Bioethanol Production from *Saccharomyces cerevisiae* through Conventional and Membrane Batch Fermentation: Experimental and Modeling Studies

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Running Title: Batch Fermentation for Bioethanol Production Using *S. cerevisiae*

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ABSTRACT

Kinetics of bioethanol production from glucose using *Saccharomyces cerevisiae* (PTCC 24860) was experimentally studied in a batch membrane bioreactor and conventional bioreactor using pervaporation process. For pervaporation, dense hydrophobic polydimethylsiloxane membrane was used. The batch membrane bioreactor resulted in increase of cell density, improved productivity and yield. A generic model was developed which can give a unique description for production of bioethanol within both batch membrane bioreactor and conventional bioreactor. Coupled describing equations of the model were solved by means of genetic algorithm approach. The logistic model considered for expression of growth kinetic and kinetic parameters calculated through genetic algorithm. The results demonstrated that this generic model is capable to describe reasonably the behavior of both conventional bioreactor and batch membrane bioreactor with highest correlation coefficient (0.979 and 0.987, respectively).

Keywords: Membrane bioreactor-Bioethanol-Genetic algorithm-Growth kinetics

INTRODUCTION

Limitations of fossil fuels as well as environmental pollution have created a great incentive for development of renewable energy sources in recent decades [1-3]. One of these renewable energy sources is bioethanol that its production is considered as a trusted energy source and suitable additive for gasoline or relevant fuels [4]. The benefits of using bioethanol as a fuel include decrease of greenhouse gas generation, higher octane number in combustion, and cheap energy which can be produced from materials including sugar [5]. Conventional bioreactor (CBR) for bioethanol production has some disadvantages such as low ethanol production due to product inhibition effect, requirement of additional purification steps in downstream, low cell densities in the cultivated broth and incomplete use of nutrients [6]. There are some processes to achieve a simultaneous separation of fermented ethanol as it is formed. The most important of them is membrane separation. Pervaporation is claimed to be one of the most efficient and promising techniques for separation of ethanol/water mixture in biological processes. That is because the process is simple and does not require extra chemicals [7]. Silicon containing polymers, especially polydimethylsiloxane (PDMS), have been widely used as the organics selective membrane for separation of organic/water mixtures via pervaporation process [8]. It is hydrophobic, and has a good chemical stability and biocompatibility in long duration time compared to other polymeric and inorganic membranes such as poly [1-(trimethylsilyl)-1-propyne] (PTMSP) and zeolite, respectively. Membrane technology represents one of the most effective and energy saving processes for ethanol production [9]. Coupling membrane separation with biological process in a single unit is very attractive configuration for the fermentations where continuous elimination of metabolites is necessary to maintain high productivity. It is designed to reduce substrate and product inhibitions, increase growth and cell density, increase ethanol productivity,

elimination of downstream processing, simple performance and improve system economic [10]. Irrespective of the process employed for fermentation, defining the kinetics and kinetic parameters of a bioprocess is an important step toward translating the considered model to industrial scales (scale-up) [11-13]. Several previous studies have considered modeling of different kinds of bioreactors for bioethanol production, such as batch [14, 15], continuous [16, 17], fed batch [18] and recycle reactors [19]. There are also some reports in the literature dealing with continuous ethanol production by integrating conventional fermentation and pervaporation [20]. Modeling of batch bioreactors including both membrane and conventional types has received some attentions, but focus has been to handle each process separately and no attempt was made to develop a unique general model for describing these two processes together. Besides, modeling of batch bioreactors performance (conventional and membrane-based) involves coupled differential equations describing biomass growth, substrate consumption and product generation which are usually difficult to solve them. Some models have been presented based on some simplifying assumptions which led to decoupled differential equations. Equations were solved separately to give the chemical species concentration involving in the bioprocess [21]. With the background mentioned above, the first aim in the present research was to enhance bioethanol production by integrating conventional fermentation with pervaporation in a batch membrane bioreactor (MBR). For this purpose, PDMS membrane was chosen for the removal of ethanol from fermentation broth which showed an improvement in ethanol yield and productivity. The second important task in this study was development of a unique general model for CBR and MBR for bioethanol production. The model developed for MBR naturally reduces to CBR model by eliminating the term including membrane flux. Coupled describing equations of the

model were solved following an optimization procedure based genetic algorithm by which the biokinetic parameters were confidently determined.

MATERIALS AND METODS

Culture Conditions. All chemicals used in present study were analytical grades and supplied by Merck (Darmstadt, Germany). The medium contained glucose, yeast extract and NH_4Cl with concentrations of 50, 3 and 5 g/l, respectively. The medium was autoclaved at 121^{o}C and 15 psig for 20 min. The sterilized medium was inoculated with 5% of pure seed culture of the microorganism (S. cerevisiae) and then the culture was cultivated in an incubator at 30^{o}C for 24 h.

Microorganism. The pure stock culture of S. cerevisiae was used. The strain was originated from Persian Type Culture Collection (PTCC 24860), supplied by Iranian Research Organization for Science and Technology (IROST).

MBR System. Figure 1 illustrates a schematic diagram of the MBR system used in this study. A set of experiments were carried out in the CBR and MBR with the same working volume of 1260 ml. Polydimethylsiloxane (PDMS) membrane (Supplied by Pervatech. Company, Netherland) with an effective thickness of 20 µm was used as a selective separation barrier to enhance substrate conversion through continuous withdrawal of ethanol from fermentation chamber [22]. The pressure on the top feed side was atmospheric, while the bottom side of membrane evacuated with a vacuum pump (E2M2-Edvards) to 1 mbar. The enriched ethanol was collected at the permeate side in liquid form using liquid nitrogen cold trap. Experiments were carried out at constant temperature of 32^{0}C. Samples were taken every 2 h from the broth and permeate and cell dry weight, glucose and produced ethanol concentration were measured by spectrophotometer, color-metric method using DNS reagent, and gas chromatograph, respectively. It should be noted that the MBR system could easily adapted to act as CBR by replacing the membrane at

the bottom of the fermentation chamber by a glass plate and eliminating cold trap at the permeate side.

MODELING

Growth Kinetics is typically categorized to structured and unstructured models or segregated and non-segregated models [14]. In this study the model was considered to be unstructured and non-segregated in logistic category. The same model was used for the CBR and MBR.

The General Model for Biomass Growth Kinetic. Most of models can describe log phase of cell growth, while few models can describe lag, exponential and stationary phases all together. In this study, the Logistic model was selected. The Logistic kinetic model is a suitable model for prediction of growth curve [23]. The specific growth rate projected by Logistic model is expressed as:

$$\mu = K.(1-\frac{X}{X_m}) \quad (1)$$

Where, μ is the specific growth rate [h^{-1}] that depends on biomass concentration, K is Logistic constant, X is concentration of S. cerevisiae [$g.L^{-1}$] and X_m is maximum concentration of S. cerevisiae [$g.L^{-1}$].

The mass balance for biomass generation can be illustrated as follows:

$$0 - F_m.X_0 + r_X.V = \frac{d(X.V)}{dt} \quad (2)$$

Rate of biomass in - rate of biomass out + generation rate of biomass = accumulation rate of biomass.

Where F_m is volumetric flow rate [$L.h^{-1}$], X_0 is concentration of S. cerevisiae at the membrane permeates side [$g.L^{-1}$], r_x is biomass growth rate [$g.L^{-1}.h^{-1}$], V is working volume of bioreactor [L] and t denotes fermentation time [h]. Considering that no biomass can pass through the membrane, X_0=0, Equation reduces to:

$$r_X.V = X\frac{dV}{dt} + V\frac{dX}{dt} \quad (3)$$

Total material balance for the MBR can be written as follows:

Rate of mass in - rate of mass out = Rate of mass accumulation

$$0 - F_m = \frac{dV}{dt} \quad (4)$$

Integration the above equation with initial condition of (t=0, V= V_0) leads to a linear relationship in variation of bioreactor volume with time:

$$V = V0 - F_m.t \quad (5)$$

V_0 is initial working volume of bioreactor [L] and r_x is given by Malthus law:

$$r_X = \mu.X \quad (6)$$

Combination of (1), (3) and (5) results in:

$$\frac{dX}{dt} = [K.(1 - \frac{X}{X_m}) + \frac{F_m}{V0 - F_{m.}t}].X \quad (7)$$

The General Model for Substrate Consumption. The Logistic model can successfully describe utilization of substrate. Mass balance for substrate utilization rate can be illustrated as follows:

Rate of substrate in - rate of substrate out – substrate consumption rate = accumulation rate substrate

$$0 - F_m.S_m - r_s.V = \frac{d(S.V)}{dt} \quad (8)$$

where S_m is the concentration of glucose at the membrane permeate side [$g.L^{-1}$], S is glucose concentration in the MBR [$g.L^{-1}$], r_s is consumption rate of glucose [$g.L^{-1}.h^{-1}$]. Knowing that membrane is not permeable to glucose, S_m=0, equation (8) reduces to:

$$- rs.V = S.\frac{dV}{dt} + V.\frac{dS}{dt} \quad (9)$$

The consumption rate of glucose can be related to the specific glucose utilization rate by the following equations:

$$r_s = \frac{\mu''.X}{Y_{x/s}} \quad (10)$$

$$\mu'' = K''.(1 - \frac{X}{X_m}) \quad (11)$$

Where the $Y_{x/s}$ is the yield of cell concentration based on the substrate consumption.

By substitution of (5), (10) and (11) into (9):

$$\frac{dS}{dt} = -\frac{\mu''.X}{Y_{x/s}} + \frac{Fm}{V0 - .Fm.t}.S \quad (12)$$

$$\frac{dS}{dt} = -K''.(1 - \frac{X}{Xm}).\frac{X}{Y_{x/s}} + \frac{Fm}{V0 - .Fm.t}.S \quad (13)$$

The General Model for Bioethanol Formation. Mass balance describing product formation in the MBR is given by following equation:

Rate of ethanol in - rate of ethanol out + formation rate of ethanol = Rate of ethanol accumulation

It should be noted that the membrane is permeable to ethanol, and therefore an output term would exist in the ethanol mass balance expression:

$$0 - F_m.P_m + r_p.V = \frac{d(V.P)}{dt} \quad (14)$$

$$- F_m.P_m + r_p.V = P.\frac{dV}{dt} + V.\frac{dP}{dt} \quad (15)$$

$$r_p = \mu'.X.Y_{p/s} \quad (16)$$

Where P_m is concentration of ethanol at membrane permeate side [$g.L^{-1}$], P is ethanol concentration in the MBR [$g.L^{-1}$], r_p is ethanol production rate [$g.L^{-1}.h^{-1}$]. $Y_{p/s}$ the yield of formed product based on substrate consumption.

By substitution of (5) and (16) into (15):

$$\frac{dP}{dt} = K'.(1-\frac{X}{X_m}).X.Y_{p/s} + \frac{F_m}{V_0 - F_m.t}.(P - P_m) \quad (17)$$

In summary, a set of differential equations consisting equations (7), (13) and (17) was obtained using Logistic kinetic model which describes *S. cerevisiae* growth, glucose consumption and ethanol production in the MBR, respectively.

Putting F_m =0, the set of equations obtained for the MBR reduces to a set of equations describing cerevisiae growth, glucose consumption and ethanol production for the CBR as follows:

$$\begin{cases} \frac{dX}{dt} = K.(1-\frac{X}{Xm}).X & (18) \\ \frac{dS}{dt} = -K''.(1-\frac{X}{Xm}).\frac{X}{Y_{x/s}} & (19) \\ \frac{dP}{dt} = K'.(1-\frac{X}{Xm}).X.Y_{p/s} & (20) \end{cases}$$

Both the sets of equations describing the MBR and CBR performances for bioethanol production consist of coupled differential equations which cannot be integrated separately to give the species concentration. Therefore, a special mathematical treatment is required to solve the problem. In this study we have used an optimization procedure based on genetic algorithm approach.

Finding the Best Parameters of Model Equations by Means of Genetic Algorithm. Expression of a modeling issue as an optimization problem is an effective way to solve many of problems including curve fitting. System identification method based on genetic algorithm is one of the ways to do this work. Genetic algorithm was used as a tool to search global optimum and it improved the parameters from the previous step to reduce the difference between experimental data and modeling results. Estimation of experimental data by means of a physical model has been done by obtaining parameters of the model so that the resulting functions would have minimum deviation compared to experimental data.

In this problem, calculations were begun with an initial guess by genetic algorithm. Then the initial guess was inserted in the differential equations in order to describe behavior of system. The system of differential equations solved by Range–Kutta method and its results were compared with experimental data. A program code was developed by means of the software package Matlab (Version 7.14) for this purpose. The calculation steps in the optimization procedure are given by the flowchart of Figure 2.

RESULT AND DISCUSION

In this study, glucose with fixed initial concentration of 50 $g.L-^{1}$ was used as substrate in the CBR and MBR. Before starting the bioprocess, separation performance of PDMS membrane was estimated separately via pervaporation of ethanol/water mixture as feed at low ethanol concentration attainable in the bioreactor. The selectivity of around 7 for ethanol over water and total flux of 0.46 $Kg.m^{-2}.h^{-1}$ was obtained which was used for modeling purposes. In the CBR at stationary phase of growth which was achieved after 22 h, ethanol and cell concentrations were approximately constant at 22.22 $g.L^{-1}$ and 13.25 $g.L^{-1}$, respectively, while glucose was almost completely consumed. The calculated yield of cell concentration based on substrate consumption, $Y_{x/s}$ and the yield of produced ethanol based on substrate consumption, $Y_{p/s}$ in the CBR were to be 0.32 and 0.54 g/g, respectively which increased to 0.41 and 0.59 g/g in the MBR and the productivity was 1.106 $g.L^{-1}.h$**.** In the MBR mode of operation at the stationary phase of growth, the cell and ethanol concentrations in the broth were 15.35 $g.L^{-1}$ and 20.02 $g.L^{-1}$ respectively. The cell concentration in the broth was higher than that of the CBR. The enriched ethanol with high concentration of 13.8 wt% was obtained at permeate side of membrane.

Figure 3 (a), (b) and (c) show variation of the cell *(S.cerevisiae*), glucose, and ethanol concentration with time in the CBR which was calculated by genetic

algorithm with simultaneous solving three equations (18), (19), (20) as coupled equations. As can be clearly seen from figures, it demonstrated that the Logistic model could successfully describe *S.cerevisiae* growth, glucose, and ethanol concentrations in CBR. Time variation of the mentioned species in the MBR was presented in Figure 4 (a), (b) and (c). Three equations (7), (13) and (17) were calculated contemporary by genetic algorithm. It was found that the Logistic model was efficiently described growth, glucose consumption, and ethanol concentration in MBR.

Experimental results obtained for the CBR and MBR were compared in Table 1 with the model predicted values.

The objective function used for optimization study based on genetic algorithm was defined as:

$$\text{Objective function} = \sum_{j=1}^{m}\sum_{i=1}^{N}(y_{j_{approximation_i}} - y_{j_{experimental_i}})^2 \quad (21)$$

Where m and N are number of equations and samples and $yj_{approximation\ i}$ is approximation solution.

In this study the convergence criterion was set based on determination coefficient. A fitting approach was used to evaluate the kinetic parameters by matching the experimental data with the equations describing for S. cerevisiae, glucose and bioethanol concentrations. The fitting results in terms of determination coefficients are shown in Table 2, while the kinetic parameters and yield of fermentations are presented in Table 1. As can be observed from Table 1, there is a close agreement between the experimental and model predicted values with respect to the maximum cell concentration, the yield of cell concentration based on substrate concentration

($Y_{x/s}$) and the yield produced ethanol based on substrate concentration ($Y_{p/s}$). Also the model predicted values using the optimized parameters were shown in Figures 3 and 4 along with experimental data. As shown in these figures the set of equations in the form of a unique general model for the CBR and MBR were capable to describe efficiently *S. cerevisiae* growth, glucose consumption and bioethanol production.

CONCOLUSION

Bioethanol production from glucose using S. cerevisiae was experimentally studied via CBR and membrane bioreactor using pervaporation (MBR) in batch mode of operation. The results demonstrated that integration of fermentation bioprocess with membrane separation enhanced the ethanol productivity at least by 27% over conventional batch fermentation. One of the most important problems for bioethanol production is still lack of a comprehensive general model to describe variation in concentration of chemical species involved in the bioprocess. In this study, a generic model was developed to describe performance of MBR which naturally reduces to a describing model for CBR by eliminating the term containing membrane flux. Coupled differential equation describing the processes were solved following an optimization procedure using genetic algorithm. Kinetic parameters were recovered through genetic algorithm fit of experimental data with model equations. It was found that general equations were efficiently described S. cerevisiae growth, glucose consumption and bioethanol production.

NOTATION

F_m	Volemetric flow rate [$L.h^{-1}$]
K	Logistic kinetic constant (biomass) [h^{-1}]
K	Logistic kinetic constant (substrate) [h^{-1}]
K	Logistic kinetic constant (product) [h^{-1}]
P	Bioethanol concentration [g. L^{-1}]
P_m	Maximum Bioethanol concentration [g. L^{-1}]
r_p	Bioethanol production rate [$g.L.^{-1}h^{-1}$]
r_s	Glucose utilization rate [$g.L.^{-1}h^{-1}$]
r_x	S.cerevisiae growth rate [$g.L.^{-1}h^{-1}$]
S_0	Initial glucose concentration [g. L^{-1}]
S	Glucose concentration [g. L^{-1}]
S_m	Outlet glucose concentration [$g.L^{-1}$]
t	Time [h]
V_0	Initial working volume of ferementor [L]
V	Working volume of ferementor [L]
X_0	Initial *S.cerevisiae* concentration [g. L^{-1}]
X_m	Maximum *S.cerevisiae* concentration [g. L^{-1}]
X	S.cerevisiae concentration [g. L^{-1}]
$Y_{p/s}$	Yield of Bioethanol concentration based on substrate utilization
$Y_{x/s}$	Yield of *S.cerevisiae* concentration based on substrate utilization
μ	Specific growth rate (*S.cerevisiae*) [h^{-1}]
μ'	Specific growth rate (glucose) [h^{-1}]
μ''	Specific growth rate (bioethanol) [h^{-1}]

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List of Tables and Figures:

Figure 1.

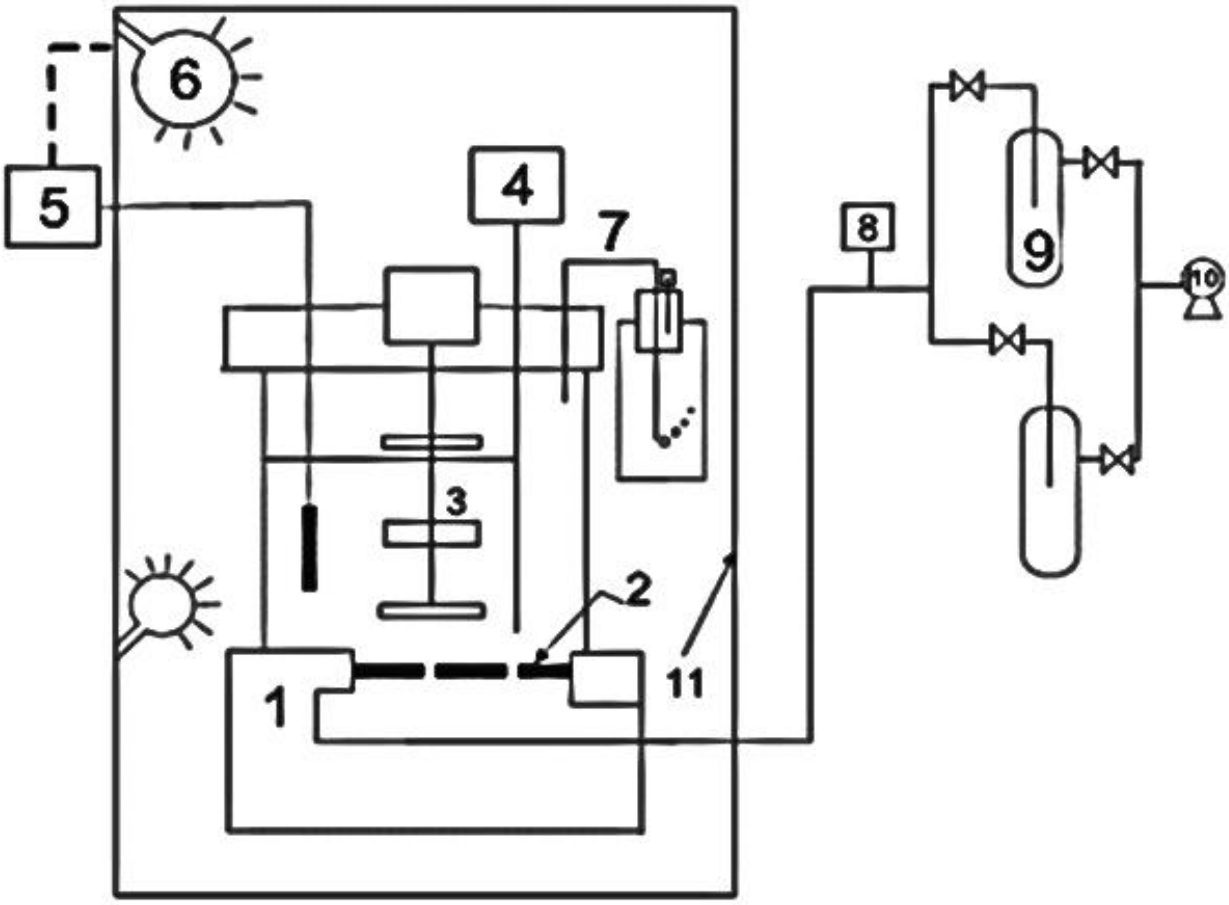
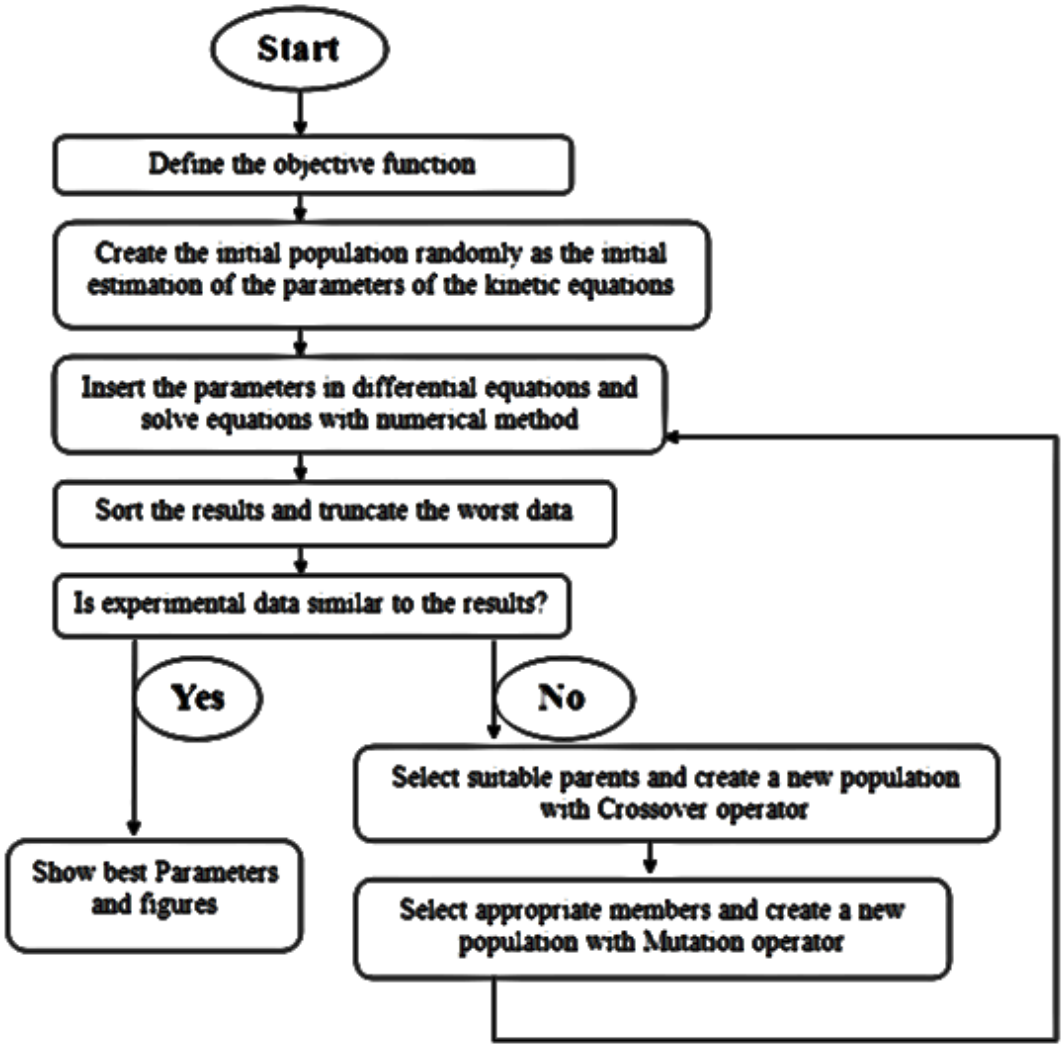


Figure 2.


Start
Define the objective function
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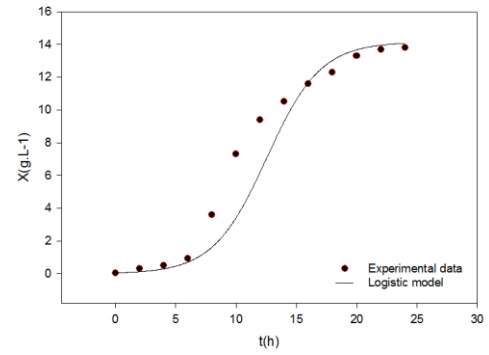
Insert the parameters in differential equations and solve equations with numerical method
Sort the results and truncate the worst data
Is experimental data similar to the results?
Yes
No
Show best Parameters and figures
Select suitable parents and create a new population with Crossover operator
Select appropriate members and create a new population with Mutation operator

Figure 3(a).



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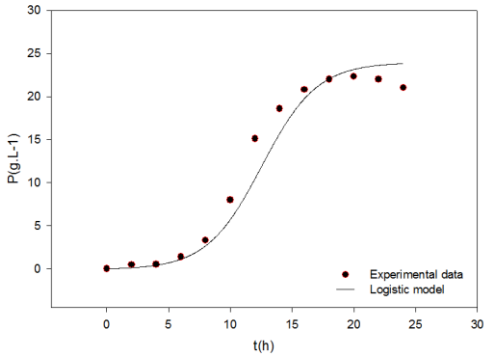

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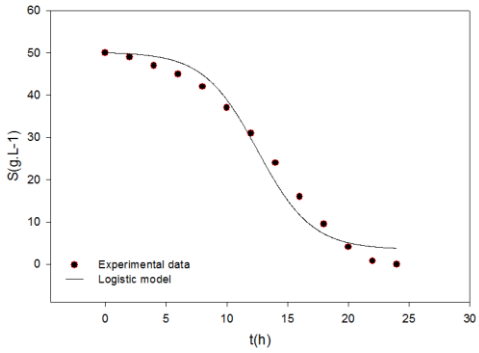

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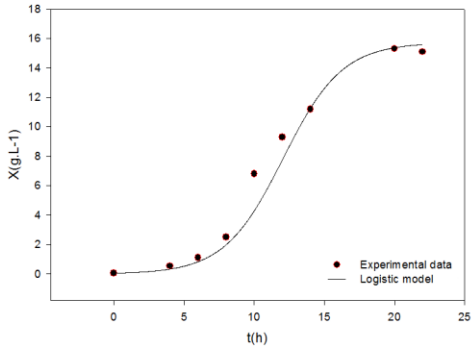

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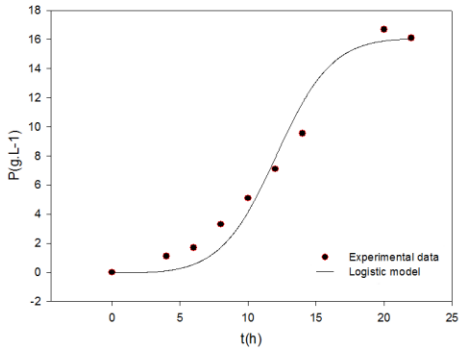

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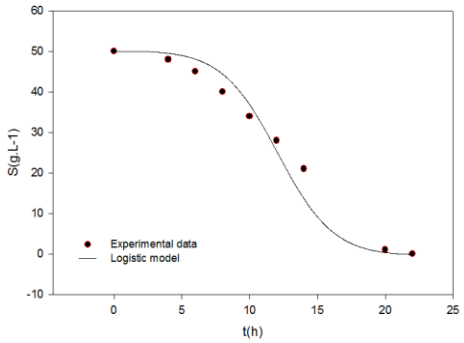

Table 1.

		K $[h^{-1}]$	X_m $[gL^{-1}]$	$K^{'}[h^{-1}]$	$K^{''}[h^{-1}]$	$Y_{p/s}$	$Y_{x/s}$
CBR	Experimental	-	13.80	-	-	0.32	0.54
	Model	0.45	14.16	2.03	0.82	0.38	0.55
MBR	Experimental	-	15.33	-	-	0.41	0.59
	Model	0.47	15.65	1.24	1.21	0.42	0.75

Table 2.

System	R-square
CBR	0.979
MBR	0.987