Proceedings of the Fifth Biochemical Engineering Symposium

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PROCEEDINGS OF THE FIFTH
BIOCHEMICAL ENGINEERING SYMPOSIUM

Report No. 66

L. T. Fan

L. E. Erickson

April 26, 1975

INSTITUTE FOR SYSTEMS
DESIGN AND OPTIMIZATION

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Kansas State University
Manhattan, Kansas
April 26, 1975

L. T. Fan and L. E. Erickson, Editors
This report presents the proceedings of the Biochemical Engineering Symposium held at Kansas State University, April 26, 1975. Since a number of the contributions will be published in detail elsewhere, only brief summaries of each contribution are included here. Requests for additional information on projects conducted at Iowa State University should be directed to Dr. Peter J. Reilly, and those at Kansas State University to the editors.


L. T. Fan
L. E. Erickson
Editors
BIOCHEMICAL ENGINEERING SYMPOSIUM
April 26, 1975
Room 103, Chemical Engineering Hall
Kansas State University

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ENZYMATIC BREAKDOWN OF HEMICELLULOSE

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The possibility of converting plant cellulosics to glucose has attracted the attention of scientists and engineers for many years. However, cellulose is only a part of the story, since with the exception of cotton, all cellulosic raw materials also contain hemicellulose in large proportions. It is toward utilization of agricultural byproducts, specifically corn cob and hull hemicellulose (xylan), that much of the ongoing bioengineering work at ISU is directed.

The three major constituents of plant structural tissues are cellulose, hemicellulose, and lignin, which may be thought of as serving structural, matrix, and encrusting roles, respectively. Wood which has been delignified and cleared of lipid materials still contains essentially the entire carbohydrate plant fraction and has been termed hollocellulose. Further treatment of hollocellulose with dilute aqueous alkali separates the insoluble cellulose from the alkali-soluble hemicellulose, as represented in Figure 1. However, the cellulose, hemicellulose, and lignin fractions exist in such close physical association that cellulose obtained in the above manner still contains considerable hemicellulose and lignin impurity.

In contrast to cellulose, which has long been known to consist of linear (unbranched) homopolymers of β-D-(1→4)-linked D-glucopyranose with an extremely high degree of polymerization (approximately 10,000), hemicellulose have been found to vary tremendously in structure and composition among species and within different tissues of the same organism. A variety of different monosaccharide constituents or residues make up the various hemicelluloses, with sometimes 5 or 6 different residues occurring in a single hemicellulose molecule. Furthermore, hemicelluloses generally have low degrees of polymerization (d.p.), ranging from 50 to 200 units only. Relatively low molecular weight, the presence in nearly all types of hemicellulose of some degree of branching, as well as the occurrence in many hemicelluloses of charged uronic acid residues together account for the greatly increased solubility of hemicelluloses over that of native cellulose.
The main constituent sugars found in plant hemicelluloses are shown in Figure 2. All are closely related structurally to D-glucose (dextrose) probably reflecting their biochemical origin. The sugar or sugars which make up the main chain or backbone of the hemicellulose molecule form the basis for nomenclature of the various hemicellulose groups. For example, those with a main chain composed of β-(1→3)-linked galactose residues are termed galactans, while hemicellulose molecules with main chains consisting of β-(1→4)-linked mannose or glucose and mannose are termed mannans and glucomannans, respectively.

Since annual plant hemicellulose is essentially devoid of mannose-based hemicelluloses, their hydrolysis is not considered further. By far the most abundant family of hemicelluloses found in the cereal grains are those based upon a main chain of β-(1→4)-linked D-xylose -- xylans. Most xylans, however, also bear side chains of other sugar residues, most commonly 4-O-methylα-D-glucopyranosyl uronic acid and α-L-arabinofuranose. The latter is somewhat unique in that it occurs in the five membered, furanose-ring form. Although the overall structures have not been determined for most xylans, the predominant linkages and common structural features are known. The uronic acid residues are apparently present solely as single unit branches on a xylosyl chain or as nonreducing end units on the xylose chains. Although most commonly linked to C-2 of D-xylose residues, C-3 linkages have occasionally been observed. L-Arabinose also occurs commonly as single-unit side chains attached directly to the xylose backbone. In contrast to the uronic acid residues, arabinose to xylose linkages are generally α-(1→3). Also, in some cases (as cob hemicellulose B), further side chains of xylose units are attached to the arabinose branch points, linked α-(1→2).

A hypothetical arabinoglucuronoxylan structure, illustrating the known modes of linkage, is shown in Figure 3.

Over the past thirty years work characterizing cereal grain (especially corn) hemicelluloses has resulted in a fairly clear picture of corn xylan constitution and structure. While all cereal grains (Graminae) hemicelluloses are thought to possess nearly identical sugar compositions, the legumes (Leguminosae) possess strikingly different hemicellulose compositions. Thus, findings concerning the hydrolysis of corn xylan would be likely to apply closely to wheat and oat straw and bagasse, but perhaps not to leguminous plants. Three major types of corn cob xylan (isolated on the basis of their differing solubilities) have been characterized, differing in d.p., constituent sugar residues, and in degrees of branching. These fundamental differences in structure are reflected in their varying solubilities. Designated linear-A, linear-B, and branched-B, each cob xylan is briefly characterized in Figure 4.

Finally, a significant point of cob xylan chemistry concerns the resistance of the three major linkages present to acidic hydrolysis. While the arabinofuranosyl to xylose linkages have been observed to be extremely labile to even mildly acidic conditions (characteristic of furanosyl linkages), the uronic acid side chains are stable to all but the most drastic treatments. The xylose to xylose bonds possess intermediate acid stability similar to that shown by cellulose.
Nature of course has provided a wide variety of enzymes capable of hydrolyzing hemicellulose. The wood-rotting fungi and cellulolytic bacteria are usually excellent sources of hemicelluloses, since hemicellulose almost always is present with cellulose. Of the two sources of the enzymes, the former is generally preferred, as the fungal enzymes have often been observed to be more stable at elevated temperatures and low pH and hence more active.

The carbohydrase enzymes are commonly classified by their specificity toward substrate and linkage within substrate. The polysaccharide hydrolases can be largely divided into 3 groups based on their action patterns on different substrates. As shown in Figure 5, the three classifications consist of the endo-hydrolases, the exo-hydrolases, and the glycoside hydrolases. The endo-hydrolases act almost entirely on interior linkages of long-chain oligomers or polymers. The exo-hydrolases, while also hydrolyzing long chain oligomers and polymers, act by removing single-unit sugar residues from the non-reducing end of the glycoside chain. Finally, the glycoside hydrolases, while also cleaving single residues from the non-reducing end of the substrate, differ from the preceding two groups in that short-chain oligomers (and sugars linked glycosidically to certain non-carbohydrate moieties) are greatly preferred over high d.p. molecules. Figure 6 lists some characteristics of the three hydrolase types and shows their primary points of distinction.

Due to the complexity of structure shown by most hemicelluloses, the presence of an endo-hydrolase acting in concert with several distinct glycoside hydrolases has been shown to be necessary for efficient, complete hydrolysis of the hemicellulose to its constituent sugars. The endo-hydrolase, xylanase in the case of xylan hydrolysis, serves to bring about the initial solubilization depolymerization of the macromolecular xylan. Since attack by xylanase is prevented within several units of branch points, the action of xylanase alone ultimately results in low d.p. oligosaccharides of widely varying structure. In the case of corn xylan, further hydrolysis is afforded by an \(\alpha\)-L-arabinofuranosidase and a \(\beta\)-D-glucuronidase which serve to remove the arabinose and glucaronic acid side chains, respectively, and by a \(\beta\)-D-xylosidase which functions to hydrolyze the short, unbranched xylosyl chains quantitatively to xylose. Oddly, even the most potent cellulolytic and hemicellulolytic organisms do not possess the full complement of hemicelluloses, as the \(\beta\)-glucuronidase is often lacking. Such organisms accumulate various acid oligosaccharides, which cannot apparently be further metabolized by the same organism.

In view of the ease of acid hydrolysis of the arabinosyl side chains, only the endo-functioning xylanase and the \(\beta\)-xylosidase are required to effect nearly complete (85-95%) hydrolysis of corn cob xylan. These enzymes obtained from fungal organisms share many common characteristics, such as low pH optima and isoelectric point (\(\sim 4\)), good thermal stability, as well as a strong physical and/or covalent association with carbohydrate. Despite these similarities, the endo-xylanase (\(M_w \sim 30,000\)) differs tremendously from the \(\beta\)-xylosidase (\(M_w \sim 200,000\)) in size and subunit structure, providing a ready means of separating the two hydrolases. The low molecular weight of the endo-xylanase presumably aids the enzyme in gaining access to the tightly-packed, insoluble xylan under naturally occurring conditions.
The major problem faced in the commercial utilization of cellulolytic and hemicellulolytic enzymes is that of allowing the enzymes access to their highly insoluble, high molecular weight substrates. Although accessibility is also a problem with acidic hydrolysis, in the case of enzymic degradation the problem is compounded by the high molecular weight of the catalyst itself. Fortunately, the cereal grasses may well be more accessible than more highly lignified tissues, such as wood.

A possible process in which the various hemicellulases could be utilized to produce free, neutral sugars is illustrated in Figure 7. In this system, the xylanase activity as well as the higher molecular weight xylan oligosaccharides would be retained by ultrafiltration. The solubilized xylan could then be completely saccharified by the xylosidase following debranching by arabinosidase and glucuronidase. These enzymes could be retained by covalent attachment to an insoluble support. Finally, removal of the glucuronic acid groups could be achieved by ion exchange. The free neutral sugars produced could be further processed for human consumption (reduction of xylose to xylitol) or used directly as an animal feed supplement or fermentation substrate, as in the production of glucose isomerase.

Acknowledgement - This work has been supported by NSF Grant APR74-20111.
Figure 1

MAJOR COMPONENTS OF PLANT CELL WALLS

Holocellulose

- 1. CELLULOSE MATRIX (alkali insoluble) Polysaccharide

- 2. HEMICELLULOSE (alkali soluble)

3. PECTIC POLYSACCHARIDE (neutral soluble)

Nonpolysaccharide

- 4. STRUCTURAL PROTEIN

- 5. LIGNIN

- 6. Other (lipid, ash, etc.)
RESIDUES:

X - XYLOSYL
A - L-ARABINOFRANOSYL
GlcA - GLUCURONOSYL (OR 4-O-METHYL)
GaLA - GALACTURONOSYL
Figure 4

CORN COB XYLANS

LINEAR A

- D - XYLOSE
- L - ARABINOSE
- D - GLUCURONIC ACID

d.p. ~ 200
UNBRANCHED

LINEAR B

- D - XYLOSE
- L - ARABINOSE

d.p. ~ 100
ESSENTIALLY UNBRANCHED
α - (1 → 2) XYLOSYL - ARABINOSE LINKAGES

BRANCHED B

- D - XYLOSE
- L - ARABINOSE
- D - GLUCURONIC ACID
- D - GALACTOSE

d.p. ~ 150
HIGHLY BRANCHED
GALACTOSE PRESENT
Figure 5

TYPES OF HYDROLASE ENZYMES

α, β - GLYCOSIDE HYDROLASES

β - D - XYLOSIDASE (XYLOBIASE)
α - L - ARABINOFLURANOSIDASE
CELLOBIASE
MALTASE

α - GLYCAN EXO-HYDROLASES

EXO-CELLULASE
GLUCOAMYLASE

α, β - GLYCAN ENDO-HYDROLASES

XYLANASE (ENDO)
CX - CELLULASE
α - AMYLASE
**Figure 6**

**COMPARISON OF HYDROLASE TYPES**

<table>
<thead>
<tr>
<th>SPECIFICITY FOR:</th>
<th>GLYCOSIDE HYDROLASES</th>
<th>EXO-HYDROLASES</th>
<th>ENDO-HYDROLASES</th>
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<tr>
<td>LINKAGE</td>
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<td>HIGH</td>
<td>HIGH</td>
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<tr>
<td>GLYCOSYL RESIDUE</td>
<td>HIGH</td>
<td>HIGH</td>
<td>ABSOLUTE</td>
</tr>
<tr>
<td>AGLYCONE</td>
<td>LOW</td>
<td>HIGH</td>
<td>HIGH</td>
</tr>
<tr>
<td>RATE OF OLIGOMER HYDROLYSIS</td>
<td>DI&gt;POLYMER</td>
<td>POLYMER&gt;DI</td>
<td>POLYMER&gt;TRI</td>
</tr>
<tr>
<td>CONFIGURATION OF ANOMERIC - OH</td>
<td>RETAINED</td>
<td>INVERTED</td>
<td>RETAINED</td>
</tr>
<tr>
<td>INHIBITION BY 1,5-GLUCONOLACTONE</td>
<td>K₁ LOW</td>
<td>K₁ HIGH</td>
<td>NO INHIBITION</td>
</tr>
</tbody>
</table>
Figure 7

Process for Obtaining Sugar from Hemicellulose

Neutalized Caustic from Pulping Stream
(Solubilized Lignin & Hemicellulose)

Solubilization
Xylanase

Phenolic Bleed

Ultrafiltration Membrane

Soluble Xylo-Oligosaccharides
(d.p. < 7)

Separation
Ion - Ex.

Saccharification
Xylosidase

Immobilized Enzyme Reactors

Debranching
Arabinosidase
Glucuronidase

Free Neutral Sugar

Acidic Sugars & Oligosaccharides
In hydrocarbon fermentation, the processes for the transport and uptake of hydrocarbon to cells are important because these processes can limit microbial growth.

Three possible pathways for hydrocarbon transport to microorganisms have been proposed. The first is the pathway via direct contact of the microorganisms with the large oil drops, the second is via contact with the fine oil droplets and the third is uptake of dissolved hydrocarbon in aqueous medium. However, it has frequently not been clear experimentally which pathway is dominant in hydrocarbon fermentation. This problem has been approached by two different methods; one is biotechnological method and another is biochemical one. This paper deals with biochemical aspects of hydrocarbon uptake by microbial cells.

Direct contact of microbial cells with large oil drops and the formation of flocs which consist of microbial cells, oil drops and air bubbles are often observed in hydrocarbon fermentation. On the other hand, good growth can be sometimes observed without the formation of flocs. These different phenomena may depend on the properties of microbial cells. Generally speaking, hydrocarbon utilizing yeasts have stronger affinity to oil than those of hydrocarbon non-utilizing yeasts, some have much stronger affinity to oil while some have comparatively weak affinity. For example, Candida petrophilum has strong affinity to oil, but the affinity of C. Lipolytica seems to be comparatively weak.

It is important for understanding hydrocarbon uptake by microbial cells to know how the flocs are formed and how fine droplets are formed from large oil drops when these droplets are utilized by microorganisms. It has been suggested that extracellular products containing lipid or fatty acids may take part in the formation of flocs.

There have been some observations that dispersion of oil in the aqueous broth is improved and the interfacial area increases as fermentation proceeds. It has been reported by Calderbank that Sauter mean diameter of oil drops strongly depends on interfacial tension. Prokop et. al. and Velankar et. al. measured the interfacial tension of culture broth and found that interfacial tension decreased during fermentation, however, their experimental results are conflicting. Prokop et. al. observed a remarkable decrease of interfacial tension at the beginning period of cultivation of Candida lipolytica and a gradual increase as fermentation proceeded. On the other hand, Velankar found that the interfacial tension decreased gradually during fermentation by Pseudomonas aeruginosa.
Erickson et. al.\(^7\) recently emphasized the importance of interfacial tension of culture broth for understanding hydrocarbon fermentation. Thus, the change of interfacial tension of culture broth of \textit{C. lipolytica} was pursued periodically. Interfacial tension was measured by du Nouy's method after separation of cells from the broth.

Fig. 1 shows growth, surface tension of aqueous phase and oil phase, and interfacial tension between aqueous phase and oil phase during fermentation. Surface tension of oil phase was almost constant through whole fermentation. On the other hand, surface tension of aqueous phase and interfacial tension decreased gradually as cultivation proceeded. Especially the decrease of interfacial tension was remarkable. After 36 hours cultivation, interfacial tension was undetectable. Although these experimental results are very interesting, further experiments are necessary in order to make sure whether these results are representative or not and also make clear what the decrease of interfacial tension depends on. Prokop et. al.\(^3\) observed the maximum accumulation of extra-cellular fatty acids in the early period of fermentation. They suggested that the extracellular long chain fatty acids might play an important part in the decrease of interfacial tension at the beginning of the growth of \textit{C. lipolytica}, but that further increase of interfacial area during fermentation might be due to cell concentration. The pH of yeast cultivation is usually slightly acidic (pH 4 - pH 6); therefore, long chain fatty acids are located in the oil phase.

Table 2 shows the effect of palmitic acid concentration in hexadecane on the average drop-volume of water in hexadecane which represents the interfacial tension between hexadecane and water. Palmitic acid did not affect the interfacial tension in the range of experiments. It is doubtful that long chain fatty acids alone play an important role in oil emulsification.

Some microorganisms excrete powerful emulsifying substances into the culture broth when using hydrocarbons as a carbon source. Suzuki et. al.\(^8\) isolated trehalose lipid from the oil phase of the culture broths of bacteria such as \textit{Arthrobacter}, \textit{Brevibacterium}, \textit{Corynebacterium}, and \textit{Nocardia}, but they could not get this sugar-lipid from hydrocarbon non-utilizing bacteria. Sophorose lipid\(^9\) and peptide lipid\(^10\) were found in the broths of \textit{Torulopsis magnoliae} and \textit{Candida petrophilum}, respectively. Hisatsuka et. al.\(^11\) found that \textit{Pseudomonas aeruginosa} S7Bl had a weak assimilation activity for hydrocarbon and produced rhamnolipid during fermentation, and that the growth of this strain was considerable stimulated by adding rhamnolipid to the culture medium.

Itoh and Suzuki\(^12\) induced a mutant which grew poorly on hydrocarbon from \textit{P. aeruginosa} KY4025 which showed good growth. The mutant lost the activity for the formation of rhamnolipid and was not able to grow well on hydrocarbon. The growth of the mutant was stimulated remarkable by rhamnolipid.

From the above results, it is clear that rhamnolipid plays an important role for the assimilation of hydrocarbons by these microorganisms.
P. aeruginosa S7B1 excreted not only rhamnolipid but also protein which activated the oxygen uptake and the growth of itself. This protein had a weak emulsifying activity, however, it could disperse hydrocarbons finely with rhamnolipid and it could make submicron oil droplets easily in the oil-water system.

Aiba et. al. first suggested the importance of submicron oil droplets in hydrocarbon uptake by yeast cells. After that, Goma et. al. reported that the concentration of submicron oil droplets in aqueous phase increased considerably during fermentation and deduced that solubilization which meant the formation of submicron oil droplets was the first step of hydrocarbon assimilation by C. lipolytica. Recently, Einsele et. al. showed by electronmicroscopy that submicron oil droplets adhere to the cell surface of C. tropicalis.

In adhering of oil to cells, hydrophobicity of cell surfaces appears to be important. Bell found that when the longer chain fatty acids dissolved in a non-metabolisable hydrocarbon such as pristane were used as a carbon source, the growth of Candida tropicalis having hydrophobic surface was improved, but that of Saccharomyces cerevisiae having hydrophilic surface was unaffected. It was deduced that the hydrophobicity of cell surface was necessary for transfer of hydrophobic substrates such as hydrocarbons and long chain length fatty acids to cells. Hydrophobicity of cell surface may be attributed to cellular lipid composition. Cells grown on hydrocarbon usually have higher lipid content than those grown on carbohydrates. Recently, several researchers have focused on the role of cellular lipid in hydrocarbon transport into the cells.

As stated above, processes for hydrocarbon transport to cells are complicated. Therefore, further investigation is necessary for understanding this problem.

Acknowledgement

This work was partially supported by NSF Grant GK-43621.
References

2) Erickson, L. E., T. Nakahara, and J. R. Guiterrez, unpublished data.
Table 1. Effect of Palmitic acid on Average Drop-Volume of Water in Hexadecane

<table>
<thead>
<tr>
<th>Interfacial Composition</th>
<th>Average Drop-Volume of Water</th>
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<tr>
<td>Hexadecane-Water</td>
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<tr>
<td>Palmitic acid</td>
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The average drop-volume of water in hexadecane was measured by using a microsyringe with an 18 gauge blunt tip needle at 30°C.
OPTIMAL CONCENTRATION PROFILES FOR BIFUNCTIONAL CATALYSTS WITH LANGMUIR-HINSHELWOOD KINETICS AND VARYING EFFECTIVENESS FACTORS

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Introduction

Two or more catalysts or enzymes are frequently employed to carry out a series of stepwise reactions. When these catalysts or enzymes are in solid form, it is possible to mix them in such a configuration within a packed bed that the extent of reaction is greater than if they were packed sequentially. 1,2

In this report numerical solutions are presented for optimal profiles for the reaction sequence

\[ A + E_1 \xrightarrow{k_1}{k-1} AE_1 \xrightarrow{k_2}{k-2} B + E_1 \]

for two situations: One where it is desired to maximize the amount of C produced, and the second where it is desired to minimize the amount of A unreacted. This is done by means of the Pontryagin maximum principle using the method of King et al.3

Rate Equations

Letting \( X_1 \) and \( X_2 \) be the amounts of A converted to B and of B converted to C, respectively, we may define the concentrations of A, B, and C as

\[ A = A_0 - X_1 \]  \hspace{1cm} (1)
\[ B = B_0 + X_1 - X_2 \]  \hspace{1cm} (2)
\[ C = C_0 + X_2 \]  \hspace{1cm} (3)
where A, B, and C are initial concentrations. If the production of C is to be maximized, it will be necessary to maximize $X_2$. If, on the other hand, it is desired to minimize unreacted A, we will wish to maximize $X_1$. Kinetic expressions for the two reactions may be derived:

$$\frac{dx_1}{dt} = f_1 U = \frac{(k_1 k_2 A - k_1 k_2 B) E_1 \kappa_1 U}{k_1 + k_2 A + k_1 A + k_2 B}$$ (4)

$$\frac{dx_2}{dt} = f_2 (1-U) = \frac{(k_3 k_4 B - k_3 k_4 C) E_2 \kappa_2 (1-U)}{k_3 + k_4 + k_3 B + k_4 C}$$ (5)

Here $\kappa_1$ and $\kappa_2$ are effectiveness factors for the two catalysts, $E_1$ and $E_2$ are their quasi-homogeneous concentrations when packed in unmixed form, and $U$ is the volume of particles containing $E_1$ divided by the total particle volume.

**Optimum Profile by the Maximum Principle**

We wish to choose $U(t)$ so that either $X_1$ or $X_2$ is maximized at $t = T$, the end of the reactor. This may be accomplished by means of the maximum principle using the following Hamiltonian:

$$H = (\lambda_1 f_1 - \lambda_2 f_2) U + \lambda_2 f_2$$ (6)

where $\lambda_1$, $\lambda_2$ are adjoint variables. 5

The Hamiltonian must be maximum and constant throughout the interval $0 \leq t \leq T$. If

$$\psi(t) = \lambda_1 f_1 - \lambda_2 f_2$$ (7)

it must follow that

$$U = 1 \quad \text{if} \quad \psi(t) > 0$$ (8)

$$0 < U < 1 \quad \text{if} \quad \psi(t) = 0$$ (9)

$$U = 0 \quad \text{if} \quad \psi(t) < 0$$ (10)

For intermediate control, $0 < U < 1$, to occur, $d\psi/dt = 0$, which with Eq. (9) has a nontrivial solution only if

$$W(t) = f_1^2 \left( \frac{\partial f_2}{\partial X_1} \right) - f_2^2 \left( \frac{\partial f_1}{\partial X_2} \right) = 0$$ (11)
In this case $U$ may be obtained from eqs. (4) and (5):

$$U = \frac{1}{\frac{f_1 \, dX_2}{1 + f_2 \, dX_1}}$$

(12)

It is obvious from the boundary conditions that $U(T)$ must be zero if $X_{2f}$, the final conversion to C, is being maximized or unity if $X_{1f}$, the final conversion from A, is being maximized. To calculate the optimum point for the switch to final control, we may define functions $r_1$ and $r_2$:

$$r_1 = \frac{\lambda_2}{\lambda_1}$$

(13)

$$r_2 = \frac{\lambda_1}{\lambda_2}$$

(14)

From these equations the following may be obtained:

When $U = 1$

$$\frac{dr_1}{dt} = -\frac{\partial f_1}{\partial X_2} + r_1 \frac{\partial f_1}{\partial X_1}$$

(15)

When $U = 0$

$$\frac{dr_2}{dt} = -\frac{\partial f_2}{\partial X_1} + r_2 \frac{\partial f_2}{\partial X_2}$$

(16)

When $U(T) < 0$ or $U(T) = 1$, $r_2$ or $r_1$, respectively, must be zero. The switch point to final control is then obtained by integrating eqs. (15) or (16) from various points until the boundary conditions are satisfied.

A typical example is shown in Fig. 1. Unless substantial amounts of B or C are present at the entrance to the reactor, $U = 1$ at that point. When enough B is produced to satisfy eq. (11), intermediate control commences. The switch to final control, where $U = 0$ or $U = 1$, is made to satisfy equations (15) or (16) and boundary conditions $r_1(T)=0$ or $r_2(T)=0$.

Typical production of $X_1$ and $X_2$ is shown in Fig. 2. When $U=0$ or $U=1$, $X_1$ or $X_2$, respectively, do not increase. However, during intermediate control both increase.

Fig. 3 shows various suboptimal policies in maximizing $X_1$ and $X_2$. Their efficiency depends largely on the equilibrium constants of the system, as shown in Table 1.
The effect of pore diffusion limitation, which becomes more severe from A to E, on optimal control policies is shown in Fig. 1. Increasing levels of pore diffusional limitation lead to lower final conversions, (Table 2) and to shorter elapsed t and higher values of U during intermediate control. At highest pore diffusional limitation (E), bang-bang operation is optimal for maximizing $x_2f$, while operation at $U=1$ throughout the bed leads to highest value of $x_1f$ when it is being maximized.

Varying residence times and catalysts loadings, insofar as they affected conversions, yielded families of optimal control profiles similar to those shown here.

Acknowledgement: - This work was supported by NSF Grant ERT72-03492.

REFERENCES

### TABLE 1

<table>
<thead>
<tr>
<th>$K_1$</th>
<th>$K_2$</th>
<th>$X_{1f}^{\text{Maximized}}$</th>
<th>$X_{2f}^{\text{Maximized}}$</th>
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<td>0.1</td>
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<td>0.993</td>
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<td>$\infty$</td>
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<td>1.000</td>
</tr>
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</table>

Conversion as a fraction of maximum conversion for the bang-bang-bang profile ($X_{1f}^{\text{Maximized}}$) and the bang-bang profile ($X_{2f}^{\text{Maximized}}$) when the reactions are carried to equilibrium. $A_0 = 1.0, B_0 = 0, C_0 = 0$. 

---

**TABLE 1**

Conversion as a fraction of maximum conversion for the bang-bang-bang profile ($X_{1f}^{\text{Maximized}}$) and the bang-bang profile ($X_{2f}^{\text{Maximized}}$) when the reactions are carried to equilibrium. $A_0 = 1.0, B_0 = 0, C_0 = 0$. 

---
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<td></td>
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</table>

\[ \delta_1 = 0, \delta_2 = 0 \\
\delta_1 = 1, \delta_2 = 2 \]
\[ \delta_1 = 3, \delta_2 = 6 \]
\[ \delta_1 = 5, \delta_2 = 10 \]
\[ \delta_1 = 10, \delta_2 = 20 \]

**TABLE 2**

The effect of pore diffusion limitation on conversion.

\( K_1 = 10, K_2 = 1 \). \( \delta_1, \delta_2 \) are pseudo-Thiele moduli.
Effect of pore diffusion limitation on $U$ for the optimal profile. Conditions are listed in Table 2.
FIG. 2
Change of conversions for the optimal profile when either $X_{1f}$ or $X_{2f}$ is maximized. Conditions are listed in Case D of Table 2.
<table>
<thead>
<tr>
<th>$X_{1f}$ MAXIMIZED</th>
<th>$X_{2f}$ MAXIMIZED</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
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<td>0.840</td>
<td>0.600</td>
</tr>
<tr>
<td>0.965</td>
<td>0.901</td>
</tr>
<tr>
<td>0.583</td>
<td>0.859</td>
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</table>

$U_{ave}$, $X_{1f}$, $X_{2f}$

$U = 1$, XXXX0<U<1, U = 0

FIG. 3

Pictorial presentation of the three types of reactors (optimal bed, uniform bed, and bang-bang-bang or bang-bang) operated under conditions listed in Case D of Table 2. // U = 1, XXXX0<U<1, \ \ \ U = 0.
SINGLE CELL PROTEIN PRODUCTION
FROM ALKANES IN TOWER SYSTEMS

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Food is an important basic need to all people. Approximately 75 grams per person per day requires a supply of 100,000,000 tons per year of protein to satisfy the world’s protein needs for food. Additional protein is needed for animal feeding. The future food supply picture is uncertain, but shortage of protein already exists. The population of the world is increasing at higher rates than the food production. As a consequence, the difference between protein production and protein required increases.

Many socio-economists have investigated this problem and have proposed many possible solutions. Among those, one which most investigators have agreed upon is the development of new sources of food.

There is nothing new in the consumption of microorganisms by men, especially yeast, either by design or by accident. It was as early as wine was discovered that men started consuming yeasts from unfiltered wines and beers.

During World War I, Germany cultured and dried Torula yeast to supplement proteins that were then in short supply. The production of single cell protein, from various sources, for use in food and feeds is receiving much attention because of the world wide protein shortage.

The history of hydrocarbon microbiology is a little different. It had its beginning in 1895 when Miyoshi observed that Botrytis cinerea attacked paraffins. But it was in the early 50’s that researchers started studying the mechanisms of hydrocarbon attack by microorganisms. Until recently the primary industrial concern of hydrocarbon microbiology was in connection with oil prospecting, corrosion problems and the formation of microbial sludges in jet fuel tanks. In 1963, Champagnat started studying the production of edible yeast from crude oil fractions (3).

The utilization of various hydrocarbons by microorganisms has recently received a great deal of attention. Moo Young et al. (2) have grown Candida Lipolytica in a medium containing n-dodecane as the sole carbon source. They used both batch and continuous fermentation. Aiba et al. (1) have grown Candida guillermondii batchwise using a mixture of n-alkanes (mainly C_{12} to C_{15}) as the sole carbon source.

The alkanes are organic compounds of the general formula CnH_{2n+2}. The alkanes which have been studied more extensively are those between
Vertical mixing is achieved by a recycle stream from the top to the bottom of the tower. This recycle stream passes through the heat exchanger. It has been found that this recycle stream and the motionless mixers provide adequate mixing so that the concentration of alkane at the top and bottom of the column is approximately the same at a given time. The same thing can be said about pH, fatty acid concentration (metabolite), and drop size distribution. With the cell concentration the same is truth until the late part of the exponential phase of growth is reached. In this period the microorganisms tend to form flocs with the oil and the oxygen and accumulate at the top of the column. This may be due to changes in surface and interfacial tensions which occur and which result in increased foaming. Proper control of foaming and adequate recycle flow rates of the fermentation broth should reduce these concentration differences.

Experience gained so far indicates that pH is important in the hydrocarbon fermentation both in the inoculum and in the tower itself. When the inoculum has a pH of 4 or lower, the lag phase of fermentation increases significantly. A plot of activity vs. pH is described by a bell shaped curve. Yeasts usually have a broad range of activity. *Candida lipolytica* has its optimum range of growth between pH 5.0 and 6.5; pH values above 6 tend to favor the growth of bacteria, which are the most important potential contaminants. Therefore, one has to restrict to pH between 5.0 and 5.5 to help avoid contaminants and at the same time have good rate of growth. When the inoculum was exposed to pH of 3.5 some microorganisms lost complete activity while others lost part of their activity. This resulted in poor growth. Microphotographs showed that a change in morphology from single (oval) yeast toward filamentous hypha occurs as the pH decreases.

Researchers have reported that the source of carbon also has an effect on the shape of microorganisms and that hydrocarbon tends to influence yeast in obtaining the filamentous form (5). However, it was our observation that when the pH was controlled the amount of filamentous organisms is much less than when the pH is not controlled. For this reason experimentation was conducted to determine the effect of pH on the morphology of the microorganisms.

The most important reason to be interested on the morphology of the microorganisms is because it affects activity. Yeast utilizes hydrocarbon by direct contact with the drops. The formation of hypha reduces the area of the microorganisms to be in contact with the oil. From the experimentation to determine the effect of pH on the change of morphology the following conclusions were made:

1. pH affect the morphology of *Candida lipolytica*
2. the effect of carbon source seems to be greater than the effect of pH and,
3. the combined effect of pH and carbon source is much greater than either by itself.
REFERENCES


ACKNOWLEDGMENT

This work was partially supported by NSF Grant GK-43621.
EFFECT OF TEMPERATURE AND pH ON THE STABILITY AND ACTIVITY OF IMMOBILIZED GLUCOAMYLASE AND GLUCOSE ISOMERASE

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Among the many commercially successful enzymes in the food industry (1) are glucoamylase and glucose isomerase. Glucoamylase (GA) attacks starch or its partially hydrolyzed form, dextrin, at the nonreducing end to produce glucose, and glucose isomerase (GI) converts glucose to a sweeter isomer, fructose.

Although the detailed action of glucoamylase on its substrate is complicated and still in dispute (2, 3), the hydrolysis of dextrin by the enzyme may be modeled as

\[
\text{Dextrin} \rightarrow (\text{DP}-2) \text{ Glucose + Disaccharide}
\]

\[
\text{Disaccharide} \rightleftharpoons 2 \text{ Glucose}
\]

where \( \text{DP} \) is an average degree of polymerization of the dextrin. The extent of glucose to be obtained from the dextrin is limited by the reversion reaction, which is catalyzed by glucoamylase. The simultaneous use of glucose isomerase and glucoamylase, however, decreases the production of reversion products, since glucose is drained to fructose.

The activity of heterogeneous catalysts was a subject of great interest to chemical engineers several decades ago and is now well understood in terms of the effectiveness factor - Thiele modulus correlation (4). To many of applied biochemists it still seems interesting, and numerous papers deal with it each year, extending the theory (5).

Since Wheeler advanced a theory to explain various types of selectivity (6), only a few experiments have dealt with such problems. Because of its practical consequences, selectivity is by far the most important characteristic of heterogeneous catalysis, and a lot more work is required.

Many hypotheses such as pore-mouth poisoning (7) and time-on-stream (8) have attempted to explain the deactivation phenomena of heterogeneous catalysts, but none of them have seen wide acceptance (9). For biocatalysts, another factor, enzyme denaturation due to physical (temperature, shear) or chemical (pH) effects, complicates the phenomena (10) and much remains to be explained.
In this paper extensive bench-scale data are presented on activity and stability of the two aforementioned enzymes. Their dependence on operating conditions was observed after they were immobilized onto separate porous glass particles by the method of Weetall (11).

Fig. 1 shows the effect of pH on the activities of the enzymes. A plot of logarithmic activity vs. pH was made, since it is well known that if the activity depends on the ionization of one functional group, the plot in case of soluble enzymes should be linear, with a slope of unity (12). Intraparticle diffusion reduces the slope to half when the enzymes are immobilized. In the case of GA and GI, it seems likely that several groups are important.

The Arrhenius type plot in Fig. 2 shows that intraparticle diffusion limitation may limit the increase of activity with temperature at higher temperatures. In addition, the effect of GA denaturation is quite evident.

Most crucial to the practicality of immobilized enzymes is their stability, which determines the operational period of a bed packed with them. To study stability, a single-pass differential reactor was employed. Feed was pumped through a gas dispersion tube for removal of impurities and then through the reactor. The next two figures (Figs. 3 and 4) show how the half-lives of the enzymes depend on operating conditions, substrate concentration, pH, and temperature.

Fig. 3 illustrates the fact that the enzymes are protected by the presence of their substrates. While the stability of GA depends linearly on starch concentration, the GI stability on the other hand shows a sigmoidal dependence on fructose concentration. This kind of dependence will be found useful when process optimization is to be considered.

The stability as a function of temperature and pH is shown in Fig. 4. To be noted is that the slope, which is proportional to the energy of activation for decay, is nearly independent of pH. With some extrapolation and interpolation it can be shown from this plot that a mixed bed of GA and GI can operate as long as a month at a pH midway between 6.0 and 6.5 and a temperature of 55°C. These conditions should prevent serious problems such as the formation of by-products or microbial contamination.

During the experiments it was also found that the GA used consisted of two isoenzymes, GA I and GA II. GA I was found four times as stable as GA II (Figs. 3 and 4). The existence of the two isoenzymes has been known to biochemists for some time.

A mixed bed of GA and GI was operated under the conditions determined from Fig. 4. As shown in Fig. 5, the combined yield of glucose and fructose is beyond 95%, which is higher than the glucose yield (90%) from GA alone. Therefore, the hypothesis that such a mixed bed would be effective in reducing the extent of the reversion reaction was confirmed.

Acknowledgement - This work was supported by NSF Grant ERT72-03492.
REFERENCES

FIGURE 1
EFFECT OF pH ON ACTIVITY OF
IMMOBILIZED GLUCOAMYLASE AND GLUCOSE ISOMERASE

Temp. 55 °C
FIGURE 2
EFFECT OF TEMPERATURE ON ACTIVITY OF
IMMOBILIZED GLUCOAMYLASE AND GLUCOSE ISOMERASE
FIGURE 3
EFFECT OF SUBSTRATE CONCENTRATION ON STABILITY OF
IMMOBILIZED GLUCOAMYLASE AND GLUCOSE ISOMERASE

EFFECT OF SUBSTRATE CONCENTRATION ON STABILITY OF IMMOBILIZED GLUCOAMYLASE AND GLUCOSE ISOMERASE
FIGURE 4
VARIATION OF STABILITY OF IMMOBILIZED GLUCOAMYLASE
AND GLUCOSE ISOMERASE WITH pH AND TEMPERATURE
FIGURE 5
BATCH PRODUCTION OF HIGH-FRUCTOSE SYRUP WITH A
MIXED BED OF IMMOBILIZED GLUCOAMYLASE AND GLUCOSE ISOMERASE

GA/Gl
Feed Flow Rate
PH Temp.
28% w/v 13 ml/min 6.5 (0.02M Mole/l)
3.2/8 55°C

GLU. + FRU. FRUCTOSE
INTRODUCTION

In 1972 Whitaker\textsuperscript{1} published a derivation of the equations of change for multiphase systems. Recently, Gray\textsuperscript{2} modified Whitaker's derivation, and the following dispersion equation for a species in the $\alpha$ phase of a two-phase system results from his work:

$$\frac{\partial C_\alpha}{\partial t} + v \frac{\partial C_\alpha}{\partial z} = D \frac{\partial^2 C_\alpha}{\partial z^2} + K_a (C^*_\alpha - C_\alpha) + P_\alpha + S_\alpha$$ (1)

The various terms are cross-sectional averages for a column and have been time smoothed. The source term $S$ contains the products involving the fluctuation quantities that remain after time smoothing. Equation (1) is valid for dilute solution, incompressible flow, and when no phase changes occur and the volume fraction of the $\alpha$ phase is independent of time.

Extending the analysis to more than two phases gives

$$\frac{\partial C_i}{\partial t} + v \frac{\partial C_i}{\partial z} = D \frac{\partial^2 C_i}{\partial z^2} + \sum K_{ij} a_j (C^*_j - C_j) + P_j + S_j$$ (2)

If the source term is assumed to be negligibly small compared to the other terms, equation (2) becomes

$$\frac{\partial C_i}{\partial t} + v \frac{\partial C_i}{\partial z} = D \frac{\partial^2 C_i}{\partial z^2} + \sum K_{ij} a_j (C^*_j - C_j) + P_j$$ (3)

Equation (3) can be used to model and design multi-phase tower systems.

In a hydrocarbon fermentor like that described by Gutierrez earlier in this symposium, production results from microbial growth, and mass transfer occurs among the gas, aqueous, and hydrocarbon phases. For the species oxygen in the aqueous phase in such a system where the hydrocarbon fraction is very small, oxygen transfer to and from the hydrocarbon phase may be neglected, and equation (3) becomes

$$\frac{\partial C_o}{\partial t} + v \frac{\partial C_o}{\partial z} = D \frac{\partial^2 C_o}{\partial z^2} + K_a (C^*_o - C_o) - rX$$ (4)
The parameters are the dispersion coefficient, volumetric mass transfer coefficient, and respiration rate; or in dimensionless form the Peclet number, Stanton number, and dimensionless respiration rate.

**DETERMINATION OF THE VOLUMETRIC MASS TRANSFER COEFFICIENT**

If a step change is introduced in the gas flow rate, and it is assumed that

$$\frac{\partial C_0}{\partial t} \gg \frac{\partial C_0}{\partial z} \quad \text{and} \quad \frac{\partial^2 C_0}{\partial z^2}$$

during the period of rapid change in the dissolved oxygen concentration, equation (4) reduces to

$$\frac{dC_0}{dt} = Ka(C_0^* - C_0) - rX$$ \hspace{1cm} (5)

If the substitution $C_0^* = C_0 - C_0 + \frac{rX}{Ka}$ is made and the cell concentration is assumed constant over the response period, the solution to equation (5) is

$$C_0^* = A \exp(-Kat)$$

where $A$ is the constant of integration. Now

$$\frac{dC_0}{dt} = \frac{dC_0^*}{dt} = -Ke A \exp(-Kat)$$

and

$$\ln\left(\frac{C_0}{dt}\right) = \ln(-Ka A) - Kat.$$

Thus a plot of the logarithm of the slope of the oxygen trace versus time will yield a curve which has a slope equal to the negative value of the mass transfer coefficient.

**DETERMINATION OF THE RESPIRATION RATE**

The respiration rate can be found by monitoring the dissolved oxygen concentration in a sample of culture broth that has been withdrawn from the column into a well mixed container in which no gas contacts the broth. In such a system

$$\frac{dC_0}{dt} = -rX.$$

Thus if $rX$ is constant during the period of measurement, the oxygen trace should be linear with time and have a slope equal to the negative of $rX$. 


NOTATION

a - interfacial area between two phases per unit volume of one phase, \( \text{cm}^2/\text{cm}^3 \)

C - concentration, \( \text{g/cm}^3 \)

D - dispersion coefficient, \( \text{cm}^2/\text{sec} \)

K - mass transfer coefficient, \( \text{cm/sec} \)

Q - flow rate, \( \text{cm}^3/\text{sec} \)

P - production rate, \( \text{g/cm}^3\text{sec} \)

R - radius of column, cm

r - specific consumption rate of oxygen by cells, \( \text{g/gsec} \)

S - source term, \( \text{g/cm}^3\text{sec} \)

t - time, secs

v - velocity, \( \text{cm/sec} \)

x - cell concentration, \( \text{g/cm}^3 \)

z - axial distance or height, cm

β - ratio of tracer feed rate to liquid feed rate, dimensionless

e - volumetric phase fraction or hold-up, dimensionless

SUBSCRIPTS

α, i - particular phase

E - exit

F - feed

O - oxygen

s - steady-state

T - tracer
SUPERSCRIPTS

i - denotes the concentration that would be in equilibrium with the concentration in the i phase in a multiphase system

* - denotes the concentration that would be in equilibrium with the concentration in the other phase in a two phase system

' - modified quantity

REFERENCES

3. Hsu, K. H., Master's Thesis, Kansas State University, 1972

ACKNOWLEDGMENT

This work was partially supported by NSF Grant GK-43621.
Fig. 1. VOLUMETRIC MASS TRANSFER COEFFICIENTS
Fig. 2 Oxygen Concentration During Respiration Rate Determination

Slope = 0.104 \text{ ppm/sec} 
= 0.375 \text{ g O}_2 / \text{L-hr}

Fig. 3 Respiration Rate
FIG. 6 Dispersion Coefficients

FIG. 7 Peclet Numbers
CONTINUOUS PRODUCTION OF GLUCOSE FROM DEXTRIN
BY GLUCOAMYLASE IMMOBILIZED ON POROUS SILICA

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Ames, Iowa 50010

In the work reported here, the feasibility of using immobilized enzymes as catalysts in the production of dextrose from corn starch was studied. The corn to dextrose industry is one of the largest currently using enzymes as catalysts. Immobilizing the enzymes offers the potential for lower enzyme costs, better quality control, and a cleaner product.

Several methods are available for the immobilization of enzymes, including gel entrapment, covalent linkage to organic and inorganic supports, adsorption and crosslinking, and membrane entrapment, using supports such as cellulosic gels, ion exchange resins, porous glass and ceramics and other inorganic supports, man-made polymers, and metals such as stainless steel.

Immobilized enzymes have been studied in a variety of reactors such as CSTR's, expanded and fluidized beds, packed beds, hollow tubes, gel slabs, and porous sheets and blocks. In our studies of glucoamylase for the production of glucose from dextrin, we have chosen to use packed bed reactors, because of their simplicity and possible industrial applications.

Our studies have characterized the immobilized enzyme covalently bound to both zirconium-coated porous glass and silica ceramic using glutaraldehyde as a coupling agent. The effects of pH, carrier pore size, liquid flow rates across the catalyst, temperature, and deactivation rates were studied and found to be almost identical for the two carriers. For the large-scale study being conducted, the porous silica ceramic was chosen because of its much lower cost. Operation at larger scale was necessary to determine several factors that were difficult to show using small-scale laboratory equipment, such as the effects produced in the enzyme column during long-term high flow production conditions, the effects of the heat of reaction, scale-up problems, and biological contamination.

The deactivation of *A. niger* glucoamylase from Novo at 65°C determined in initial laboratory studies is shown in Fig. 1, and a plot of enzyme half-life versus inverse temperature is shown in Fig. 2. These tests showed that a linear correlation exists on an Arrhenius type plot. The data were extrapolated to show a possible enzyme half-life of up to four years at 40°C.

Fig. 3 shows a process flow sheet for the immobilized enzyme pilot plant during the current work, concerned with studying the effects of the method of starch thinning on the concentration of glucose in the immobilized enzyme reactor effluent. The immobilized enzyme reactor is a 1 cubic foot packed
volume column, 6 ft tall and 6 inches in diameter, employing Corning silica ceramic carrier of 30-45 mesh particle size with a pore diameter of 400 Å. The immobilization procedure is shown in Table I. Following enzyme immobilization, the column was washed with a saturated solution of chloroform in water to sterilize it. Feed dextrin for the enzyme column is prepared by slurrying Pearl corn starch in water and adding 0.05-0.1% (dissolved solids basis) Novo Thermamyl 60 α-amylase and running the slurry through a steam jet starch cooker at 105°C, holding the resulting liquified starch until the desired amount of cooking is attained, and stopping the α-amylase reaction by dropping the pH.

The dextrin is fed through a sterilizer unit where it is heated to 120°C and held for 4 min to kill most of the microorganisms present, followed by cooling to reaction temperature, filtering and feeding to the enzyme column. The initial studies in the pilot plant were carried out using an acid-converted cornstarch feed dextrin of 24 DE (dextrose equivalent) obtained from Staley in dry powder form. Process details and results of these tests are shown in Tables II and III. The powdered dextrin was used in an 80-day continuous run to show feasibility and enzyme stability. During the course of the run (at a rate of 1000 lbs dry weight glucose for 40 days and 500 lbs per day for 40 days), no deactivation of the enzyme in the column was noted. Contamination of the column by microorganisms was at a minimum until an upset occurred 70 days after start-up, resulting in very high bacteria counts. The column was flushed with a saturated chloroform solution which brought the bacteria count to a normal level.

Since a glucose concentration in the product stream above 90.4% dry substance basis could not be obtained, laboratory tests were conducted (Table IV) which showed that the conversion in the column depended on the DE of the feed dextrin and the method of liquifying the starch. These tests demonstrated that for higher conversions, we needed to use enzyme-thinned starches of low DE. It was also shown that for these dextrins, the enzyme column employing immobilized glucoamylase yielded from 1 - 2% lower conversion than the free enzyme, which is to be expected from analysis of the effectiveness factors for heterogeneous catalysts inside pores.

To study these effects in the pilot plant column, a continuous steam jet starch cooker was installed to enable the manufacture of α-amylase thinned cornstarch. Tests using Novo Thermamyl 60 were conducted on GPC pearl corn starch, with the results shown in Tables V and VI and Fig. 4. Using the Novo α-amylase, it was found that cooking the starch to produce a dextrin in the range of 22-26 DE resulted in the maximum concentration of glucose in the enzyme column. Longer cooking to higher DE's results in formation of unhydrolyzable products which lower yields, and very low feed DE does not enable the column to operate at a reasonably fast flow rate because of the amount of very long chain dextrins to be broken down, and the longer diffusion time into the carrier pores required. The maximum concentration of glucose in the product was 94.0% (D.S.B.) produced when 26 DE feed was used in the column, and was 96.4 DE.

Acknowledgement - This work was supported by NSF Grant ATA73-07783.
Figure 1. The deactivation of enzyme activity under continuous operation.
Figure 2. Log of enzyme half life vs. inverse of absolute temperature.
Figure 3
PROCESS FLOW DIAGRAM
IMMOBILIZED ENZYME PILOT PLANT
Figure 4. EFFECT OF FEED DE ON PRODUCT DE AND GLUCOSE CONCENTRATION
### TABLE I

**ENZYME IMMOBILIZATION**

**Enzyme Support:** SiO$_2$, 30-45 Mesh, 400 Å Pore

**Enzyme:** Glucoamylase, Novo
- 60,000 units/g, \( 1 \text{ unit} = 1 \mu \text{mole of glucose/min.} \)

**Enzyme Offered:** 4 lbs to 31.8 lbs of carrier (1 cubic foot bulk volume)

- Bulk enzyme activity before immobilization = 12,700 units/ml
- Bulk enzyme activity after immobilization = 1,400 units/ml

**Enzyme Attached:** 3.56 lbs/31.8 lbs of carrier or 112 mg/g carrier

**Immobilized enzyme activity:** 3,000 units/g carrier

**Enzyme bonding method:** silanization of SiO$_2$ followed by glutaraldehyde coupling of enzyme

SiO$_2$ was silanized in a batch and glutaraldehyde and enzyme solutions were recirculated through the reactor.
TABLE II

PROCESS DETAILS

Reactor: Dimension = 6" i.d. x 5' length  
Volume = 0.98 cu. ft.  
Estimated Reactor Void Volume with Packing = 0.3 cu. ft.  
(Pore volume excluded)

<table>
<thead>
<tr>
<th>Production Rate (lbs Glucose/day)</th>
<th>Flow Rate (gpm)</th>
<th>*Nominal Residence Time(min)</th>
<th>**Real Residence Time(min)</th>
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</thead>
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<tr>
<td>1,200</td>
<td>0.30</td>
<td>24.4</td>
<td>7.48</td>
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<tr>
<td>1,000</td>
<td>0.25</td>
<td>29.3</td>
<td>8.99</td>
</tr>
<tr>
<td>800</td>
<td>0.20</td>
<td>36.6</td>
<td>11.22</td>
</tr>
<tr>
<td>500</td>
<td>0.125</td>
<td>58.4</td>
<td>17.98</td>
</tr>
</tbody>
</table>

*Reactor volume divided by flow rate  
**Reactor void volume divided by flow rate

Sterilization:
Steam heat exchanger  
Process stream inside the 1/4" tube coil  
Steam pressure = 12 psig  
Sterilizer outlet temperature = 117°C  
Soaking coil holding volume = 0.13 cu. ft.  
Soaking time = 3.2 min @ 0.30 gpm  
3.9 min @ 0.25 gpm
TABLE III
ANALYSIS OF PILOT PLANT FEED AND PRODUCT UNDER VARYING CONDITIONS

<table>
<thead>
<tr>
<th>Feed Analysis (Staley Star-Dri 24-R)</th>
<th>Flow Rate* lb/day</th>
<th>Degree of Polymerization</th>
<th>Calc. DE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1100</td>
<td>3.8% 0.6% 0.5% 0.5% 1.0% 3.8% 89.7% 92.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>900</td>
<td>3.3% 0.6% 0.5% 0.5% 0.7% 4.4% 90.0% 93.0</td>
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<tr>
<td></td>
<td>700</td>
<td>2.8% 0.6% 0.5% 0.5% 0.7% 4.5% 90.4% 93.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>2.6% 0.7% 0.5% 0.5% 0.9% 4.7% 90.1% 93.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>1.7% 0.6% 0.5% 0.4% 1.0% 7.7% 88.1% 92.7</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Feed Analysis (Staley Star-Dri 35-R)</th>
<th>Flow Rate* lb/day</th>
<th>Degree of Polymerization</th>
<th>Calc. DE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>500</td>
<td>3.1% 1.2% 1.1% 1.1% 1.2% 5.3% 87.0% 91.0</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Feed Analysis (Staley Star-Dri 42-R)</th>
<th>Flow Rate* lb/day</th>
<th>Degree of Polymerization</th>
<th>Calc. DE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>900</td>
<td>3.3% 1.2% 1.3% 0.7% 1.8% 5.2% 86.5% 90.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>2.9% 1.2% 1.2% 0.7% 1.7% 5.7% 86.6% 91.0</td>
<td></td>
</tr>
</tbody>
</table>

*BASED ON DRY PRODUCT

Temperature 38°C
Solids Content 30%
### TABLE IV

**MAXIMUM GLUCOSE YIELD AND DE OBTAINED IN A RECIRCULATED BATCH REACTOR OF IMMOBILIZED GLUCOAMYLASE AND IN A FREE GLUCOAMYLASE BATCH REACTOR**

<table>
<thead>
<tr>
<th>Origin</th>
<th>D.E. of Substrate</th>
<th>Enzyme type</th>
<th>Max. glucose yield (wt%, dry basis)</th>
<th>Max. DE</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPC**</td>
<td>10</td>
<td>IMMOb.</td>
<td>92.8</td>
<td>95.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FREE</td>
<td>95.1</td>
<td>97.1</td>
</tr>
<tr>
<td>GPC**</td>
<td>15</td>
<td>IMMOb.</td>
<td>92.0</td>
<td>94.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FREE</td>
<td>93.0</td>
<td>95.8</td>
</tr>
<tr>
<td>P&amp;F**(25 wt%)</td>
<td>18</td>
<td>IMMOb.</td>
<td>91.3</td>
<td>94.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IMMOb.</td>
<td>92.1</td>
<td>95.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FREE</td>
<td>94.5</td>
<td>96.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GPC**</td>
<td>20</td>
<td>IMMOb.</td>
<td>92.2</td>
<td>94.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FREE</td>
<td>93.4</td>
<td>95.8</td>
</tr>
<tr>
<td>STALEY</td>
<td>24</td>
<td>IMMOb.</td>
<td>90.5</td>
<td>93.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FREE</td>
<td>90.7</td>
<td>93.8</td>
</tr>
<tr>
<td>GPC**</td>
<td>25</td>
<td>IMMOb.</td>
<td>92.8</td>
<td>95.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FREE</td>
<td>93.0</td>
<td>95.4</td>
</tr>
<tr>
<td>HUBINGER</td>
<td>25</td>
<td>IMMOb.</td>
<td>89.5</td>
<td>92.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>55°C</td>
</tr>
<tr>
<td>STALEY</td>
<td>35</td>
<td>IMMOb.</td>
<td>88.4</td>
<td>91.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FREE</td>
<td>88.8</td>
<td>92.5</td>
</tr>
<tr>
<td>STALEY</td>
<td>42</td>
<td>IMMOb.</td>
<td>87.2</td>
<td>91.3</td>
</tr>
<tr>
<td>GPC</td>
<td>42</td>
<td>IMMOb.</td>
<td>86.1</td>
<td>90.8</td>
</tr>
</tbody>
</table>

*All reactions at 45°C and 30% by wt. starch unless otherwise indicated.

**Mainly α-amylase liquefied**
TABLE V
PEARL CORN STARCH HYDROLYZED WITH A JET-COOKER
AND NOVO THERMAMYL 60 α-AMYLASE AT 105°C

<table>
<thead>
<tr>
<th>Enzyme Concentration, g/100 lb. Starch</th>
<th>Cooking Time, Hr</th>
<th>Initial Feed DE*</th>
<th>Final Feed DE**</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>3 3/4</td>
<td>16.0</td>
<td>16.0</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>16.4</td>
<td>16.4</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>15.7</td>
<td>15.7</td>
</tr>
<tr>
<td>45</td>
<td>8½</td>
<td>19.4</td>
<td>22.5</td>
</tr>
<tr>
<td>55</td>
<td>6</td>
<td>16.8</td>
<td>22.0</td>
</tr>
<tr>
<td>65</td>
<td>8</td>
<td>22.4</td>
<td>24.0</td>
</tr>
<tr>
<td>75</td>
<td>11½</td>
<td>26.0</td>
<td>26.0</td>
</tr>
<tr>
<td>150</td>
<td>21</td>
<td>32.7</td>
<td>32.7</td>
</tr>
</tbody>
</table>

* Sampled after cooking was completed and pH adjusted to 4.4.

** Sampled after autoclaving and filtering at column inlet.

Starch slurry was made up to 30% by weight and the pH adjusted to 6.5. Calcium chloride at 75g/100 lbs. starch was added.
# TABLE VI

**IMMOBILIZED GLUCOAMYLASE COLUMN RESULTS USING α-AMYLASE THINNED PEARL CORN STARCH FEED**

<table>
<thead>
<tr>
<th>Feed DE</th>
<th>Column Feed Rate, ml/min</th>
<th>Final Glucose Conc, %</th>
<th>Final Product DE</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>600</td>
<td>87.2</td>
<td>89.6</td>
</tr>
<tr>
<td></td>
<td>435</td>
<td>90.9</td>
<td>93.4</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>93.0</td>
<td>95.4</td>
</tr>
<tr>
<td>22.5</td>
<td>960</td>
<td>90.0</td>
<td>92.6</td>
</tr>
<tr>
<td></td>
<td>830</td>
<td>91.8</td>
<td>94.1</td>
</tr>
<tr>
<td></td>
<td>620</td>
<td>92.6</td>
<td>95.0</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>93.4</td>
<td>96.0</td>
</tr>
<tr>
<td>24</td>
<td>820</td>
<td>92.7</td>
<td>95.1</td>
</tr>
<tr>
<td></td>
<td>620</td>
<td>92.8</td>
<td>95.3</td>
</tr>
<tr>
<td>26</td>
<td>820</td>
<td>92.8</td>
<td>94.9</td>
</tr>
<tr>
<td></td>
<td>610</td>
<td>93.8</td>
<td>95.9</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>94.0</td>
<td>96.4</td>
</tr>
<tr>
<td>32.7</td>
<td>820</td>
<td>91.5</td>
<td>94.9</td>
</tr>
<tr>
<td></td>
<td>620</td>
<td>92.0</td>
<td>95.4</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>91.2</td>
<td>94.9</td>
</tr>
</tbody>
</table>

All runs were conducted with the column operating at 40°C and downward flow at pH 4.4.


*Copies of these reports can be obtained by writing to:

Prof. L. T. Fan, Director
Systems Institute
C/o Dept. of Chemical Engineering
Kansas State University
Manhattan, Kansas 66506