Modeling of enzymatic synthesis of fructooligosaccharides in continuous membrane reactors

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Outline

1. Inroduction
2. Reaction kinetics
   - Network of the reaction mechanism
   - Enzyme screening
   - The reaction model
3. Membrane-assisted Continuous Reactor
   - Experimental
   - Process design equations
   - Operation strategies
4. Summary

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Introduction

Motive of the study:

- Economic significance: 33 billion USD estimated world market for functional foods
- Non-nutritive sweeteners providing 30-50% of the sweetness of table sugar
- Non-digestible dietary fibers
- Encouragement of the growth of beneficial bacteria in the colon
- Health benefits: anticancerous effects, reduction of serum cholesterol level, synthesis of B-complex vitamins, enhance absorption of dietary calcium.
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- Experimental investigation on batch and continuous FOS production
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Network of the reaction mechanism

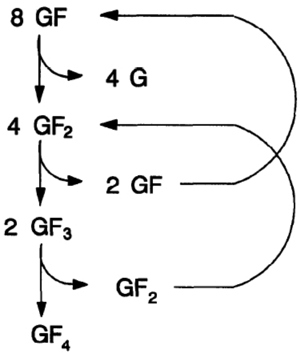


Figure: Production of fructooligosaccharides from sucrose catalyzed by fructosyltransferase derived from *Asp. pulluans.*

[Jung et al., Enzyme Microb. Technol. 11 (1989) 491-494]

Enzyme screening

FTase activity* of commercial enzyme preparations used in fruit juice processing

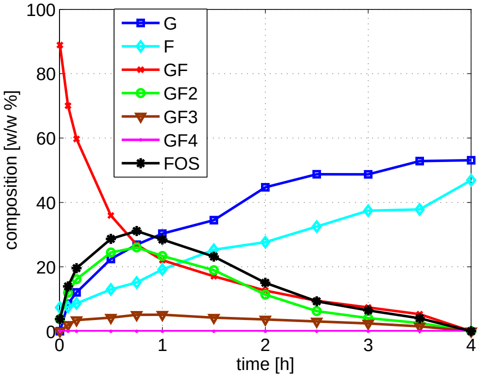
Enzyme Preparation	Enzyme activity (units/ml)	Specific activity (units/mg protein)
Pectinex Ultra SP-L(Novo)	44.8	2.71
Pectinex 3XL(Novo)	9.1	0.98
Novozym 188(Novo)	32.9	0.62
Celluclast 1.5L(Novo)	0	0
Rohapect D5L Special(Rohm)	15.7	1.48
Rohapect DA6L(Rohm)	14.7	1.58
Rohapect VRSL(Rohm)	6.9	0.67
Rohapect MA plus(Rohm)	0.39	0.12
Rohapect B1L(Rohm)	10.7	1.14
Rohament CL(Rohm)	1.87	0.06
Rohament MAX(Rohm)	0.52	0.06
Rapidase Liqplus(Gist-Brocades)	20.6	0.79
Rapidase Pomaliq(Gist-Brocades)	10.5	0.18
Rapidase Vino Super(Gist-Brocades)	10.7	1.99
Klerzyme 200(Gist-Brocades)	19.6	1.96
Cytolase CL(Gist-Brocades)	0	0
Cytolase M102(Gist-Brocades)	3.5	0.13
Cytolase PCL5(Gist-Brocades)	6.3	0.49
Cytolase 2AL(Gist-Brocades)	1.74	0.04
Clarex 5XL(Solvay)	10.6	0.94
Clarex ML(Solvay)	4.88	0.39
Pectinase AT(Solvay)	12.2	0.87

*1U is defined as the amount of FTase that transfered 1 umole of fructose per min under assay conditions (0.6M GF, pH 5.6, 40^{o}C, 15 h).

[Y. D. Hang and E. E. Woodams, Biotechnology Letters 7 (1995) 741-744]

Enzyme screening

Fructozyme L™

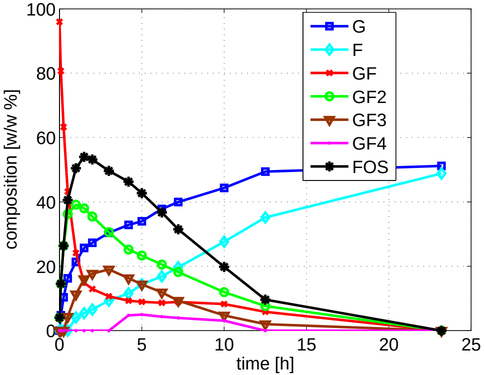


Product Characteristics:

Inulinase (EC 3.2.1.7)
from *Aspergillus niger*
Producer: Novozyme Corp.
Supplier: Sigma-Aldrich Inc.

Enzyme screening

ULTRA-*Fruit* ™

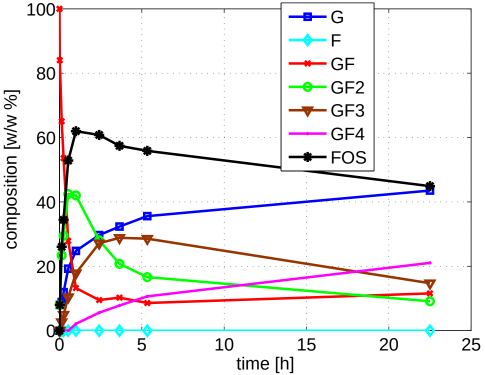


Product Characteristics:

Pectinase
from *A. niger*
and *Tr. Longibrachiatum*
Supplier: ReKru GmbH
FAO/WHO food-grade purity

Enzyme screening

Pectinex Ultra SP-L ™



Product Characteristics:

Polygalacturonase
form submerged fermentation
of *Aspergillus aculeatus*
FAO/WHO food-grade purity
Physical form: Liquid
Density (g/ml): 1.16
Stabilisers: Glycerol, KCl
Preservatives: None
Colour: Brown
Producer: Novozyme Corp.
Supplier: ReKru GmbH

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The reaction model:

$$GF + GF \xrightarrow{r_1} GF_2 + G$$
$$GF_2 + GF_2 \xrightarrow{r_2} GF_3 + GF$$
$$GF_3 + GF_3 \xrightarrow{r_3} GF_4 + GF_2$$
$$GF + GF_2 \xrightarrow{r_4} GF_3 + G$$
$$GF + GF_3 \xrightarrow{r_5} GF_4 + G$$
$$GF_2 + GF_3 \xrightarrow{r_6} GF_4 + GF$$

The Cauchy problem:

$$\frac{d[G]}{dt} = r_1 + r_4 + r_5$$
$$\frac{d[GF]}{dt} = -2r_1 + r_2 - r_4 - r_5 + r_6$$
$$\frac{d[GF_2]}{dt} = r_1 - 2r_2 + r_3 - r_4 - r_6$$
$$\frac{d[GF_3]}{dt} = r_2 - 2r_3 + r_4 - r_5 - r_6$$
$$\frac{d[GF_4]}{dt} = r_3 + r_5 + r_6$$

[Nishizawa et al. Food Sci. Technol. Res. 7 (2001) 39-44]

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Assuming a two-substrate random bi-bi model (Price & Stevens, 1989), rate equations are obtained as follows:

$$r_1 = \frac{V_{m,GF}[GF]^2}{K_{m1,GF}K_{m2,GF}+K_{m2,GF}[GF]+[GF]^2+K_{m2,GF}[GF][G]/K_{i,n}+K_{m1,GF}K_{m2,GF}[G]/K_{i,n}}$$

$$r_2 = \frac{V_{m,GF2}[GF_2]^2}{K_{m1,GF2}K_{m2,GF2}+K_{m2,GF2}[GF_2]+[GF_2]^2}$$

$$r_3 = \frac{V_{m,GF3}[GF_3]^2}{K_{m1,GF3}K_{m2,GF3}+K_{m2,GF3}[GF_3]+[GF_3]^2}$$

$$r_4 = \frac{V_{m4}[GF][GF_2]}{K_{m1,GF}K_{m2,GF-GF2}+K_{m2,GF-GF2}[GF]+K_{m2,GF2-GF}[GF_2]+[GF][GF_2]}$$

$$r_5 = \frac{V_{m5}[GF][GF_3]}{K_{m1,GF}K_{m2,GF-GF3}+K_{m2,GF-GF3}[GF]+K_{m2,GF3-GF}[GF_3]+[GF][GF_3]}$$

$$r_6 = \frac{V_{m6}[GF_2][GF_3]}{K_{m1,GF2}K_{m2,GF2-GF3}+K_{m2,GF2-GF3}[GF_2]+K_{m2,GF3-GF2}[GF_3]+[GF_2][GF_3]},$$

where V_m is the maximum transfructosylation rate, K_{m1} and K_{m2} are dissociation constants.

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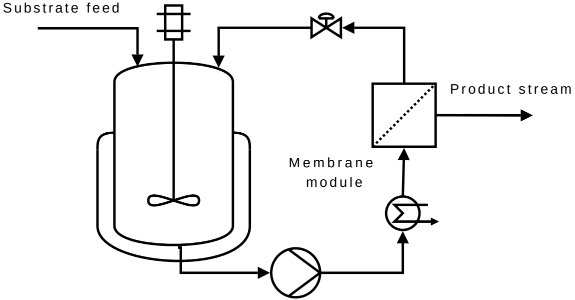
Membrane-assisted Continuous Stirred Tank Reactor



Manufacturer	Membrane	MWCO	Membrane material	pH range	Max. temp.
atech	UF-TiO_2	20 kD	Al_2O_3/TiO_2	0-14	121

Coupling MBR process design equations with enzyme kinetics model

General Mole Balance for a Continuous Chemical Reactor:

$$\frac{d}{dt}\int_V c_j dV = q_{in}c_{j,in} - q_{out}c_{j,out} + \int_V R_j dV$$

If the reactor volume is constant, the inlet and outlet flows are equal, the reactor is well stirred:

$$\frac{dc_j}{dt} = \frac{q}{V}(c_{j,in} - c_{j,out}) + R_j,$$

where τ=V/q is the residence time.

The membrane filtration is dictated by the biochemistry and not vice versa.

$$q = q(A, \mu, T, c_{prot}, \Delta P)$$

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The active enzyme E undergoes an irreversible transformation to inactive form E_i:

$$E \xrightarrow{r} E_i$$

The rate of deactivation is a first order reaction:

$$-\frac{dc_E}{dt} = r_d = k_d c_E$$

Integration gives the active enzyme concentration as a function of time:

$$c_E = c_{E0} e^{-k_d t}$$

Because the enzyme reaction rate depends on the amount of active enzyme present, we can write:

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Resting stability

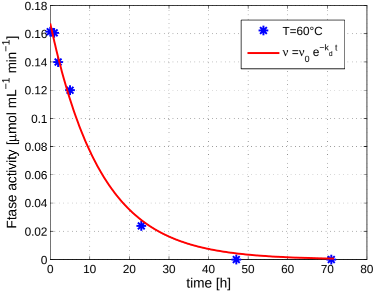

Table: Temperature dependence of deactivation rate constant and catalyst half-life

T [^{o}C]	pH	k_d [h^{-1}]	t_h [h]
30	6.0	0.111	6.2
40	6.0	0.004	156.2
50	6.0	0.003	223.8
60	6.0	0.078	8.9
60	4.5	0.122	5.7

Assay conditions: 500g/L GF, 100 mL/L enzyme preparation, 60^{o}C, pH 5.8, KH_2PO_4-NaOH buffer

MBR operation strategies for compensating catalysts deactivation

- Addition of fresh enzyme into the reactor to raise the concentration of active enzyme
  - requires an increase in the transmembrane pressure
- Increasing the residence time τ by decreasing the permeate flow-rate q
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- Mixed-policy

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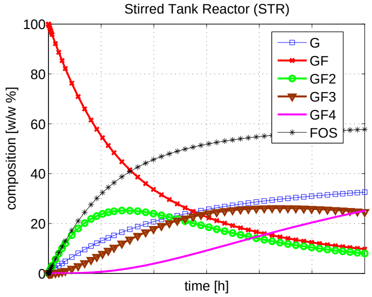
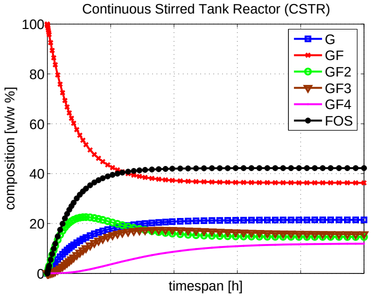
Typical operational profiles

Dynamic evaluation of a batch FOS synthesis:



Steady-state operation performance of a continuous FOS production:



Summary

- Mathematical model for production of FOS by a membrane-assisted CSTR is provided.
- Mathematical framework is built by coupling the design equations of the membrane reactor configuration with the equations describing the enzyme kinetics.
- Pectinex Ultra SP-L can be utilized in an CSTR to produce high-content FOS syrup.
- Recommended for applications where partial sucrose conversion is aimed.
- Simple configuration, easy scale-up and simple control of residence time.

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Reference I

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