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POLYSACCHARIDES AND BACTERIAL PLUGGING

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Objective

The objectives of this research are to elucidate and model bacterial transport in porous media, to determine the importance of polysaccharides bridging as a retentive mechanism, and to identify key parameters that influence porous media plugging.

This project has been subdivided into three tasks: Task 1 is the determination of the growth kinetics of the *Leuconostoc* bacteria and how they are affected by 1) the nutrient feed, and 2) surface effects; Task 2 will quantify the importance of polysaccharide production as a cell retention mechanism; and Task 3 is the elucidation of the rate of polysaccharide production and the combined effect that polysaccharide production and cell growth has upon plugging.

Summary of Technical Progress

Batch growth experiments have been conducted to determine the rate of polysaccharide production (dextran) during cell growth. In addition, culture studies have been conducted for the verification of the growth model.

Effect of Yeast Extract Concentrations on Cell Growth

The further verification of the two parameter model, as presented in the past quarterly reports, was the focus of these batch experiments. Three series of batch culture experiments, labelled as experimental series KE-20, KE-21 and KE-23, were conducted with varying yeast extract concentrations and with no saccharides. These experiments were conducted according to the procedure detailed in earlier quarterly reports, with the exception that the inoculum comprised of cells directly cultivated from a stock cultures purchased from ATCC 14935, ie. new inoculum. Cells used in the past kinetic experiments were originally from a stock of ATCC 14935 cells However, they were continuously cultivated as inoculum for the past kinetic experiments, ie. a cultured inoculum.

The results from these experiments and the past growth experiments having yeast extract as the sole substrate are presented in Table 1. As can be seen, the specific rate constants for the cells for the new inoculum are higher than the rates found from the past experiments using cultured cells as inoculum. This indicates that the cells used in the original experiments have undergone a phenotypic alteration. Thus, the model developed could not be verified and requires additional data. In future experiments, the growth of the inoculum will be controlled by minimizing the number of cell transfers before use in kinetic experiments.

Polysaccharide Production

Two additional experimental series have been completed to determine the rate of cellular production of dextran. The first set of experiments consisted of two batch reactors containing 5 and 36 g/L of sucrose and 10 g/L of yeast extract in each. These reactors were inoculated with cells that were continuously cultured as discused earlier. The second experimental series consisted of cells grown in feed containing 5, 10, 30 or 50 g sucrose/L and 10 g yeast extract/L. The inoculum used in the second series of experiments were new cells. In both series the respective inoculums were grown in a glucose-fructose feed for a

24 hour period, centrifuged, and then resuspended in their respective feed at the time of inoculation.

Table 1. Specific growth rate as determined by batch cultures.

Yeast Extract Conc. (g/L)	Experimental Series					
	KE 09	KE 11	KE 16	KE 20	KE 21	KE 23
	Specific Growth Rate, μ (hr^{-1})					
1		0.046	0.105			0.45
5			0.208		0.506	0.55
10	0.205	0.332		0.551	0.548	0.630
20				0.596	0.864	0.680
30				0.555	0.713	

Note that Experimental Series KE 09, KE 11, and KE 16,were inoculated with cells that were continuously cultured.

Cellular dextran production was determined by using the phenol-sulfuric assay[1], while cell counts where determined by Culture Counter, as detailed in the last report. Figure 1 illustrates a growth curve and dextran production curve for cells grown in a feed containing 36 g/L sucrose and 10 g/L yeast extract. The curve demonstrates a lag between dextran production and cell growth, with cells growth always preceding polymer production. This result was typical for all batch experiments. The difference in the duration of time before dextran production and after cell growth indicates that dextran synthesis is a Type III product as typified by Garden.[2] Type III products are known to be produced only when cells reach maturity. Hence the difference between the growth lag and the dextran synthesis lag is the time require for the cell to reach maturity. The corresponding growth rate, growth lag, dextran synthesis lag, and final dextran yields are presented in Table 2.

Two interesting results are presented by the data which should be discussed. The first being the ability of the cells to produce dextran after reaching maturity. Note that time required to reached maturity depends on the concentration of sucrose in the feed. However, at this time there does not seem to be any correlation between the length of this maturity period and the sucrose concentration. The second result is the difference in the cell growth rate and final dextran production yields when inoculated with continuously cultured cells or new cells. The cell's growth rate for both types of inoculums were found to depend on the sucrose concentration in the feed; the rates for the continuously cultured cells were comparatively lower. The lower cell growth rates were accompanied by an increase in the final dextran production yields. This result is expected since cell production and dextran synthesis are competing for sucrose for growth. Hence, even though the data from past experiments inoculated with continuous cultured cells can not be used to model the cells growth, because the cell's have undergone a phenotypic alteration, the data does provide us with information with respect to the ability of manipulating the feed and cells to control the cell's ability to produce polysaccharides. This manipulation will possibly enable us to eventually influence cell transport in porous media by controlling polysaccharide production relative to cell production.

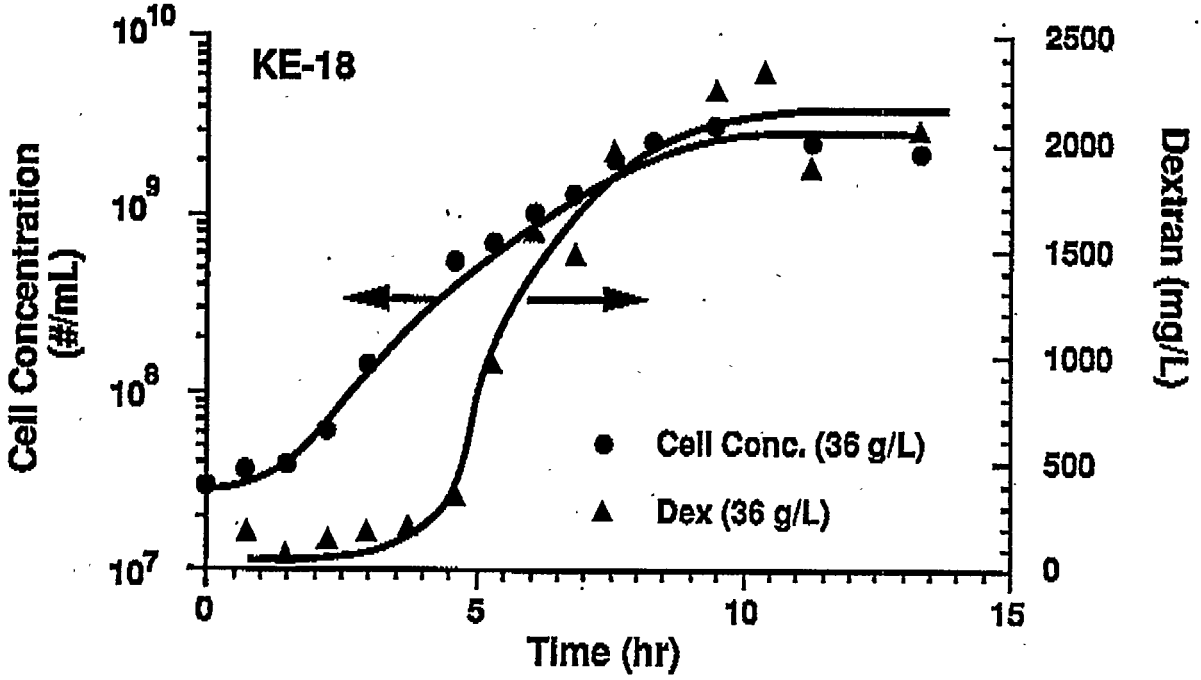

Figure 1. The resulting cell and dextran concentration when Leuconostoc cells are inoculated into a media containing 36 g sucrose/L and 10 g yeast extract/L.

Table 2. Cellular growth rate, growth lag, dextran synthesis lag, and final dextran yields as a function of initial sucrose concentration.

Sucrose Conc. (g/L)	Specific Growth Rate (μ) (hr^{-1})	Growth Lag (hr)	Maturity Time (hr)	Final Dextran Conc. (mg/L)
Inoculum - cultured cells				
5	0.684	1.2	1.9	200
20	0.782	1.4	2.7	2200
36	0.746	1.1	2.6	2200
Inoculum - new cells				
5	0.649	0.27	2.73	138
15	0.832	0.9	2.1	522
30	0.907	0.89	1.6	700
50	0.782	0.51	4.5	1333

References

1) Chaplin, M.F. and J.F. Kennedy, Carbohydrate Analysis, IER Press, Oxford England 1986

2) Bailey, James E. and David F. Ollis, Biochemical Engineering Fundamentals, McGraw Hill Book Co., 1977