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POLYSACCHARIDES AND BACTERIAL PLUGGING

First Annual Report
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Abstract

Before any successful application of a MEOR process can be realized, an understanding of the cells' transport and retentive mechanisms in porous media is needed. Cell transport differs from particle transport in their ability to produce polysaccharides, which are used by cells to adhere to surfaces. Cell injection experiments have been conducted using *Leuconostoc* cells to illustrate the importance of cellular polysaccharide production as a transport mechanism that hinders cell movement and plugs porous media.

Kinetic studies of the *Leuconostoc* cells, carried out to further understand the plugging rates of porous media, have shown that the cells' growth rates are approximately equal when provided with monosaccharide (glucose and fructose) or sucrose. The only difference in cell metabolism is the production of dextran when sucrose is supplied as a carbon source. Experimentally it has also been shown that the cells' growth rate is weakly dependent upon the sucrose concentration in the media, and strongly dependent upon the concentration of yeast extract. The synthesis of cellular dextran has been found to lag behind cell generation, thus indicating that the cells need to reach maturity before they are capable of expressing the detransucrase enzyme and synthesizing insoluble dextran. Dextran yields were found to be dependent upon the sucrose concentration in the media.

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Executive Summary

Bacterial growth and transport in reservoirs can greatly influence enhanced oil recovery. Bacteria can enhance oil recovery by producing gases such as carbon dioxide and hydrogen that increase reservoir pressures and decrease the crude's viscosity. In addition, *in situ* biosurfactant production has been shown to be a viable technique for enhanced oil recovery.by reducing the interfacial tension for the oil-water interface.[1,2] Also, controlled bacterial growth in high permeability regions for the purpose of plugging these regions, can be used to control diversion fluids and thus increase sweep efficiencies. However, all of these Microbial Enhanced Oil Recovery, or MEOR, techniques require control of cell transport within the reservoir. Cell transport can be thought of as being similar to particle transport in porous, that is hydrodynamic, Electrokinetic, Van der Waal's, and Born Repulsion forces effect cell transport in a manner similarly to particles transport. However, bacterial cells also have the additional ability to produce polysaccharides which is known to increase cells' adhesion to surfaces. An understanding of the influence of cellular polysaccharide production on transport is thus needed to implement any MEOR process.

The objective of this work is to focus on the investigation of live bacteria transport and plugging in porous media. This study has started with core plugging experiments where live *Leuconostoc m.* cells were injected into ceramic cores, followed by the injection of a specific nutrient media. *Leuconostoc m.* cells have the ability to produce dextran when provided with sucrose, while any other saccharides will only support reproduction. Thus *in situ* growth of cells in different saccharide media provide a means for comparative experimentation examining the effect of cellular polysaccharide production on cell transport and retention in porous media.

The experiments were conducted on both high and low permeability cores. The high permeability cores had a permeability of 14.7 Darcies and a median pore size of 34 μm, a pore size that is an order of magnitude greater than the bacteria. The results from these experiments have indicated that cellular polysaccharide production is responsible for core plugging and cell retention. Low permeability core experiments, 98 milliDarcies with a median pore size of 4.6 μm demonstrated plugging due to cell growth and polysaccharide production. However, in these experiments, the plugging was more pronounced for the core which was injected with a sucrose-based feed. In summary, the core plugging experiments using these cells have demonstrated that polysaccharides can hinder cell transport and plug the porous media.

Cell growth kinetic experiments have demonstrated equivalent cell growth rates for stoichiometrically equivalent saccharide feeds; sucrose or glucose-fructose feed solutions were found to have approximately equal growth rates. Furthermore, experimentation has demonstrated that the cells' growth rate constant was weakly dependent upon the concentration of sucrose, and strongly dependent upon the yeast extract concentration. This data is currently being used to model cell growth by the implementation of a new kinetic model which assumes cell growth rates to be dependent on two nutrients, with only one nutrient limiting growth. In addition, dextran production yields were dependent on the initial concentration of sucrose in the feed media. Dextran production was found to be a Type III product, a metabolic product that is only produced after a lag period when cells reach the exponential growth phase.

1. Introduction

Bacterial products such as bio-gasses, surfactant, and organic acids are all metabolic products that can enhance oil recovery during water flooding.[1,2] Exploiting the ability of the cells to produce these products is typically called Microbial Enhanced Oil Recovery, or MEOR. However, accompanying cellular production of these beneficial metabolic products are several detrimental effects created by the generation of cells. These detrimental effects include increased biomass (cells and polysaccharides) production which can reduce the permeability of the reservoir, and hydrogen sulfide production, which can damage recovery-well equipment and sour the crude oil. Hence, an understanding of cell interaction within the reservoir is needed, since bacteria are indigenous to the reservoir and will proliferate if provided with the proper growth conditions.[3]

The transport and retention of cells within a reservoir is an important aspect when considering the utilization of any MEOR process. This need for the proper placement of the cells into the reservoir has been a focus of the authors. The importance of polysaccharides, a cellular polymer used by cells for adhesion to surface and for nutrient storage, has been demonstrated as a mechanism that controls cell transport. In addition, it has been demonstrated that cellular production of polysaccharides reduces porous media permeability.

2. Core Plugging Experiments

Research has shown that cellular polysaccharide production is a dominating factor affecting the loss of reservoir permeability. This was demonstrated by conducting *in situ* core plugging experiments using the bacteria *Leuconostoc mesenteroides*. *Leuconostoc m.* bacteria have the unique characteristic of producing dextran, a polysaccharide, only when supplied with the disaccharide sucrose. No other saccharide is known to induce the production of this polysaccharide.[4] Hence, by comparing the permeability reduction of ceramic cores due to *in situ* growth of *Leuconostoc m.* cells in various nutrient media, the importance of polysaccharide production as a cell retentive mechanism, in addition to being a plugging agent has can be demonstrated. Figures 1 and 2 illustrate the results of the plugging experiments for high and low permeability ceramic cores, 14.7 D and 98 mD respectively. The high permeability cores had a median pore size of 33 μm, an order of magnitude larger than the cells' diameter, while the low permeability cores had a median pore size of 4.4 μm, which is the relative size of the *Leuconostoc m.* cells which are typically 0.5 to 1.3 μm in diameter.

The plugging of the ceramic cores resulted from inoculating the cores with cells grown in a glucose-fructose media, followed by the injection of a glucose-fructose or sucrose feed at a rate of 0.1 mL/min (1.18 cm/hr). The pressure drop across the cores was monitored to determine the overall permeability reduction of the cores. The differences in the plugging of the cores as a function of time might be attributed to differences in the cell growth rate due to the use of difference nutrient sugars, or to the ability of the cells to produce polysaccharide. However, batch growth experiments, which are detailed in this report, have demonstrated that the cell's replication rate is roughly equivalent for the two different feeds; hence, the core plugging was attributed to the production of polysaccharides. In addition, when the respective cores are sterilized by gamma radiation, cut axially and stained with crystal violet, the resulting stain pattern demonstrates a hindrance of cell transport due to the cells' ability to secrete polysaccharides. Axial cell density profiles for the high permeability cores are presented in Figure 3. Note that the cell

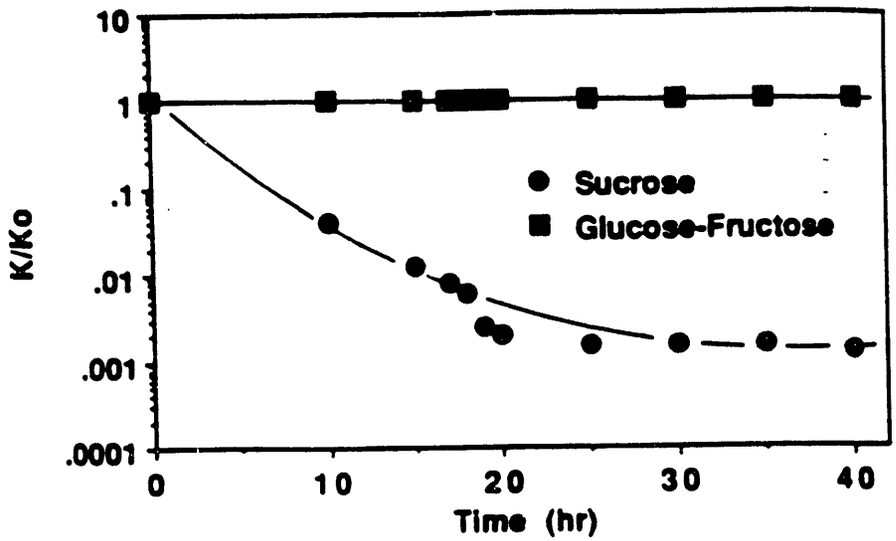


Figure 1: Permeability reduction due to dextran synthesis in high permeability core (14.7 Darcies)

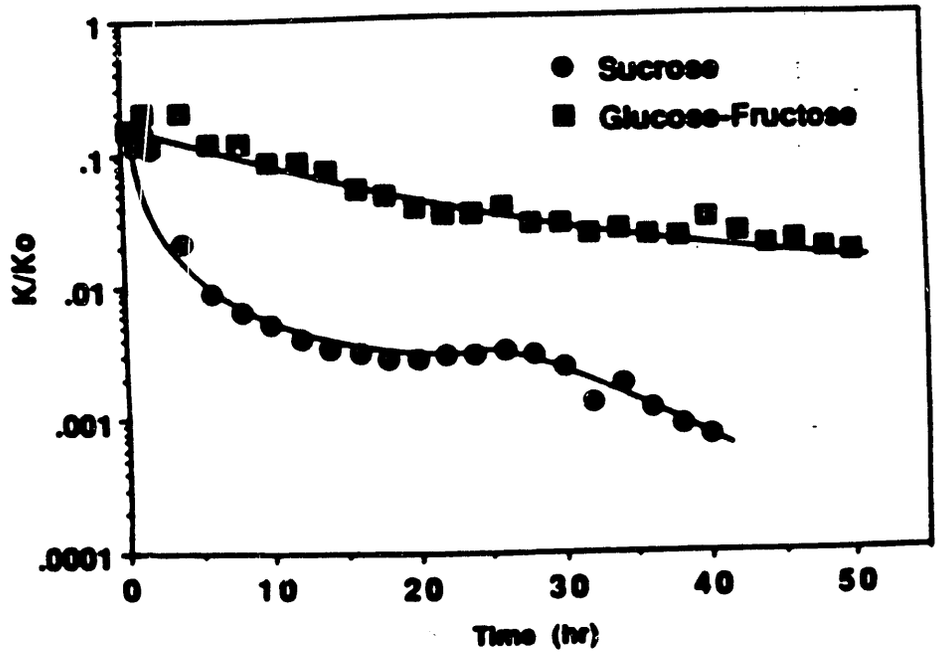


Figure 2: Permeability reduction due to dextran synthesis in low permeability core (98 milliDarcies)

densities for the cores as illustrated by the figure, are presented on a relative scale with the basis being the injection face cell density for the sucrose fed core. Such an observation indicates that polysaccharide production is an important cellular mechanism that can retard cell transport.

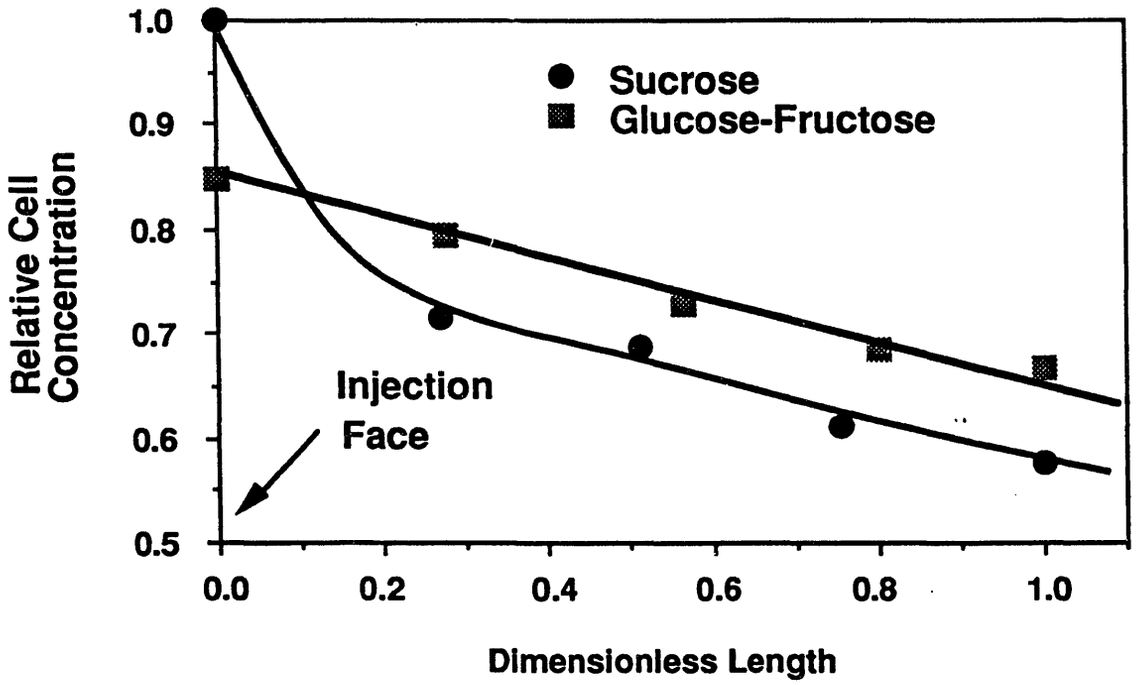


Figure 3. The axial cell concentration for high permeability core.

3. Cell Kinetics

Figure 4 is an illustration of a typical growth curve for bacteria grown in a batch reactor. As presented in the figure, cells experience four phases, of which only exponential growth accounts for actual growth.

3.1 Lag Phase

The first phase experienced by bacteria when inoculated into a new media is the lag phase. The lag phase is the duration of time required for by a cell to produce the needed enzymes for the metabolism of nutrients provided by the media. During this phase the actual number of cells within the batch culture does not change. For this reason the cells are said to be in an unbalanced growth phase.[5]

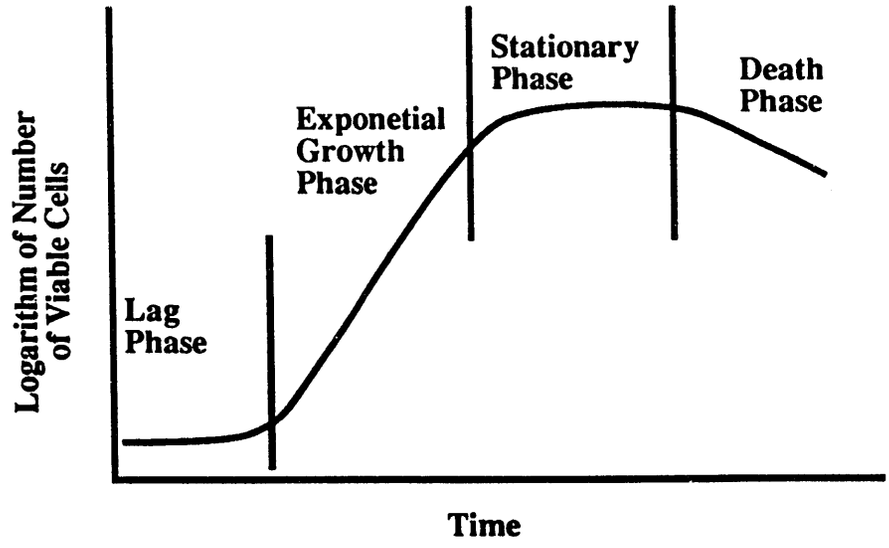


Figure 4: Typical batch growth curve of a microbial culture.

The four factors that can control the duration of lag time are:

- changes in nutrient composition,
- age of the cells,
- loss of vitamins, cofactors and activators from cells due to diffusion,
- volume of inoculum, result in a net loss of metabolic intermediates in the feed.[6]

All of these factors affect the length of time needed by the cell for assimilation, by influencing its ability to metabolize the media nutrients. Changes in the nutrient composition of the media may affect the time before cells can replicate, by forcing cells to first produce the new enzymes needed to metabolize the nutrients.

Cell age affects the duration of the lag time because cellular concentrations of growth enzymes are dependent upon which phase of growth the cells are experiencing when transferred into the new media. Cells have all the required growth enzymes when they are in their exponential growth phase. Hence, cells transferred during the time when they are experiencing exponential growth will have a comparatively shorter lag time relative to cells transferred during a lag, stationary, or death phase.

The volume of the inoculum is another factor affecting the length of the lag phase. Dilution of the inoculum, when suspended in the growth media, causes both the loss of necessary cofactors, vitamins, and activators from the cells by diffusion and the loss of intermediates from the media. The cells must then compensate for this loss before cell replication can occur.

3.2 Exponential Growth

The exponential growth is the phase experienced by the cells when they are capable of constantly assimilating nutrients and replicating. For this reason this phase is also

referred to as the period when balanced growth is occurring, i.e. biomass increase is proportional to increases of all measurable properties of the population such as protein, RNA, DNA, and intracellular water.[5]

During exponential growth the cells are typically modelled by first-order kinetics with respect to cell concentration. However, the specific rate is dependent upon the concentration of the limiting growth nutrient. The Monod equation is typically used to correlate the dependence of the growth rate on the concentration of the limiting nutrient.[6]

3.3 Stationary Phase

The stationary phase of growth is experienced by the cells during depletion of nutrients and/or the accumulation of metabolic toxins causes the deceleration of cell growth. It might be expected that the remaining unmetabolized limiting nutrients will still provide the cells with enough nutrients for replication until the nutrients are completely exhausted. However, nutrient demands by cells for maintenance become significant during this phase because of their high density, thus preventing further cell replication.

Toxin accumulation is the second factor that can force cells into stationary growth. Toxins inhibit cell growth by retarding the enzymatic pathways used by the cells to assimilate the nutrients into cellular material.[6]

3.4 Death Phase

The death phase occurs when the limiting nutrient is completely exhausted and the build up of toxins is high enough to cause the death of cells. According to Bailey and Ollis,[1971] relatively few studies have been conducted on the death phase experienced by cells. Death kinetics are typically modelled by assuming a first order decay.[6]

3.5 Product Production

According to Garden,[1955] all metabolic products can be categorized into one of three groups, as presented in Table 1. The first group includes all products that are the result of direct energy metabolism. For the Leuconostoc mesenteroides, Type I products include lactates, ethanol, carbon dioxide, acetates, and mannitol.[5,7] These products are the result of glucose, fructose, and/or sucrose metabolism. In addition, all of the products are stoichiometrically related to the consumption of sugars.

Type II products include all metabolic intermediates that are the indirect result of cell metabolism, while Type III products include large biomolecules. Type III products tend to be synthesized when the nutrient consumption rate (on a volumetric basis) is a minimum and cell growth has nearly stopped.[6] Polysaccharide (dextran) synthesis most likely exhibit Type III behavior.

Table 1: Fermentation Classifications [6]

Type I	Type II	Type III
Main products appear as a result of primary energy metabolism; desired product may result from direct oxidation of carbohydrate substrate.	Main product again arises from energy metabolism, but now indirectly; reaction rates complex in behavior; several maxima may appear as with Type I reaction	Biosynthesis of complex molecules not resulting directly from energy metabolism; cell and metabolism activities reach maximum early; at later stages formation of desired product occurs

4. Bacterial Kinetics

To date, batch experiments have been conducted to determine the rates of cellular and polysaccharide (dextran) production, and the lag time for growth and polymer production. These experiments consisted of mixing the desired media for cellular growth, sterilizing this media by autoclaving, and then inoculating the media with *Leuconostoc m.* cells. Initially the inoculum consisted of cells originating from a stock of ATCC 14935 cells (American Type Culture Collection). These cells were kept viable for the purpose of growth experiments by continuously transferring the cells into a 10 g yeast extract/L and 15 g sucrose/L medium. For this reason, the inoculum was considered to be continuously cultivated. These cells were used for all experiments coded KE-01 to KE-19. After which all kinetic experiments (KE-20 and on) were inoculated with cells recently purchased from ATCC, and therefore are considered to be a new inoculum. These cells experienced a maximum of two or three transfers from the parent source before being used as inoculum for kinetic experiments. These differences in the inoculum preparation were not expected to influence the cells' growth rate. However, the results from growth experiments indicate that the continuously cultured cells underwent phenotypical alteration, making the data derived from kinetic experiments KE-01 to KE-19 unusable for the estimation of the growth kinetics parameters for the original parent cells. This data, as presented in Appendix I, are valuable in understanding the dependence of cellular polysaccharide production and cell growth on media composition.

4.1 Effect of Carbohydrate

For the growth of *Leuconostoc m.* bacteria, a complex media is typically used, consisting of yeast extract, mono or disaccharides, trace minerals, and a buffer (see Appendix II). Saccharides are provided to the cells by the feed as an energy and carbon source. Hence, providing the cells with different types of saccharides may cause the cells to grow at different rates.

The effect of the type of saccharide provided to the cells was examined by conducting batch experiments. One batch reactor was charged with a media containing yeast extract and sucrose, while the other reactor was provided with a media containing yeast extract, glucose and fructose. It should be noted that sucrose is a disaccharide composed of two monosaccharides, glucose and fructose, connected together by a glycosidic bond. Appendix II lists the recipe used for this kinetic experiment.

Each reactor was inoculated with cells in their stationary phase of growth (approximately 1% volume transfer). Figure 5 is a plot of the cell density as a function of time for two batch cultures. Cell densities were determined spectrophotometrically.

As seen from Figure 5, the growth rate constants, μ (first order rate constant), are comparable: 0.472 hr^{-1} for sucrose feed, and 0.488 hr^{-1} for the glucose-fructose feed. This result was expected, since the two feeds used in the batch experiment were stoichiometrically equivalent. In addition, this result further reinforces the thesis that cellular polysaccharide production was responsible for core damage, as was detailed in the Core Plugging Experiment.

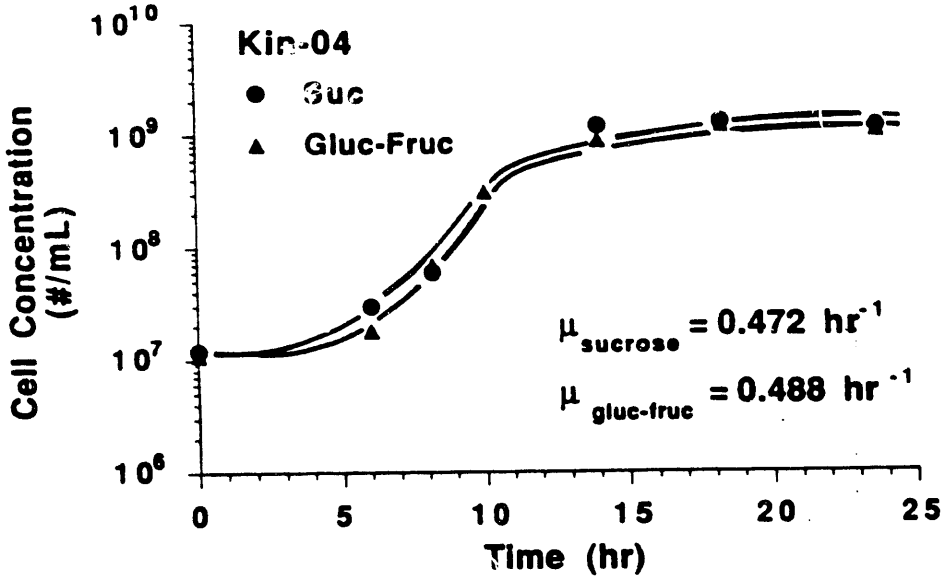


Figure 5: The dependency of the cell's growth rate on a sucrose and glucose-fructose medium (15 g/L sucrose, 7.9 g/L glucose and fructose)

4.2 Effect of Sucrose Concentration

It is a well documented fact that the growth rate of cells is dependent upon the concentration of the limiting nutrient in the feed. For the *Leuconostoc m.* bacteria, it was believed that sucrose was limiting the growth. Hence, a series of batch experiments, each reactor containing a varied initial sucrose concentrations and inoculated with cells, were conducted to determine how the specific rate constant is affected by the sucrose concentration. The inoculation procedure for this experimental phase was altered from procedures typically used for culture experiments; the cells were separated from their original media before they were transferred into the batch reactors. This step was used to emulate the conditions the cells would experience when applied to well injection. For these cultures cells were grown for 18 to 24 hours; which means that the cells were in the stationary phase for growth. For these experiments, and all others following, the cell concentrations were determined by employing a Coulter Counter.

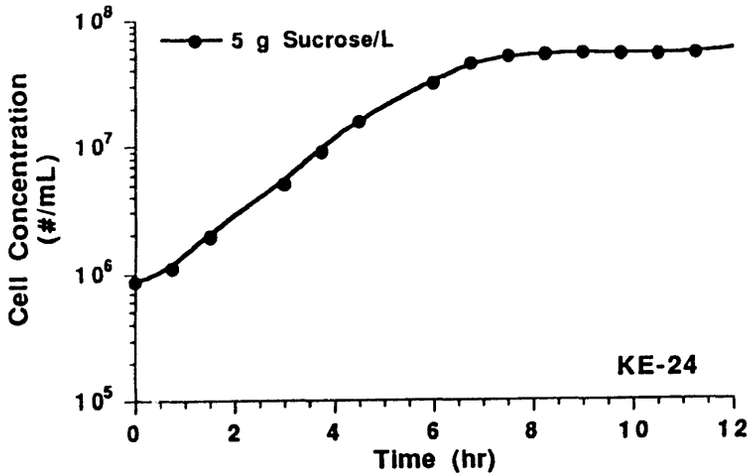
Figure 6 illustrates the results of four batch reactor experiments (KE-24). These batch reactors containing 5, 15, 30, or 50 g sucrose/L and 10 g yeast extract/L. Table 2 lists the respective specific rate constants for the cells estimated from the results of KE-24. In addition, the results of a second set of batch experiments using a medium that contained the same varying saccharide concentrations of 5, 15, 30 and 50 g sucrose/L, but only 0.5 g yeast extract/L are presented in Table 2. The results in Table 2 illustrates, for either series KE-24 or KE-25, the dependency of the growth rate on the sucrose concentration in the media. In addition, both series demonstrate a reduction in the growth rate at high sucrose concentrations. Further experimentation is needed to determine whether this retardation is due to inhibition from sucrose at high concentration, or merely a competition between cell growth and sucrose utilization for dextran synthesis.

Table 2. Specific growth rate as determined by batch culture experiments.in media composed of varying sucrose concentrations and 10 or 0.5 g yeast extract/L.

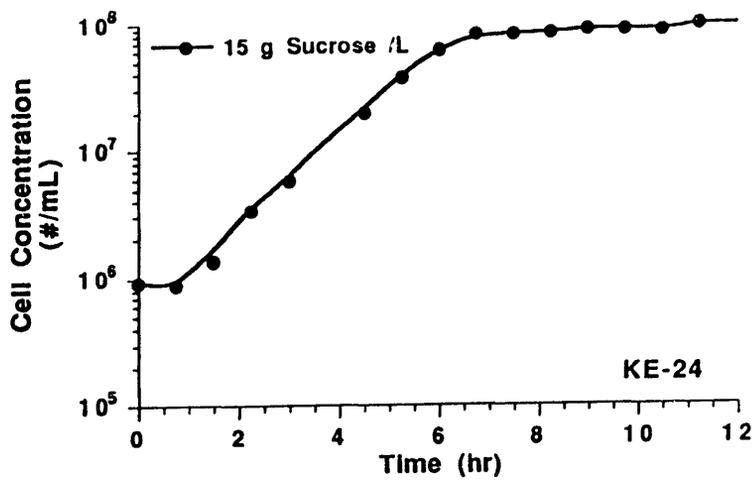
Experimental Series	KE 24	KE 25
Sucrose Conc. (g/L)	Yeast Extract Conc. (10 g/L)	Yeast Extract Conc. (0.5 g/L)
	Specific Growth Rate, μ (hr^{-1})	
5	0.649	0.377
15	0.832	0.447
30	0.907	0.425
50	0.785	0.334

Comparing the results from KE-24 to KE-25 demonstrates that the cells' growth rate is not only affected by the sucrose concentration, but also by the yeast extract concentration in the media. This dependence suggests that yeast extract is also a limiting growth nutrient. By employing the results from KE-09, (growth experiments using continuously cultured cells) the dependence of the growth rate on the sucrose concentration and presence of yeast extract is demonstrated. Three batch experiments were conducted using feed solutions that contained no sucrose, 1.25, and 6.2 g sucrose/L. All three growth media contained 10 g/L yeast extract as detailed in Appendix II. Figure 7 presents the cell concentrations for the three batch experiments as a function of time.

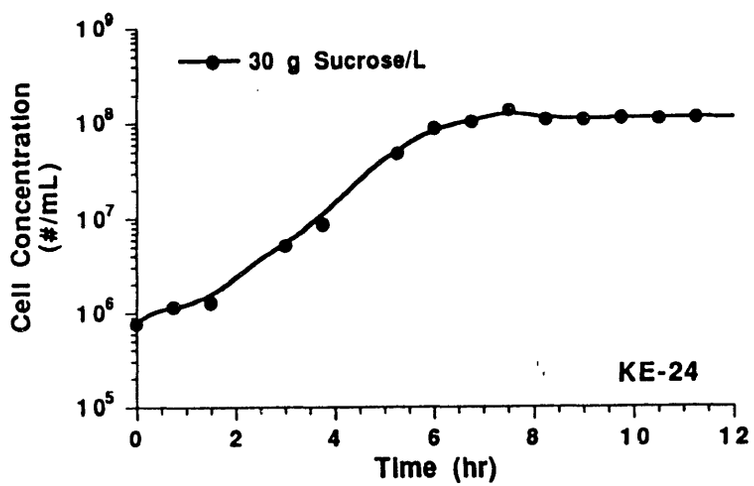
The results from this series of experiments indicate that cell growth rate is dependent upon the presence of both yeast extract and sucrose, and that cell growth can be sustained by the presence of yeast extract alone. For this reason additional batch growth experiment were conducted to determine the importance of yeast extract on the growth rate.



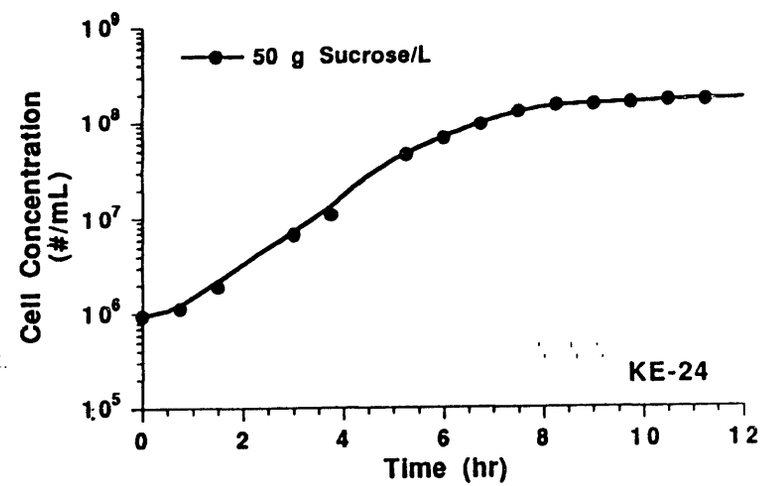
a) Initially 5.0 g Sucrose/L



b) Initially 15 g Sucrose/L



c) Initially 30 g Sucrose/L



d) Initially 50 g Sucrose/L

Figure 6. The dependency of the cell's growth rate on the initial sucrose concentration of 5, 15, 30 and 50 g sucrose/L and 10 g yeast extract/L

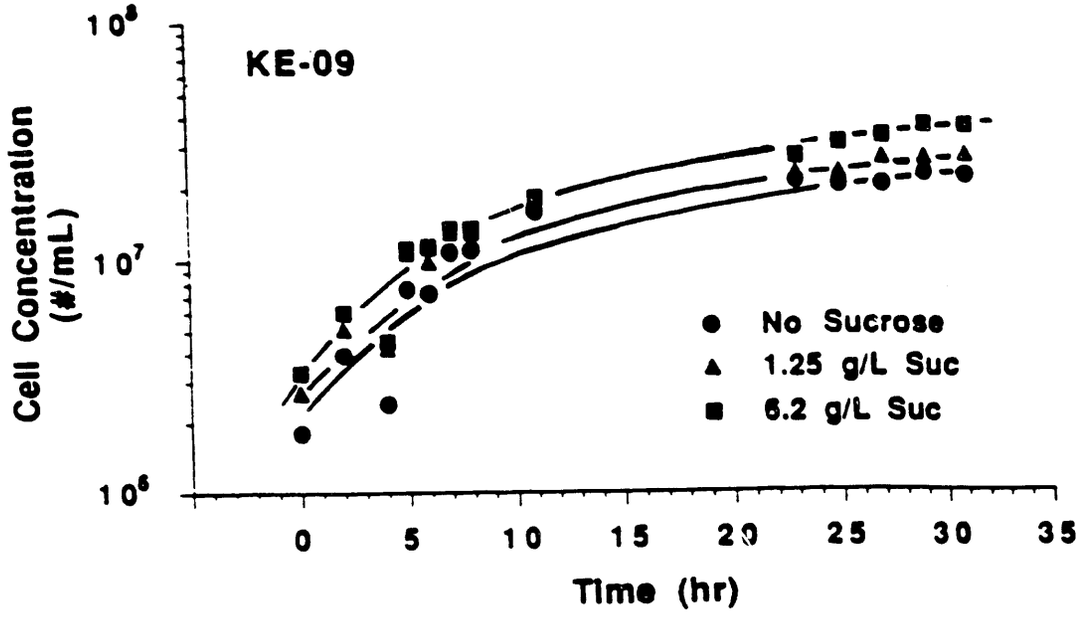


Figure 7: Cell densities for batch reactors containing 0, 1.25, and 6.25 g sucrose/L and 10 g/L yeast extract.

4.3 Effect of Yeast Extract Concentration

The growth of cells on a media containing yeast extract alone indicate that the yeast extract is a critical nutrient component affecting the cells' growth rate. Table 3 presents the growth rate constants for three experimental series. Each batch reactor contained a media of trace mineral, as detailed in Appendix II, and varied yeast extract concentrations. The results indicate that the maximum growth rate of the cells on yeast extract alone is approached when the yeast extract concentration is greater than 5 g/L.

Table 3. Specific growth rate as determined by batch cultures.

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

4.4 Model Development

The ability of the cells to grow on yeast extract alone, without any sucrose, is a unique observation that was not anticipated. This has serious implications with regards to fitting the specific rate constant to a Monod model. The Monod model states that the growth rate is dependent upon the limiting growth nutrient, and can be expressed as presented below.

A cell balance for batch growth follows:

$$\frac{dX}{dt} = \mu X \tag{1}$$

and the specific rate constant (assuming a Monod model) is defined as:

$$\mu = \mu_{max\ s} \frac{C_s}{K_s + C_s}. \tag{2}$$

The constants:

X	- represents the cell concentration,
μ	- represents the specific rate constant,
$\mu_{max\ s}$	- is the maximum growth rate, and
C_s	- is the sucrose concentration.

However, the Monod model does not correctly predict the specific growth rate constant at low sucrose concentrations. Thus, a new model is under development in which yeast extract and sucrose concentrations are the parameters affecting the specific rate constant. This model lends its origins to the two limiting nutrients Monod model, with the exception that sucrose is not truly a limiting nutrient, but its presence in the media is known to accelerate bacterial growth. The proposed model is as follows:

$$\mu = \mu_{max}\, F(C_y)\, G(C_s) \tag{3}$$

where

$$F(C_y) = \frac{C_y}{K_y + C_y} \tag{4}$$

and

$$G(C_s) = \frac{C_s + B}{K_s + C_s}. \tag{5}$$

The constants:

C_y	- is the the concentration of yeast extract, and
K_y	- is the Michaelis-Menten constant for yeast extract.

An additional constant B is incorporated into $G(C_s)$ to describe the dependency of cell growth on the yeast extract when sucrose is not present in the feed. Thus the constant B is defined to be:

$$B = \frac{Ks\ \mu\ max\ y}{\mu\ max} \tag{8}$$

where $\mu_{max\ y}$ is the maximum growth rate of the cells on yeast extract alone i.e.. $C_S=0$ and $C_y >> K_y$, and

μ_{max} is the maximum growth rate on yeast and sucrose i.e.. $C_S>>K_S$ and $C_y >> K_y$.

Note that $G(C_S)$ does not account for retardation of growth due to high sucrose concentrations. Since further experimentation is needed to prove that inhibition is occurring, and due to the increased complexity of a model incorporating inhibition, the current model will be fitted to data obtained from batch experiments where sucrose concentrations were maintained at 30 g sucrose/L or less.

From this model it is noted that K_y must also be estimated. To determine this parameter, and K_S, cell growth experiments using a CSTR (Continuous-Stirred Tank Reactor) will have to be conducted. Initial estimates of the Michaelis-Menten parameters can be determined by batch experiments, since batch reactors are experimentally less complicated to operate then a CSTR. The estimates for K_S and K_y are 2.5 g sucrose/L and 0.5 g/ yeast extract/L, respectively. CSTR experiments are needed for final determination of the Michaelis-Menten parameters, since the nutrient concentration in batch reactors are changing with cell growth.

Estimates of μ_{max} and $\mu_{max\ y}$ can be determined from the batch experiments were the medium contains a high concentration of sucrose and yeast extract, and yeast extract only, respectively. The maximum growth rate on yeast extract alone, as presented in Table 3, indicates that $\mu_{max\ y}$ is approximately 0.7 hr^{-1}. The maximum growth rate on yeast extract and sucrose tends to be approximately 0.98 hr^{-1}. These results indicate that cell growth rates are strongly dependent upon the yeast extract concentration and weakly dependent upon the addition of sucrose to the media.

4.5 Dextran (Polysaccharide) Production

The core plugging experiments have demonstrated the importance of polysaccharide production a core plugging agent. To explain these plugging results and to model the plugging phenomena in the near future requires a thorough understanding of the polymer production kinetics. Thus batch experiments have also been used to determine the rate and occurrence of dextran synthesis by the cells.

These batch experiments were conducted by inoculating the reactor with *Leuconostoc m.* cells, followed by sample withdrawal for the purpose of assaying for cell density, and dextran concentrations. Dextran was assayed by using dextranase to break the insoluble dextran into soluble oligosaccharides.[9] The concentration of these oligosaccharides was then determined by employing the phenol-sulfuric acid assaying technique, as detailed by Chaplin.[8]

Figure 8 illustrates the growth and dextran production curves for a typical batch experiments, while Figure 9 present the dextran production curves for four batch reactors. As presented by Figure 8, a lag occurred before cell growth and dextran production commenced. The production of dextran always followed cell replication. The difference in the duration of the lags, dextran synthesis lagging behind cell replication, indicates that dextran is a Type III product. Type III products are known to be produced only when

cells reach maturity [8], and are modelled by the following equation as proposed by Blanch and Rogers [1971]:

$$\frac{dP}{dt} = Y_{p/x} \frac{dX_p}{dt} \qquad (9)$$

$$\frac{dX_p}{dt}_t = \frac{dX}{dt}_{t-t_{mat}} \qquad (10)$$

where :

$Y_{p/x}$ - is the dextran to cell yield,

t_{mat} - is the time period require for the cells to reach maturity, i.e. the time difference between cell replication and dextran production,

P - is the product concentration, and

X_p - is the concentration of the mature cells.

Table 4 lists the cell growth, dextran lag times, and the time duration required for the cells to reach maturity. From the data collected to date, there appears to be no correlation between the start of dextran synthesis and the concentration of sucrose present in the medium. However, for KE-24, there does seem to be a correlation between the growth rate and the duration of the time to reach maturity. As the growth rate increases, the time required for the cells to reach maturity decreases. This result may be explained by the fact that as more cells are produced, the number of cells reaching maturity increases and thus dextran synthesis can begin. This hypothesis will have to be further studied before any final relationship can be drawn.

Table 4. 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 - new cells (KE-24)				
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
Inoculum - cultured cells (KE-15 and KE-18)				
5	0.684	1.2	1.9	200
20	0.782	1.4	2.7	2200
36	0.746	1.1	2.6	2200

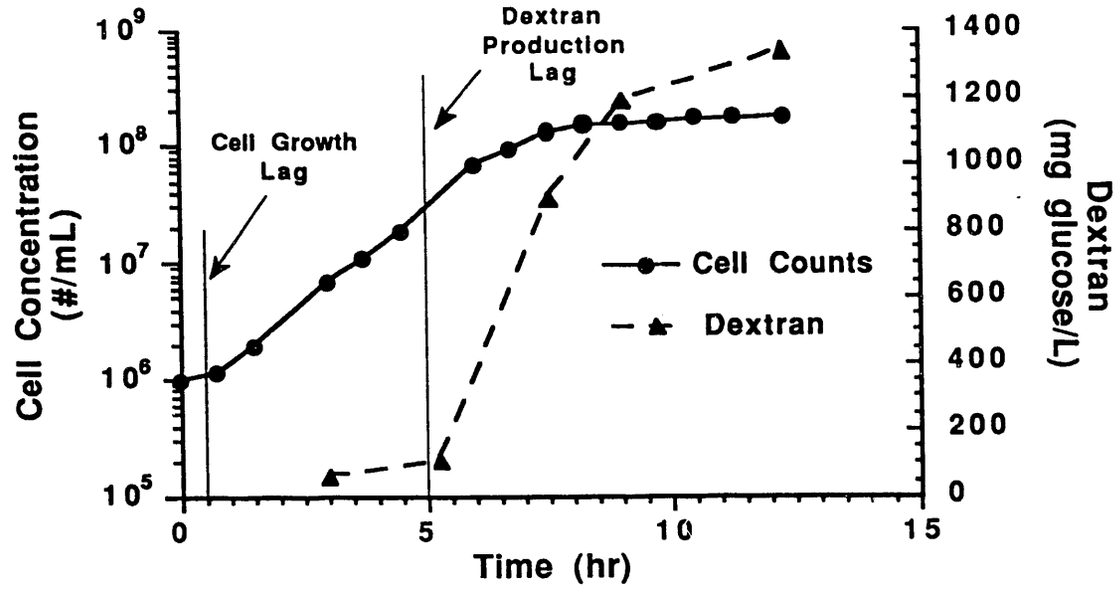


Figure 8. Cell and dextran production curves on media containing 50 g Sucrose/L and 10 g yeast extract/L.

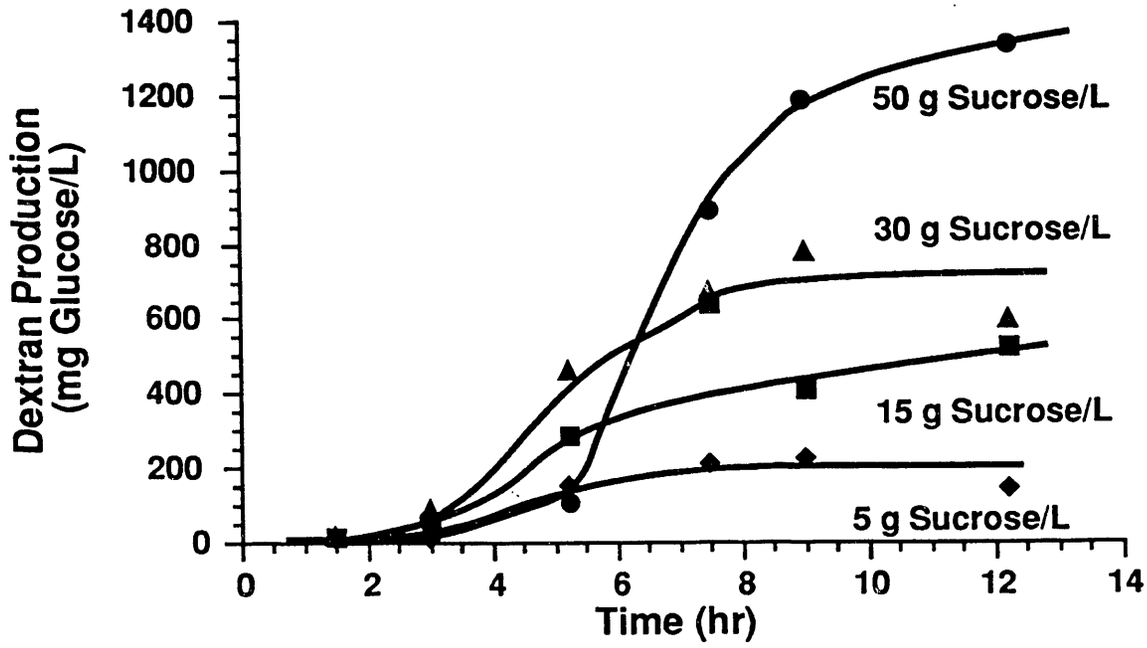


Figure 9. Dextran production on media containing 5, 15, 30 and 50 g sucrose/L and 10 g yeast extract/L

Table 4 also lists the final dextran yields for the four batch experiments. As can be seen, the reactor which was charged with a low concentration of sucrose yielded 138 mg dextran/L. As the concentration of sucrose in the feed increased, the final amount of dextran increased. The kinetic results for a series of batch experiments that were inoculated with continuously cultured cells are also present in Table 4. Comparing these results (KE-15 and KE-18) with the results based on the new inoculum provides interesting information regarding the difference in the cell growth rate with relation to the final dextran production yields. Note again that KE-15 and KE-18 experimental runs used cells which were continuously cultured, while KE-24 used cells which were new. The cells' 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; an expected result since cell production and dextran synthesis are competing reactions. Hence, the data from past experiments inoculated with continuous cultured cells can not be used to model the cells' growth rate because the cells have undergone a phenotypic alteration. The data do provide relevant information with respect to the manipulation of the feed to control the cells' ability to produce polysaccharides. This manipulation will possibly enable us to influence cell transport in porous media by controlling polysaccharide production relative to cell production.

5. Conclusions

The results of past core plugging experiments show that cellular polysaccharide production is a significant factor in formation plugging and hindering cell transport. The kinetic study has aided in the development of a new specific rate law model. The rate law is similar to a Monod model that characterizes growth as being dependent upon sucrose and yeast extract. Yeast extract concentration was found to have the most profound influence on cells' growth rate. The dextran production experiments have demonstrated that cells need to reach maturity before they can produce dextran. The time needed for the cells to reach maturity was found to depend on the initial concentration of sucrose in the feed, although at this point there doesn't seem to be any correlation. In addition, the final polymer production yields have shown that dextran production is dependent upon the availability of sucrose in the feed.

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Appendix I

Appendix I provides three tables summarizing the result of the initial kinetic experiments which used continuously cultured cells for the inoculum. Tables I.1 and I.2 also present results based on experiments that used new cells as the inoculums. These results are provided for comparison purposes, illustrating how the continuously cultured cells underwent a phenotypical alteration. In summary, the comparison demonstrates that the continuously cultured cells' growth rates were slower, and the lag times before cell growth started was longer. Table I.3 presents the earlier estimates of the parameters for the proposed model, which are based on the batch results using continuously cultured cells as inoculum.

Table I.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 continuously cultured cells.

Table I.2 Specific growth rate as determined by batch culture.in media composed of varying sucrose concentrations and 10 g yeast extract/L.

Sucrose Conc. (g/L)	Experimental Series				
	KE 03	KE 15	KE 18	KE 24	KE 25§
	Specific Growth Rate, μ (hr^{-1})				
1	0.350				
5	0.652		0.684	0.649	0.377
10	0.70				
15	0.699			0.832	0.447
20		0.785			
30				0.907	0.425
36			0.746		
50				0.785	0.334

Note - that Experimental Series KE 03, KE 15, and KE 18 were inoculated with continuously cultured cells.

§ - KE-25 contained only 0.5 g yeast extract/L

Table I.3: Kinetic Growth Rate Parameters

μ max	0.7 hr^{-1}
μ max y	0.205 hr^{-1}
K_s	2.43 g/L
B	0.712 g/L
K_y	1 g/L

Appendix II

Table II.1: Medium for Carbohydrate Experiments

Tap Water	1000 mL
Tryptone	20 g
Yeast Extract	5 g
NaCl	4 g
Sodium Acetate	1.5 g
Ascorbic Acid	0.5 g
Trace Elements (Ca, Mn,Fe,Mg)	
Carbohydrates	
Sucrose	15 g
or	
Fructose	7.9 g
Glucose	7.9 g

Yeast extract (and tryptone) are supplied to the cells as the sources of amino acids, purines, pyrimidines, and vitamins, which are needed by the cells for growth since *Leuconostoc* cells can not synthesis these compounds for themselves.[5]

Table II.2: Medium for Kinetic Experiments

Tap Water	1000 mL
Yeast Extract	10 g
NaCl	4 g
Sodium Acetate	5 g
Ascorbic Acid	0.5 g
Potassium Phosphate dibasic	1 g
Trace Elements Ca, Mn,Fe,Mg	
Sucrose	

END

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