from deactivation and degradation of the cells. The use of multilayer coatings and printing technology provides a suitable matrix for embedding of bacteria in a thin, adhesive and nano-porous latex biocatalytic coating. The nano-porous structure is generated by latex polymer particle coalescence and can contain 50% (v/v) of non-growing metabolically active microorganisms. It has recently been demonstrated that the aerobic bacteria *G. oxydans* can be entrapped in this thin nano-porous latex coating and carry out the bioconversion of D-sorbitol to L-sorbose with high efficiency [1]. Freeze fracture CRYO-FESEM images of the previously mentioned coating have been obtained (Figure 1) [1].

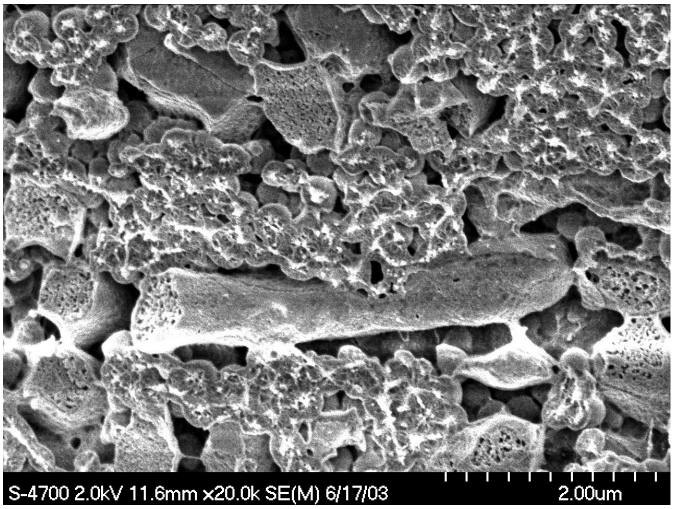

Figure 1: Freeze fracture CRYO-FESEM of nano-porous structure and G. Oxydans fractured cells.

The main objective of this project was to create model micro-channels for the study of *G. oxydans* biocatalytic latex coating. Biocatalytic micro-channel reactors can serve as a model for process intensification in the biotechnological industry. Process intensification is a very popular concept, which refers to the miniaturization and integration of many systems into one. This concept brought us computer micro-chips and, in the biotechnological industry, allows for processes to run at higher temperatures, with shorter residence time, with faster kinetics and with smaller reaction volumes, all these providing for a more efficient and cost-effective process.

Experimental Procedure:

We initially wanted to test the bioconversion capability of *G. oxydans* inside the latex coating. To do so we grew a fresh culture of *G. oxydans* cells in Petri dishes for ~ 72 hours until isolated colonies could be detected. We later transferred one colony of these cells to SYE growth medium and incubated it at 30°C and 90 rpm until an OD_{600} ~ 1.5 was obtained. These cells

where later centrifuged and 2.5 cm^2 coated strips where created using the method developed by Lyngberg et. al [2]. These strips where placed in 60 mm x 15 mm Petri dishes containing the sorbitol rich SPP medium and incubated at 30°C for around 150 hours. Samples where taken every 24 hours to measure the accumulation of sorbose using an analytical HPLC machine.

After the viability of the cells inside the biocatalytic latex coating was determined, we proceeded to create a ~ 450 μm wide micro-channel by stacking a series of ~125 μm polyester sheets and separating them (in order to create the channel) with a previously measured metal spacer. These channels where coated and imaged through phase microscopy (Figure 2).

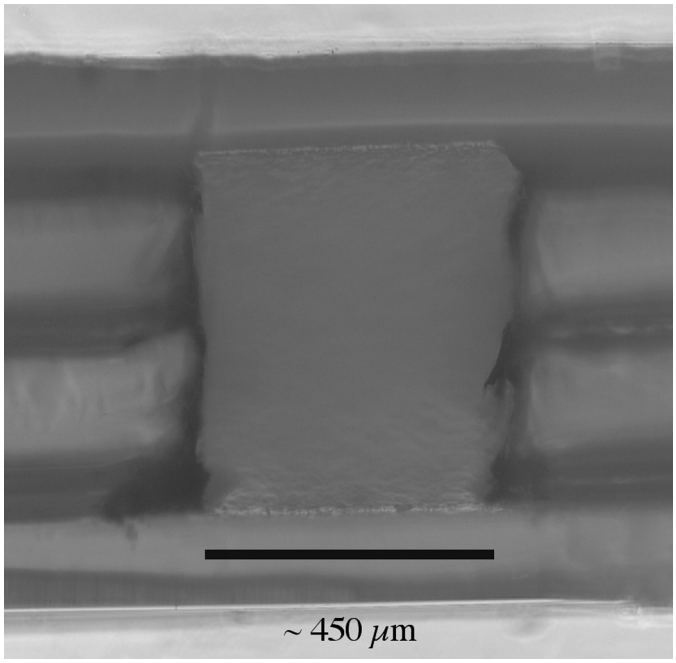

Figure 2: Phase microscopy of uncoated micro-channel.

In order to model the behavior of the ~ 450 μm micro-channels, a macro-channel (10 mm wide) where coated strips of *G. oxydans* can be placed was created. Inside these novel macro-channel 7.5 cm^2 coated strips where placed, and a three phase flow was achieved by the assembly of two peristaltic pumps (air and media) connected simultaneously to the channel inlet. SPP at ~ 27°C was continuously flowed through the channel and the outlet liquid was collected in a flask kept at 4°C. This product was also measured for accumulation of sorbose with and analytical HPLC.

Results and Conclusions:

Top-coated and non-top-coated latex strips were prepared for the bioconversion studies in Petri plates. As observed in Figure 3, bioconversion or accumulation of sorbose increased linearly with time, therefore showing that our strip can remain catalytically active for more than 150 hours. These encouraging results led to the creation of micro-channels for bioconversion studies. Microscopic images of these channels showed a nano-porous latex coating inside the novel channel. No measurable bioconversion was obtained with these channels. In the macro-channel, created to model the micro-channel, accumulation of sorbose was recorded with time. Despite this, more experiments need to be carried out with these macro-channels before results can be presented.

These results serve as an example to the great suitability of using nano-porous coating techniques as an immobilization matrix. Novel micro- and macro-channels where these coating technology for aerobic bacteria can be effectively tested where efficiently developed and may serve in the near future as great examples and models for process intensification purposes.

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References:

[1] Fidaleo, M. et. al Determination of the rate of oxidation of D-sorbitol to L-sorbose by thin bi-layer latex coatings of Gluconobacter Oxydans in microbioreactors.

[2] Lyngberg, O.K. et al. Biotechnol. Bioeng. 62: 44-55 (1999).

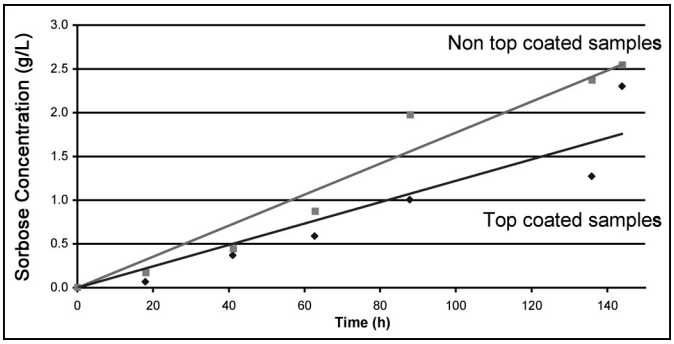

Figure 3 : Accumulation of sorbose with time for 2.5 cm^2 top coated and non-top coated strips in Petri dishes bioreactors.