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Model of Continuous Cheese Whey Fermentation by *Candida Pseudotropicalis*

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***Abstract*—**The utilization of cheese whey as a fermentation substrate to produce bio-ethanol is an effort to supply bio-ethanol demand as a renewable energy. Like other process systems, modeling is also required for fermentation process design, optimization and plant operation. This research aims to study the fermentation process of cheese whey by applying mathematics and fundamental concept in chemical engineering, and to investigate the characteristic of the cheese whey fermentation process. Steady state simulation results for inlet substrate concentration of 50, 100 and 150 g/l, and various values of hydraulic retention time, showed that the ethanol productivity maximum values were 0.1091, 0.3163 and 0.5639 g/l.h respectively. Those values were achieved at hydraulic retention time of 20 hours, which was the minimum value used in this modeling. This showed that operating reactor at low hydraulic retention time was favorable. Model of bio-ethanol production from cheese whey will enhance the understanding of what really happen in the fermentation process.

***Keywords*—**Cheese whey, ethanol, fermentation, modeling.

I. Introduction

CURRENTLY, the shortage of fossil fuel has encouraged the investigation on some alternative energy sources. One of the alternative energy sources is bio-ethanol, which can be produced from fermentation. The raw materials that are usually fermented to produce ethanol come from crop products such as corn, sweet sorghum, and sugar cane. Hence, to produce ethanol, it always needs land opening for plantation which ultimately will result in deforestation. To reduce land utilization for plantation, and to eliminate the land competition between food and energy orientation, the use of alternative non-crop raw materials needs to be explored. Such raw materials could be from industrial waste.

Cheese whey is waste from cheese production. Whey is watery portion that separates from the curds during conventional cheesemaking or casein manufacture. There are two types of cheese whey, i.e. sweet and acidic whey. Sweet whey is produced from ripened cheese with pH 5.9 to 6.3. While, acidic whey is produced from unripened fresh cheeses with pH 4.4 to 4.6. About nine kilograms of whey are usually produced from one kilogram of cheese production [8]. Generally, cheese whey still contains some nutrients for growth which consist of 5-6% lactose, 0.8-1% protein, and 0.06% fat [5]. There is more lactic acid, calcium, phosphorous, and lactose in acid whey.

In Canada, about 0.22 million t/year cheese whey is produced of which over half is discarded as waste [2]. In Brazil, production of cheese whey is estimated to be around 3 millions t/year [3]. In USA more than 1.7×10^{10} kg of whey are generated annually [4].

There have been some researches concerning the use of cheese whey fermentation to produce ethanol [1],[2],[5],[8]. The effect of operating parameters such as initial pH, cheese whey powder (CWP) concentration, and external nutrient (N,P) supplementation on the cheese whey powder (CWP) fermentation has been investigated by Kargi and Ozmihci [5]. They used cheese whey powder as the substrate of batch fermentation and found that initial pH of 5 was the most suitable for producing maximum final ethanol concentration and ethanol formation rate. The external addition of N and P source did not improve the ethanol formation. The final ethanol concentration and ethanol formation rate increased with sugar concentration. The ethanol production from batch fermentation of crude whey by *Kluyveromyces marxianus* has been investigated by Zafar and Owais [8]. They reported that the specific cellular growth rate and product formation rate reached maximum values of 0.157 and 0.046 1/h at exponential phase. Ghaly and Taweel [1],[2] have studied the kinetic of batch and continuous fermentation of cheese whey by yeast *Candida pseudotropicalis*. They produced kinetic parameter from batch fermentation and recommended the operating parameters for continuous cheese whey fermentation that gave the maximum ethanol concentration are 150 g/l inlet substrate concentration and 42 h hydraulic retention time. Ethanol production from sweet whey permeate and sweet whey permeate-grain batch fermentation has also been investigated [4]. The yeast cells used were *Kluyveromyces fragilis* and *Saccharomyces lactis*. The ethanol concentration produced from 24 h whey permeate fermentation was 20 g/l. As much as 97 and 94 g/l of ethanol was produced from whey permeate-grain fermentation using yeast *K. fragilis* and *S. cerevisiae* respectively in 36 h.

Mathematical models are necessary for the design, scale-up, optimal control and economic analysis of ethanol fermentation process. These models may lead to the development of better strategies of the fermentation optimization to ensure its economic viability. The aim of this study is to provide model

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of cheese whey fermentation for the above reasons.

This paper is organized as follows. Section II derived mathematical models for continuous cheese whey fermentation process by *Candida pseudotropicalis* based on mass balance. Section III presents simulation results of the proposed models using the available kinetic parameters. Finally, some conclusions are presented in Section IV.

II. Mathematical Modeling

In a continuous fermentation system as shown in Fig. 1, cheese whey substrate inlet flow with initial lactose concentration S_i enters the fermenter at constant volumetric flowrate of Q. Ethanol will appear in the product outlet with concentration P_o as a result of lactose fermentation by the cell (*Candida pseuditropicalis*). Concentration of the cell in the inlet and outlet are denoted as X_i and X_o respectively and the remaining latctose at the outlet is S_o. At certain inlet volumetric flowrate, the ethanol product concentration will depend on the volume of the fermenter.

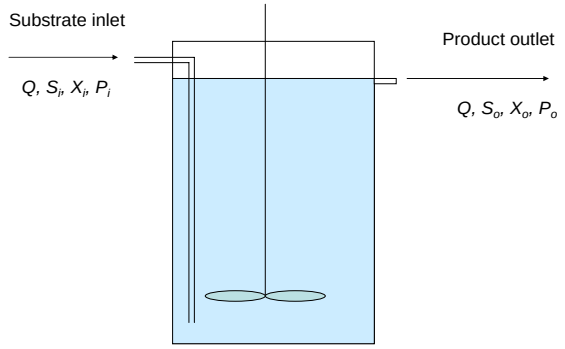

Fig. 1 A Continuous cheese whey fermenter

A. Growth Kinetic

Optimal expression of growth kinetics depends on the transport of the necessary nutrients to the cell surface, the rate of mass transfer from the medium into the cells and the environmental parameters (temperature and pH) being optimally maintained. The kinetic of microbial cell growth can be modeled mathematically as follows [6],[7].

$$R_X = \mu X \tag{1}$$

Where: μ = specific growth rate, 1/h
X = cell concentration, g/l

At high substrate concentration, cell growth rate could be inhibited by the substrate. Fermentation product can also cause cell growth inhibition. Ethanol as a fermentation product is well known to be inhibitory to both yeast cell growth and ethanol production. The effect of inhibition must be accounted in the growth model. One of the models of specific growth rate which involve the effect of substrate and product inhibition is shown below:

$$\mu = \frac{\mu_m S}{K_s + S}\frac{K_s'}{K_s' + S}\frac{K_p}{K_p + P} \tag{2}$$

where: μ_m = specific growth rate, 1/h
S = substrate concentration, g/l
P = ethanol concentration, g/l
K_s = saturation constant, g/l
K_s' = substrate growth inhibition concentration, g/l
K_P = ethanol growth inhibition concentration, g/l

B. Cell Mass Balance

The cell mass balance in continuous fermenter can be formulated as follows:

[cell accumulation rate] = [cell input rate]
+ [cell growth rate]
– [cell death rate]
– [cell output rate] (3)

Mathematically, the above mass balance can be rewritten below:

$$\frac{dX}{dt}V = QX_i + \mu XV - K_d XV - QX_o \tag{4}$$

or

$$\frac{dX}{dt} = \frac{1}{R}(X_i - X_o) + \frac{\mu_m S}{K_s + S}\frac{K_s'}{K_s' + S}\frac{K_p}{K_p + P}X - K_d X \tag{5}$$

where: K_d = spesific cell death rate, 1/h
V = fermenter volume, l
Q = volumetric feed rate, l.1/h
R = hydraulic retention time, h

$$R = \frac{V}{Q} \tag{6}$$

Assuming that the feed is sterile ($X_i = 0$) and the fermenter is at the steady-state condition, the above equation can be written:

$$\frac{1}{R} = \frac{\mu_m S}{K_s + S}\frac{K_s'}{K_s' + S}\frac{K_p}{K_p + P} - K_d \ . \tag{7}$$

C. Substrate Mass Balance

Using similar formula of cell mass balance, the mass balance of substrate in continuous fermentation can be written as follows:

[Substrate accumulation rate] = [input rate of substrate] – [substrate utilization rate for growth] – [substrate utilization rate for maintenance]- [substrate utilization rate for product] [output rate of substrate] (8)

$$\frac{dS}{dt}V = QS_i - (R_{SX} + R_{Sm} + R_{SP})V - QS_o \quad (9)$$

where: S_i = substrate concentration at the inlet concentration of the fermenter, g/l

S_o = substrate concentration at the outlet concentration of the fermenter, g/l

R_{SX} = substrate utilization rate for cell growth, g/l.1/h

R_{Sm} = substrate utilization rate for maintenance, g/l.1/h

R_{SP} = substrate utilization rate for product formation, g/l.1/h

At steady-state condition, we can modify (9) into the following expression:

$$(S_i - S_o) = R\left[\frac{\mu X}{Y_{X/S}} + m_S X + \frac{\alpha R_X + \beta X}{Y_{P/S}}\right] \quad (10)$$

where: $Y_{X/S}$ = yield coefficient for cell on substrate

m_S = maintenance energy coefficient, 1/h

α = growth associated product formation constant

β = non-growth associated product formation constant, 1/h

$$\alpha = \frac{Y_{P/S}}{Y_{X/S}} \quad (11)$$

The value of β can be neglected because ethanol is a growth associated product. Hence, we can simplify (10) into the following:

$$(S_i - S_o) = R\left[\frac{\mu X}{Y_{X/S}} + m_S X + \frac{\alpha R_X}{Y_{P/S}}\right] \quad (12)$$

D. Product Mass Balance

Finally, the mass balance for ethanol as the product of the fermentation can be derived:

[Ethanol accumulation rate] = [input rate of ethanol] + [ethanol production rate] – [ethanol output rate] (13)

or

$$\frac{dP}{dt}V = QP_i + [\alpha R_X + \beta X]V - QP_o \quad (14)$$

At steady state and no product in the input flow the above equation can be simplified as follows:

$$P = RX\left[\alpha \frac{\mu_m S}{K_s + S}\frac{K_s'}{K_s' + S}\frac{K_p}{K_p + P}\right] \quad (15)$$

The kinetic data for the fermentation of cheese whey by *Candida pseudotropicalis* are given by Ghaly and Taweel (1994) and shown in the Table I.

TABLE I
KINETIC PARAMETERS

Parameter	Initial Substrate concentration [g/l]		
	50	100	150
μ_m	0.0510	0.0510	0.0510
K_s	1.9000	1.9000	1.9000
K_p	20.6500	20.6500	20.6500
$Y_{X/S}$	0.0480	0.0480	0.0380
$Y_{P/S}$	0.4260	0.4420	0.4240
K_d	0.0022	0,0032	0.0041
m_S	4.2100	4,0400	4.1800
K_s'	112.5100	112.5100	112.5100

III. RESULT AND DISCUSSION

The mathematical model of continuous fermentation process of cheese whey by *Candida pseudotropicalis* has been derived as shown in (2), (7), (12) and (15). The main difference between the above models and the previously published (Ghaly and Taweel, 1997) are in substrate and product mass balance models ((12) and (15)). Here we used μX to express cell kinetic term (R_X) in subsrate and product mass balance models which is more apropriate, while Ghaly and Taweel used $\frac{dX}{dt}$ as expressed in (5) in their models.

The continuous fermentation is influenced by the value of hydraulic retention time (R). At the certain value of R, the specific growth rate can be determined by using (7) and the

concentration of remaining substrate, cells and ethanol produced then can be evaluated by solving (2), (12), and (15) simultaneously.

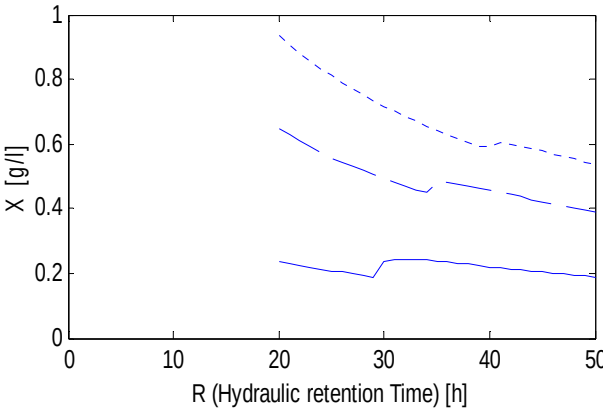

Fig. 2 Effect of hydraulic retention time on the cell concentration within reactor

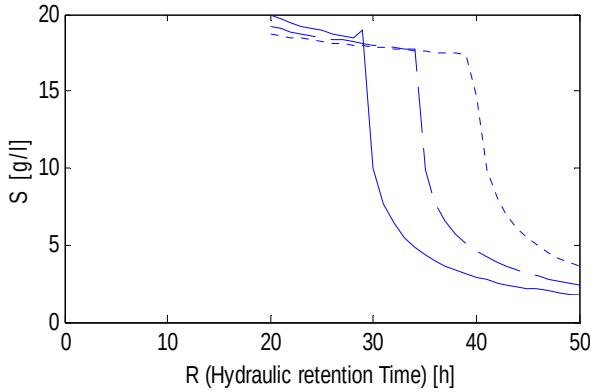

Fig. 3 Effect of hydraulic retention time on the substrate concentration within reactor

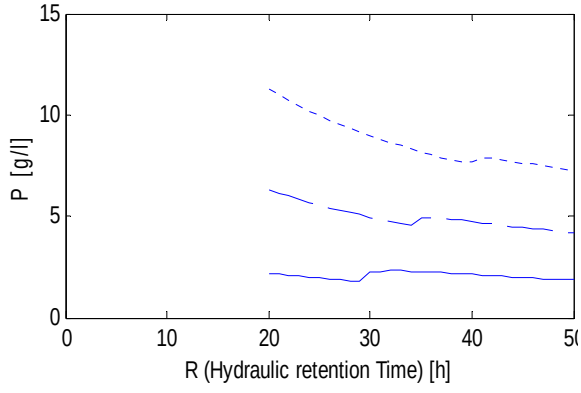

Fig. 4 Effect of hydraulic retention time on the ethanol concentration within reactor

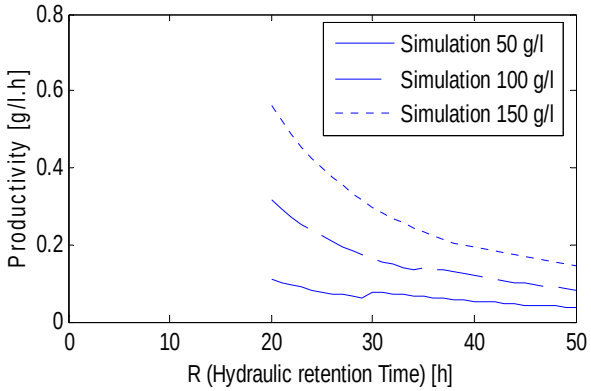

Fig. 5 Effect of hydraulic retention time on the ethanol productivity

Fig. 2 depicts the effect of hydraulic retention time and inlet substrate concentration on the cell concentration in the reactor. The increasing substrate concentration would increase cell concentration at the outlet flow. According to (2), the higher substrate concentration resulted in higher specific growth rate. This would cause the cell concentration increase. At low inlet substrate concentration (50 g/l), the higher values of hydraulic retention time would increase the substrate conversion, and hence rose the cell concentration. At higher substrate inlet concentration (100 and 150 g/l), the cell concentrations tended to decrease at increasing hydraulic retention time, although the substrate conversion still remained increasing as also shown at low inlet concentration. This fact was due to the effect of substrate and product inhibition.

The profile of substrate concentration in the reactor as the hydraulic retention is varied is shown on Fig. 3. The higher values of hydraulic retention time caused the declining of substrate concentration or the increase of substrate conversion. At higher inlet substrate concentration (100 and 150 g/l), the increase of substrate conversions with hydraulic retention time seemed to be caused by the higher demand of cell maintenance to cope with the inhibition effect of substrate and ethanol product.

As shown on Fig. 4, the increasing substrate concentration would also increase the outlet ethanol concentration. This because ethanol was a growth-associated product and therefore the increase in cell concentration would result in the increase in ethanol concentration. Inline with cell concentration, at low inlet substrate concentration (50 g/l), the higher values of hydraulic retention time would increase the substrate conversion, and hence rose the ethanol concentration. At higher substrate inlet concentration (100 and 150 g/l), the ethanol concentrations tended to decrease at increasing hydraulic retention time, although the substrate conversion still remained increasing as also shown at low inlet concentration. The higher values of ethanol concentration produced at higher substrate concentration could cause the product inhibition on growth and hence resulted in lowering cell concentration with hydraulic retention time.

Productivity is defined as the ratio of ethanol concentration to the hydraulic retention time. From Fig. 5 it can be seen that at low inlet substrate concentration (50 g/l), the ethanol productivity increased up to the maximum value and then declined with hydraulic retention time. However, the increasing amount was not significant. Therefore, low hydraulic retention time was preferred as this would require smaller reactor size. The ethanol productivity tended to decrease at increasing hydraulic retention time at higher substrate inlet concentration (100 and 150 g/l). As summarized in Table II, the ethanol productivity showed maximum value of 0.1091, 0.3163, and 0.5639 g/l.h which were achieved at hydraulic retention time of 20 hours and inlet substrate concentration of 50, 100 and 150 g/l, respectively. This showed that operating reactor at low hydraulic retention time was favorable. However, it was not recommended to operate the reactor at very low hydraulic retention time since this would cause the cell not able to grow (wash out) and hence no ethanol would be produced.

From Table II, it can be seen that the maximum ethanol of 2.1829, 6.3263 and 11.2783 g/l were obtained at 20 h hydraulic retention time by using inlet substrate concentration of 50, 100 and 150 g/l respectively.

TABLE II
SUMMARY OF SIMULATION RESULTS

Si	Recommended R value	P [g/l]	Productivity [g/l.h]
50	20	2.1829	0.1091
100	20	6.3263	0.3163
150	20	11.2783	0.5639

IV. CONCLUSION

Mathematical model of continuous cheese whey fermentation based on mass balance was established. Steady state simulation results for various hydraulic retention time and inlet substrate concentration showed that maximum ethanol productivity was achieved at low value of hydraulic retention time. The higher the inlet substrate concentration the higher the ethanol concentration and productivity would be. The hydraulic retention time of 20 hours resulted in maximum ethanol productivity in the range of inlet substrate concentration used. Therefore it can be concluded that operating reactor at low hydraulic retention time is favorable.

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