Modeling of single cell protein production from cheese whey using tanks-in-series model

Marjan Varedi Kolaei[1], Ramin Karimzadeh[2*], Seyed Abbas Shojaosadati[2], Jafar Towfighi[2]

[1]Department of Biotechnology, Faculty of Science, University of Tehran, P.O. Box 1198-16765, Tehran, I.R. Iran [2]Department of Chemical Engineering, Faculty of Engineering, Tarbiat Modarres University, P.O. Box 14155-4843, Tehran, I.R. Iran

Abstract
In this work, mathematical modeling of microbial (*Trichosporon* sp.) biomass production in a stirred tank bioreactor and in an external airlift bioreactor has been investigated. A model based on a tanks-in-series model without back-flow has been used to simulate the production of single cell protein in the external airlift bioreactor under an unsteady condition and without oxygen limitation, utilizing cheese whey as a substrate. The kinetic parameters of cell growth and substrate consumption including μ_m(maximum specific growth), K_s(growth associated parameter), γ(saturated constant) and λ(non growth associated parameter) were determined based on experimental data derived from the batch process in the stirred tank reactor and the kinetic model, which resulted in 0.59 h^{-1}, 46.84 g/l, 0.383 and 1.275, respectively. Estimated biokinetic parameters were applied to find the profiles of biomass and lactose in the airlift bioreactor. MATLAB software was used to find kinetic parameters and solve the equations of the tanks-in-series model. The number of stages of the tanks-in-series obtained equals 16.
Keywords**:** Modeling; Cheese whey; External airlift bioreactor; Single cell protein; Tanks-in-series model

INTRODUCTION

The batch aerobic process has been used successfully for the production of single cell protein (SCP) from cheese whey using the yeast *Trichosporon* sp. The genus *Trichosporon represents* a taxon comprising microorganisms with a unique set of enzyme capabilities for aerobic biodegradation of diverse organic compounds including cheese whey (Spanning and Neujahr, 1990; Gholson and Gough, 1980). Cheese whey fermentation for the production of single cell protein SCP using the yeast *Trichosporon* sp. can be described as a biochemical reaction of cells and lactose to produce microbial cells as the main product. Microbial growth kinetics, i.e., the relationship between the specific growth rate (μ) of a microbial population and the substrate concentration (s), is an important tool in microbiology and biotechnology. Traditional kinetics is based on the assumption that a single compound (e.g. lactose in this present model) is controlling the rate of growth of a microbial cell.

For design, operation and control purposes, an accurate simulation of a reactor performance is essential (Camarasa *et al*., 2001). The production process can be performed in different kinds of vessels including stirred tank reactors (STRs) and airlift reactors. Various models are used for simulating the performance of airlift reactors depending on whether flow is close to plug or mixed. The tanks-in-series models can be used for any extent of mixing in a reactor. It is a combination of a series of theoretical and well-mixed reactors. The tanks-in-series model provides a set of first order differential equations, which can be solved using rather simple numerical techniques (Levenshpiel, 1999).

Znad *et al*., (2004) applied the tanks-in-series model for mathematical modeling of the unsteady per-

Correspondence to: **Ramin Karimzadeh**, Ph.D.
Tel: +98 21 82883315; Fax: +98 21 88006544
E-mail: ramin@modares.ac.ir

formance of a semi-batch operation in an internal loop airlift bioreactor for production of gluconic acid by fermentation. Zuo *et al*. (2006) used a modified tanks-in-series model to describe the cultivation of *Acetobacter xylinum* for bacterial cellulose production in a modified airlift reactor with wire-mesh draft tubes. In this work, cheese whey was used as a substrate for biomass production in a stirred tank bioreactor and in an external airlift bioreactor. A mathematical model based on a tanks-in-series model without back-flow has been used to simulate the production of SCP in the external airlift bioreactor under an unsteady condition and without oxygen limitation.

MATERIALS AND METHODS

Microorganism and cultivation: After sampling from several cheese making plants, the favored microorganism was selected and enriched. The selection was based on the microorganisms' ability for chemical oxygen demand (COD) reduction and cell dry weight (CDW) production. During this procedure, *Trichosporon* sp. was selected as the best microorganism for this purpose (Shafaghi, 2000).

The yeasts were maintained on potato dextrose agar (PDA) slants. The cultures were incubated at 30ºC for 24h and thereafter preserved at 4ºC. For preparing the culture medium, fresh cheese whey was adjusted to pH 4.5 and then boiled at 100ºC for 15 min. After cooling, denatured proteins were separated by filtration. For removing smaller proteins, ultrafiltration was carried out. The resulting cheese whey was green to yellowish in color and was stored at 4ºC until further use. Ammonium sulfate was added as nitrogen source, pH value adjusted to 3.5 and finally sterilized in an autoclave. In order to prepare the seed culture, 350 ml of ultra-filtrated cheese whey was transferred to 1-liter flasks and sterilized at 12ºC for 15 min. 10-20 ml of sterile medium was transferred to the slants. The cell suspension was then added to the flasks which were incubated at 30ºC, with shaking at 200 rpm for 24h. The prepared seed culture was used for inoculation in bioreactors.

Batch fermentation in the stirred tank reactor: A 2-liter stirred tank bioreactor (INFORS, Switzerland) with a working volume of 1 liter was employed in this experiment. The bioreactor and all accessories (mixing system, tubing, etc.) were sterilized in an autoclave before use. 900 ml of sterilized cheese whey medium was transferred to the bioreactor which was then inoculated with 100 ml of microbial suspension. The optimum culture condition was determined using several experiments (Shafaghi, 2000). In each optimizing experiment all of the variables (pH, temperature, agitation rate, and aeration rate), except one, were kept constant. The initial value of each constant variable was selected according to references. The bioreactor was maintained in optimum operational conditions: 30ºC, pH 3.5, aeration 2 v.v.m, and 800 rpm. After inoculation the bioreactor was set to work for 24h and samples were collected every 2 h.

Batch fermentation in the external-loop airlift bioreactor (ELAB): The external airlift reactor used in this study was made of glass. It was 1500 mm in height with a 100 mm diameter riser, and a 50 mm diameter downcomer. The gas sparger in the airlift was located just above the pipe connecting the riser and the downcomer. It consisted of a cross with 32 holes, each 1 mm in diameter, in a triangular arrangement. Figure 1 shows a schematic of the external loop air lift reactor used in this work.

The bioreactor and all accessories (mixing system, tubing, etc.) were sterilized for one hour by steam. Temperature and pH as effective factors in microbial growth and metabolism were set at optima of 30ºC and 3.5, respectively. On the other hand, after choosing the sparger, several experiments with cheese whey as

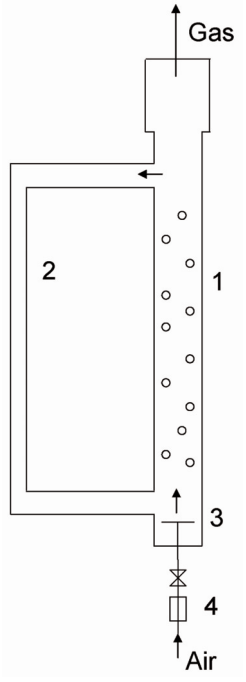

Figure 1. Schematic of the external loop airlift bioreactor, 1) Riser, 2) Downcomer, 3) Gas sparger, 4) Flowmeter.

Table 1. Basic parameters of the ELAB (in ISI scale).

V_b (m^3)	V_t (m^3)	V_d (m^3)	V_r (m^3)	V_L (m^3)	A_r (m^2)	A_d (m^2)	h_r (m)	h_d (m)	D_d (m)	D_r (m)
3.92E-4	3.92E-4	1.67E-3	5.89E-3	8.34E-3	7.85E-3	1.92E-3	0.75	0.85	0.05	0.1

Please see nomenclature for description of the above parameters.

medium, without the microorganism, were carried out to optimize the ratio of downcomer's diameter to riser's diameter, liquid level in the gas separator, and aeration rate. The optimum aeration rate, temperature, liquid level in the gas separator, and pH were 2.5 v.v.m, 30ºC, 3, and 3.5, respectively. The basic parameters of the ELAB is given in Table 1.

Analytical methods: Samples were collected every 2 h during a 20 h fermentation period in the STR and 24 h in the airlift during batch culture and were then analyzed. In order to measure the amount of biomass, samples were centrifuged at 1900×*g* for 15 min; supernatants were transferred to other tubes for the purpose of measuring lactose. After washing the biomass with Ringer serum, biomass was precipitated for a second time before measuring dry weight. The results are displayed in Table 2. Lactose concentration was determined by the Somogyi-Nelson method (Shafaghi 2000).

Model development

Tanks-in-series model for external-loop airlift reactor: In this simulation, the mixing characteristics are described by a tanks-in-series model. In the tanks-in-series model, the flow in the airlift bioreactor is considered as flow through a series of equally sized, well-mixed stirred stages or tanks and the parameter describing non-ideal flow is the number of stages. The mixing characteristics of the riser, downcomer, top and bottom sections in the airlift bioreactors are different (Verlaan *et al*., 1989). For example, in the computer simulation model of Merchuk and Stein (1981), the mixing characteristics in the riser and the downcomer were postulated as plug flow and the head space was considered to be well mixed. An extension for the incorporation of micro-mixing effects into the model can be carried out by introducing back-flow and lateral-flow (Zuo *et al*., 2006). In our model, the bottom (i=1) and top (i=N/2) sections are treated as well-mixed stages. The riser and the downcomer top sections, with i = 2, …, N/2-1 and i = N/2+1,…N respectively, are described as tanks-in-series. Also it is possible to modify the number of stages in each part.

Table 2. Concentration of biomass and lactose, cell number (N) versus time in the stirred tank reactor.

Time(h)	Biomass(g/l)	Lactose(g/l)	Cell No.	$\ln(N/N_0)$
0	0.028	28.5	2.25E+06	0.000
2	0.037	28	2.95E+06	0.271
4	0.055	27	4.40E+06	0.671
6	0.304	23	2.43E+07	2.380
8	1.501	19	1.20E+08	3.977
10	3.32	15.5	2.66E+08	4.773
12	6.45	12	5.15E+08	5.433
14	7.52	8.5	6.01E+08	5.588
16	7.55	8.3	6.03E+08	5.591
18	7.57	8	6.05E+08	5.594
20	7.52	8	6.57E+08	5.677

$P \leq 5\%$

At the top section, most of the gas bubbles passing upward in the riser disengage and only the rest is entrained downward by liquid recirculation into the downcomer. On the other hand, the flow in the downcomer is almost single-phase and relatively well defined. Therefore, the backmixing in the downcomer is neglected. It has been assumed that the oxygen concentrations in the gas phase are uniform and that there is no oxygen limitation for cells during SCP production. Consequently the oxygen balance is not taken into account; this is due to the premise that the fluid was saturated with oxygen. At the bottom section, the gas feed and the recycle flow from the downcomer are introduced. It is assumed that the fermentation has a good temperature control and the temperature is constant. Consequently, in this study, energy balances are not taken into account, as well as in the work of Luttman *et al*. (1983), Kanai *et al*. (1996), Znad *et al*. (2004).

The tanks-in-series model without back-flow provides simultaneous first order ordinary differential equations, which are material balances of the microorganism and substrate in hypothetical well-mixed tanks or stages. The unsteady state material balances of these components can be written as follows:

For the microorganism (biomass), x, substrate (lactose), s:

Bottom section (i = 1):

$$dx/dt = Q_l(x_N - x_i) / (v_b(1 - \varepsilon_{gr})) + \mu_m s_i x_i / (K_s x_i + s_i) \quad (1)$$

$$ds/dt = Q_l(s_N - s_i) / (v_b(1 - \varepsilon_{gr})) - \mu_m s_i x_i / (K_s x_i + s_i) - \lambda x_i \quad (2)$$

Riser section (i = 2, . . ., N/2-1):

$$dx/dt = Q_l(x_{i-1}-x_i)(N/2 - 2) / (v_r(1 - \varepsilon_{gr}))+ \mu_m s_i x_i/(K_s x_i + s_i) \quad (3)$$

$$ds/dt = Q_l(s_{i-1} - s_i)(N/2 - 2)/(v_r(1 - \varepsilon_{gr})) - \mu_m s_i x_i / (K_s x_i + s_i) - \lambda x_i \quad (4)$$

Top section (i = N/2)

$$dx/dt = Q_l(x_{i-1}- x_i) / (v_t(1 - \varepsilon_{gr})) + \mu_m s_i x_i / (K_s x_i + s_i) \quad (5)$$

$$ds/dt = Q_l(s_{i-1}- s_i) / (v_t(1 - \varepsilon_{gr})) - \mu_m s_i x_i / (K_s x_i + s_i) - \lambda x_i \quad (6)$$

Downcomer section (i = N/2 + 1, . . ., N)

$$dx/dt = Q_l(x_{i-1} - x_i)(N/2) / (v_d(1 - \varepsilon_{gd})) + \mu_m s_i x_i / (K_s x_i + s_i) \quad (7)$$

$$ds/dt = Q_l(s_{i-1} - s_i)(N/2) / (v_d(1 - \varepsilon_{gd})) - \mu_m s_i x_i/(K_s x_i + s_i) - \lambda x_i \quad (8)$$

Kinetic model:The kinetic model presented by Ghaly *et al*. (2004) has been used in this simulation to describe the biomass production from cheese whey:

$$dx_i/dt = \mu_i x_i \quad (9)$$

$$ds_i/dt = -\gamma(dx_i/dt) – \lambda x_i \quad (10)$$

where the specific growth rate is defined by:

$$\mu_i = \mu_m s_i / (K_s x_i + s_i) \quad (11)$$

and depends on one limiting substrate, i.e., lactose. Kinetic coefficients of growth and lactose consumption processes of *Trichosporon* sp. were estimated from the batch STR fermentation data using the MATLAB software by solving the differential equation set (9) and (10). In order to obtain the best fit of the experimental data the program determined parameter values resulting in the minimum total residual sum of squares.

RESULTS

Kinetic parameters: Biomass and substrate concentration profiles are shown in Figure 2. During the exponential growth phase, the biomass concentration increased exponentially with cultivation time and lactose concentration depleted rapidly.

Firstly $\mu_m$was determined as ln (N/N_0) versus time (Fig. 2). Maximum specific growth rate was obtained from logarithmic phase of this curve as 0.59 h^{-1}. To determine K_s, γ and λ in the model mentioned in part 3.2, MATLAB fitting tools were used which resulted in 46.84 g/l, 0.383, and 1.275, respectively.

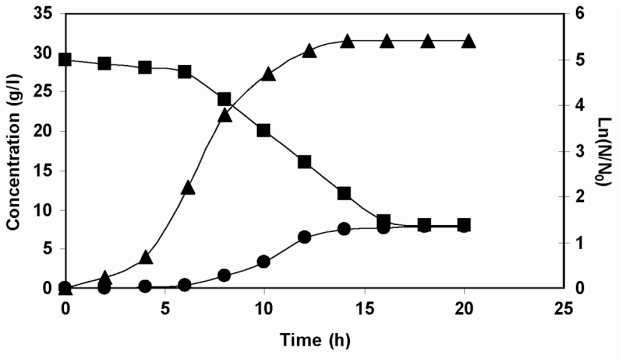

Figure 2. Growth curve versus time according to ln(N/N_0), and concentration of biomass & lactose in stirred tank reactor, ● Biomass, ■ Lactose, ▲ Ln (N/N_0).

Hydrodynamic parameters: In modeling of the reactors, it is necessary to understand their hydrodynamic behavior, in particular gas hold-up and liquid circulation velocity. These two parameters have been extensively studied because of their influence on transfer phenomena.

To solve the equations 1-4 the values of ε_{gr} and ε_{gd} are needed. The overall gas hold-up was calculated during the experiments by:

$$\varepsilon_g = (h_D – h_L) / h_D \quad (12)$$

where h_D and h_L are liquid level with and without aeration, respectively.

The gas holdups in the riser and downcomer are related to the overall gas hold-up by the analytical equation (Chisti, 1989)

$$\varepsilon_g = (A_r \varepsilon_{gr} + A_d \varepsilon_{gd}) / (A_r + A_d) \quad (13)$$

Using the following equation (Chisti, 1989)

$$\varepsilon_{gd} = 0.89 \varepsilon_{gr} \quad (14)$$

The calculated values for ε_{gr} and ε_{gd} are 0.078 and 0.069, respectively. The measured value of Q_l is 5.865 m^3h^{-1}.

Model parameters: Having estimated biological & hydrodynamic parameters, the remaining indefinite values were the model parameters: the number of stages which is an indicator of the extent of longitudinal mixing in the reactor and back-flow ratio in the riser including the top and the bottom stages. To specify the number of stages and back-flow ratio, the objective was minimizing the error between the model-predicted value and experimental data. We set

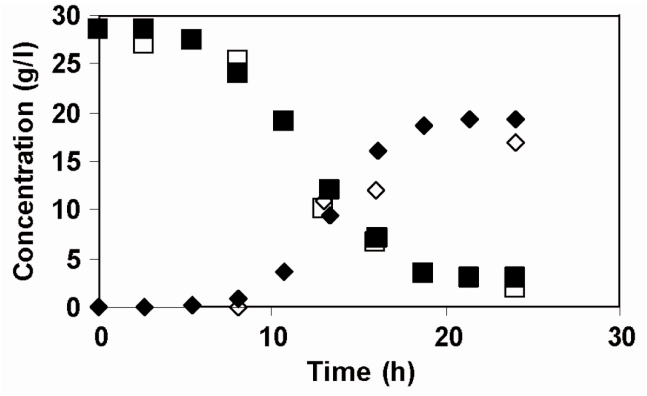

Figure 3. Concentrations of biomass and lactose in the external airlift bioreactor: Theoretical data, and experimental data, ◆ Biomass-Model, ◇Biomass-Exp., ■ Substrate-Model, □ Substrate-Exp.

back-flow at zero and then predicted N, the model parameter, in a way to give the best fitting between experimental and theoretical data. The value of N obtained equals 16. The experimental and theoretical data profiles are shown in Figure 3.

DISCUSSION AND CONCLUSION

The decrease in lactose and increase in biomass occurred in parallel and growth rate also increased simultaneously with the increasing lactose consumption rate (Figure 2). This phenomenon justifies our assumption that lactose is the growth controlling substrate. The kinetics of the growth of *Trichosporon* sp. using lactose as limiting substrate is satisfied by the Monod model. By finding the kinetics parameters, the growth of *Trichosporon* sp. was considered as a chemical reaction in ELAB.

Tanks-in-series model with a parameter of the number of tanks, gives the ability to fit the model with the experimental data. The type of reaction, operation system, and reactor definitely affect the extent of backmixing and reactant concentrations and therefore model parameters. We neglected the effect of oxygen (one of the reactants) for simplification, but it can be included simply in case of necessity. N tanks-in-series model, which for the first time has been applied to the external airlift bioreactor, predicted properly the experimental results in this reactor. In fact, the experimental data and the predicted results are in good agreement. But some deviations from experimental data in the later stages of fermentation were observed. This is due to lower oxygen transfer in the liquid phase of the bioreactor after addition of antifoam reagent and consequently reduction of biomass toward the last stages of fermentation.

NOMENCLATURE

t	time (h)
A_d	cross-sectional area of the downcomer (m^2)
A_r	cross-sectional area of the riser (m^2)
V_b	volume of the bottom section (m^3)
V_d	volume of the downcomer section (m^3)
V_L	working volume of the reactor (m^3)
V_r	volume of the riser section (m^3)
V_t	volume of the top section (m^3)
D_r	riser diameter (m)
D_d	downcomer diameter (m)
h_L	height of gas-free liquid (m)
h_D	height of aerated liquid (m)
h_r	height of the riser (m)
h_d	height of the downcomer (m)
N	number of stages in the bioreactor
Q_l	liquid flow rate (m^3h^{-1})
ε_{gd}	gas hold up in the downcomer
ε_{gr}	gas hold up in the riser
s	substrate concentration (kg m^{-3})
x	biomass concentration (kg m^{-3})
K_S	Saturation constant (kg m^{-3})
γ	growth associated parameter in the Luedeking-Pirt-like equation for substrate uptake (g substrate/g biomass)
λ	non-growth associated parameter in the Luedeking-Pirt-like equation for substrate uptake (g substrate/g biomass)
μ	specific growth rate (h^{-1})
μ_m	maximum specific growth rate (h^{-1})

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