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Modeling and validating *Pseudomonas aeruginosa* kinetic parameters based on simultaneous effect of bed temperature and moisture content using lignocellulosic substrate in packed-bed bioreactor

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ABSTRACT

The aim of this study was to propose kinetic parameters of a bacterial growth and its products formation as functions of environmental conditions. Hence, the effect of temperature and initial moisture content on bacterial growth and rhamnolipid biosurfactant production by *Pseudomonas aeruginosa* were investigated in the first step. A mixture of corn bran and corn germ was used as substrate in small glass packed-bed laboratory bioreactors. Results showed the highest rhamnolipid production rate was observed in stationary phase and the highest growth and rhamnolipid production were at 35 °C and initial bed moisture of 70%. Then, kinetic parameters of the bacteria were determined using the fitting of kinetic models to experimental results of cumulative CO_2, rhamnolipid amount and metabolic water production. After that, the kinetic parameters m_{CO_2}, m_{RL}, m_w and $Y_{X/W}$ were considered with constant values while μ_m, X_m, Y_{X/CO_2} and $Y_{RL/X}$ were presented as functions of environmental conditions, by modifying a model and using 3-dimensional curve fitting. Finally, in order to validate the obtained kinetic parameters and functions, transport phenomena in a packed-bed bioreactor were modeled in 2-dimensions. The model results were verified by the experimental results obtained from a 3-liter glass packed-bed bioreactor and the validation was satisfactory.

1. Introduction

Solid-state fermentation (SSF) is the process of microbial growth on solid culture in absence of free water, although, for metabolic activities of microorganisms, enough water content in solid bed is necessary. During last two decades, SSF has attracted attentions widely. Cheap substrate (usually agricultural waste), less energy consumption, higher yields, less sewage and being more eco-friendly, are the advantages of SSF over submerged fermentation (SmF). However, low conductive heat coefficient and the heterogeneity of solid cultures are drawbacks of SSF (Pandey et al., 2008; Singhania et al., 2009).

Mathematical models by considering various occurred phenomena in a bioreactor are significant tools for optimization of design and operation in SSF bioreactors. These models should describe the growth kinetic of microorganism, the effect of environmental conditions on the growth and vice versa. Because of changes in bed temperature and moisture content of SSF, quantitative description for effect of bed temperature and moisture content on microbial growth is essential factor in modeling and optimization of SSF processes (Hamidi-Esfahani et al., 2004). Different models to describe the influence of environmental conditions on kinetic parameters have been presented in SSF. Some researchers have used modified logistic equation to describe effect of temperature on growth kinetic, according to amount of a physiological factor in microorganism (Dalsenter et al., 2005; Fanaei and Vaziri, 2009). Also some others have described growth kinetic parameters as a function of temperature by using Arrhenius, ratkowski and polynomial equations (Hamidi-Esfahani et al., 2007; Ikasari et al., 1999; Weber et al., 2002). Von Meien and Mitchell presented a 4-parametric model to

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Nomenclature

a, b, c, d	Antoine equation constants of air saturation, dimensionless
a_1, a_2, b_0, b_1, b_2	Fit parameters, dimensionless
BW	Mass of water in bed, g water kg^{-1} IDS
CCP	Cumulative CO_2 production, g CO_2 kg^{-1} IDS
CCP_0	Initial cumulative CO_2 production, g CO_2 kg^{-1} IDS
CPR	CO_2 production rate, g CO_2 kg^{-1} IDS h^{-1}
C_{pa}	Heat capacity of moist air, J kg^{-1} °C^{-1}
C_{pb}	Heat capacity of bed, J kg^{-1} °C^{-1}
C_{ps}	Heat capacity of substrate, J kg^{-1} °C^{-1}
$\frac{dH_{sat}}{dT}$	Change in the water-carrying capacity of air with a change in temperature, g water kg^{-1} air °C^{-1}
h	Convective heat transfer coefficient for thermal jacket, J m^{-2} s^{-1} °C^{-1}
IDS	Initial dry substrate, kg
IWS	Initial wet substrate, kg
K_a	Thermal conductivity of moist air, J h^{-1} m^{-1} °C^{-1}
K_b	Thermal conductivity of bed, J h^{-1} m^{-1} °C^{-1}
K_s	Thermal conductivity of substrate, J h^{-1} m^{-1} °C^{-1}
KP	A kinetic parameter as a function of environmental conditions
m_P	Maintenance coefficient on product, g product g^{-1} biomass h^{-1}
m_{CO_2}	Maintenance coefficient on CO_2, g CO_2 g^{-1} biomass h^{-1}
m_{RL}	Maintenance coefficient on rhamnolipid, g rhamnolipid g^{-1} biomass h^{-1}
m_w	Maintenance coefficient on water, g water g^{-1} biomass h^{-1}
P	Product formation, g product kg^{-1} IDS
PR	Product formation rate, g product kg^{-1} IDS h^{-1}
p	Air pressure, Pa
R	Bed radius of 3-liter packed-bed bioreactor, m
R_{evap}	Evaporation rate, g water h^{-1}
RL	Rhamnolipid production, g rhamnolipid kg^{-1} IDS
RL_0	Initial rhamnolipid, g rhamnolipid kg^{-1} IDS
r	Radial position, m
T	Bed temperature, °C
T_0	Initial bed temperature, °C
T_{min}	Minimum temperature for growth, °C
T_{max}	Maximum temperature for growth, °C
T_J	Thermal jacket temperature, °C
T_a	Inlet air temperature, °C
t	Time, h
V	Volume of bed, m^3
V_z	Superficial velocity of moist air, m h^{-1}
W	Metabolic water production, g water kg^{-1} IDS
W_0	Initial metabolic water, g water kg^{-1} IDS
X	Biomass concentration, g biomass kg^{-1} IDS
X_0	Initial biomass concentration, g biomass kg^{-1} IDS
X_m	Maximum biomass concentration, g biomass kg^{-1} IDS
Y	Bed moisture content, g water kg^{-1} IWS
Y_0	Initial bed moisture content, g water kg^{-1} IWS
Y_Q	Metabolic heat yield coefficient, J g^{-1} biomass
$Y_{P/X}$	Yield coefficient for product, g product g^{-1} biomass
$Y_{RL/X}$	Yield coefficient for rhamnolipid, g rhamnolipid g^{-1} biomass
$Y_{X/W}$	Yield coefficient for water, g biomass g^{-1} water
Y_{X/CO_2}	Yield coefficient for CO_2, g biomass g^{-1} CO_2
Z	Bed height of 3-liter packed-bed bioreactor, m
z	Axial position, m
ε	Porosity of the bed, dimensionless
λ	latent heat of evaporation of water, J g^{-1} water
μ	Specific growth rate, h^{-1}
μ_m	Maximum specific growth rate, h^{-1}
ρ_a	Air density, kg m^{-3}
ρ_b	Bed density, kg m^{-3}
ρ_s	Substrate density, kg m^{-3}

describe the effect of moisture content on specific growth rate (μ) for two fungal species (von Meien and Mitchell, 2002). Hamidi-Esfahani et al. (2004) presented equations to describe simultaneous effect of temperature and moisture content on growth kinetic parameters of *Aspergillus niger*. On the other hand, most of the mentioned studies have been about fungi and yeast that in some cases, by using artificial substrate, SSF condition was simulated for the growth of microorganisms. However, although bacterial cultures in SSF have been recently used widely in order to produce valuable biological products, but less attention is paid to growth kinetic of bacterial systems in SSF (Thomas et al., 2013). In addition to this, knowing both the kinetics of bacterial growth and products formation is important for modeling bioreactors to predict and scale them up; However, most researchers have only reported the results of a bacterial growth or production of a specific metabolite in bacterial fermentation processes in SSF (Mazaheri and Shojaosadati, 2013). And few researches have been conducted into product formation models.

Some presented product models are according to mass balance (Hashemi et al., 2011) and microorganism stoichiometry (Mazutti et al., 2010); and usually, Luedeking–Piret (Luedeking and Piret, 1959) equation is used to model growth associated or non-growth associated product. In the majority of researches, kinetic parameters of models for products formation are reported at optimum conditions of temperature and moisture content. However, in reality, these parameters are dependent upon environmental conditions that mostly vary during a SSF process.

An important factor in better designing of SSF bioreactors, is to provide the models in which transport phenomena and kinetic models are coupled to each other (Mazaheri and Shojaosadati, 2013). Transport phenomena are any of the phenomena involving the movement of various entities, such as oxygen, CO_2, water and energy through a medium. In many researches in SSF, transport phenomena are merely coupled to growth kinetic (Fanaei and Vaziri, 2009; Sangsurasak and Mitchell, 1998; Weber et al., 2002); although in a few researches (Meeuwse et al., 2012), transport phenomena are coupled to kinetic model of product formation.

Measuring the amount of biomass is essential in determining microbial growth and kinetic study of it. Direct measurement of biomass in SSF is generally difficult due to the attachment of microorganisms to the substrate (Singhania et al., 2009). In some studies, researchers have tried to simulate SSF by using nutrient carriers (Hamidi-Esfahani et al., 2004; Lareo et al., 2006) and hydrogel (Zolfaghari-Esmaeelabadi and Hejazi, 2018) in order to ease the biomass measurement. Respiratory measurement is one of the most important and useful methods for indirect measurement of biomass that in many researches (de Carvalho et al., 2006; Karimi et al., 2014) have been used. Measurement of O_2 consumption or CO_2 production rate in output air is a fast and practical way of providing a large amount of data that are directly related to microorganisms metabolism (Auria et al., 1993; Saucedo-Castañeda et al., 1994).

Recently, a group of biosurfactants called rhamnolipids, commonly produced by *P. aeruginosa*, because of their vast applications in decomposition of hydrocarbon contaminants, removal of heavy metals from soil, anti-microbial activity, the synthesis of nanoparticles by the Greener method, biodegradability and low toxicity have been widely considered (Kiran et al., 2016; Wu et al., 2017; Zhu et al., 2012). Production of biosurfactants by SmF method involves problems such as high foam production and a high increase in the viscosity of the medium due to the production of exopolysaccharides. Hence, studies have been conducted into production of biosurfactants in SSF (Thomas et al., 2013).

So far, some studies have been carried out to investigate the growth kinetics of *P. aeruginosa* and rhamnolipid production in SmF. Study of the effect of temperature on *P. aeruginosa* growth and production of rhamnolipid, modeling of growth kinetic and rhamnolipid production in constant environmental condition, are the reported cases in SmF (Lotfabad et al., 2009; Silva et al., 2010; Zhu et al., 2012). In a study, the kinetics of rhamnolipid production for *P. aeruginosa* has been investigated in SSF (Camilios Neto et al., 2008). But, the kinetic parameters of rhamnolipid production have not been determined. Lately, in our last study, growth kinetics of *P. aeruginosa* has been described as a function of substrate and biomass concentration in the biofilm in SSF (hydrogel) (Zolfaghari-Esmaeelabadi and Hejazi, 2018). The study reported that the concentration of biomass in biofilm is the merely factor of growth inhibition. However, enough knowledge about kinetic parameters of *P. aeruginosa* as a function of various environmental conditions for rhamnolipid production has not been presented in SSF.

The aim of this study was to determine kinetic parameters of a bacterial growth and its products formation as functions of environmental conditions and validate them by coupling transport phenomena and kinetic models to each other in a higher scale. In this study, first, respiration and products formation for *P. aeruginosa* in various conditions of bed temperatures and initial moisture contents on mixture of corn bran and corn germ as natural substrate were investigated in laboratory small packed-bed bioreactors. Next, the kinetic parameters were determined by non-linear curve fitting of corresponding equations to the experimental results of CO_2, rhamnolipid and metabolic water production. Then, by modifying and applying model of Hamidi-Esfahani et al., kinetic parameters were presented as functions of environmental conditions. Although the kinetic parameters of different microorganisms have been determined in SSF (Hamidi-Esfahani et al., 2004; Hashemi et al., 2011), they have been less studied for their accuracy in other bioreactor scales. Hence, in this study, in order to evaluate the accuracy of obtained bacterial kinetic parameters, a 3-liter packed-bed bioreactor were modeled by using obtained kinetic parameters and equations of transport phenomena. Finally, the results of this modeling were validated using experimental results obtained from the 3-liter packed bed bioreactor.

2. Material and methods

2.1. Materials

All the chemicals were of analytical grade and the solutions were prepared with distilled water. The corn germ and corn bran were obtained from Glucosan Company (Ghazvin, Iran).

2.2. Microorganism

P. aeruginosa IRMD-2010 was used in this study. The microorganism was isolated and identified in the Biotechnology Laboratory of Iran University of Science and Technology. A loop of the bacteria from 24 h incubated nutrient agar was added to LB (Luria broth) culture. The culture was incubated in shaker-incubator for 15 h at 30 °C and 150 rpm until the optical density of 4 in 600 nm and after that used as seed culture.

2.3. Substrate

The mixture of the dried extracted oil-corn germ meal and corn bran as oily (10–14%) and cellulosic substrates with the proportion of 70:30 (%w/w) was applied in SSF. For setting the pH and the moisture content of substrate, the mixture of distilled water and NaOH 0.4 N was used. The sterilization operation was carried out at temperature of 121 °C and pressure of 1.2 bar for 20 min. The substrate was inoculated with 10% (v/w) of seed culture.

2.4. Bioreactors: set-up and fermentation conditions

In order to investigate respiration and products formation of *P. aeruginosa*, five small packed-bed bioreactors were applied at different conditions of temperature and initial bed moisture content. After that, a 3-liter packed-bed bioreactor was used to validate the parameters and kinetic functions obtained from experiments involving small packed-bed bioreactors. Fig. 1 represents schematic diagrams of packed-bed bioreactors setup and the detail description is as follows:

2.4.1. Small packed-bed bioreactors

Fig. 1(a) shows the schematic diagram of laboratory small packed-bed bioreactors set up. Each bioreactor was an autoclavable glass tubular column with the height of 12 cm and the internal diameter of 2.5 cm and each bioreactor contained 10 g IDS (initial dry substrate). Each bioreactor was connected to the humidifier at a flow rate of 0.5 L h^{-1} g IDS filtered inlet air (with a 0.2-μm pore size hydrophobic PTFE filter). The fermentation time was considered 120 h. Every 24 h, one of the bioreactors was removed and the fermented bed was used for analysis of products formation. The output air of a bioreactor was dried and connected to a CO_2 sensor (Biosense, Switzerland) until the end of the fermentation process. CO_2 results were logged on-line by a computer every hour. The bioreactors were placed in an incubator equipped with forced air flow to adjust the fermentation temperature.

An extra small packed-bed bioreactor with no inoculation (filled with substrate) was applied as control sample (not shown in Fig. 1a). This bioreactor was used for two aims: First, no microbial contamination penetrated the bioreactors; second, the inlet saturated air did not affect the initial moisture content of substrate during 120 h fermentation.

General factorial design of experiments was carried out for two variables of bed temperature (20, 25, 30, 35, 40 and 45 °C) and initial moisture content (60, 65, 70, 75 and 80%). The initial moisture content of the bed was expressed as percentage and based on the weight of water per weight of the initial wet substrate (g-water kg^{-1} IWS). All experiments in this section were carried out 2-times, and the results were presented in the mean and with the corresponding standard deviations.

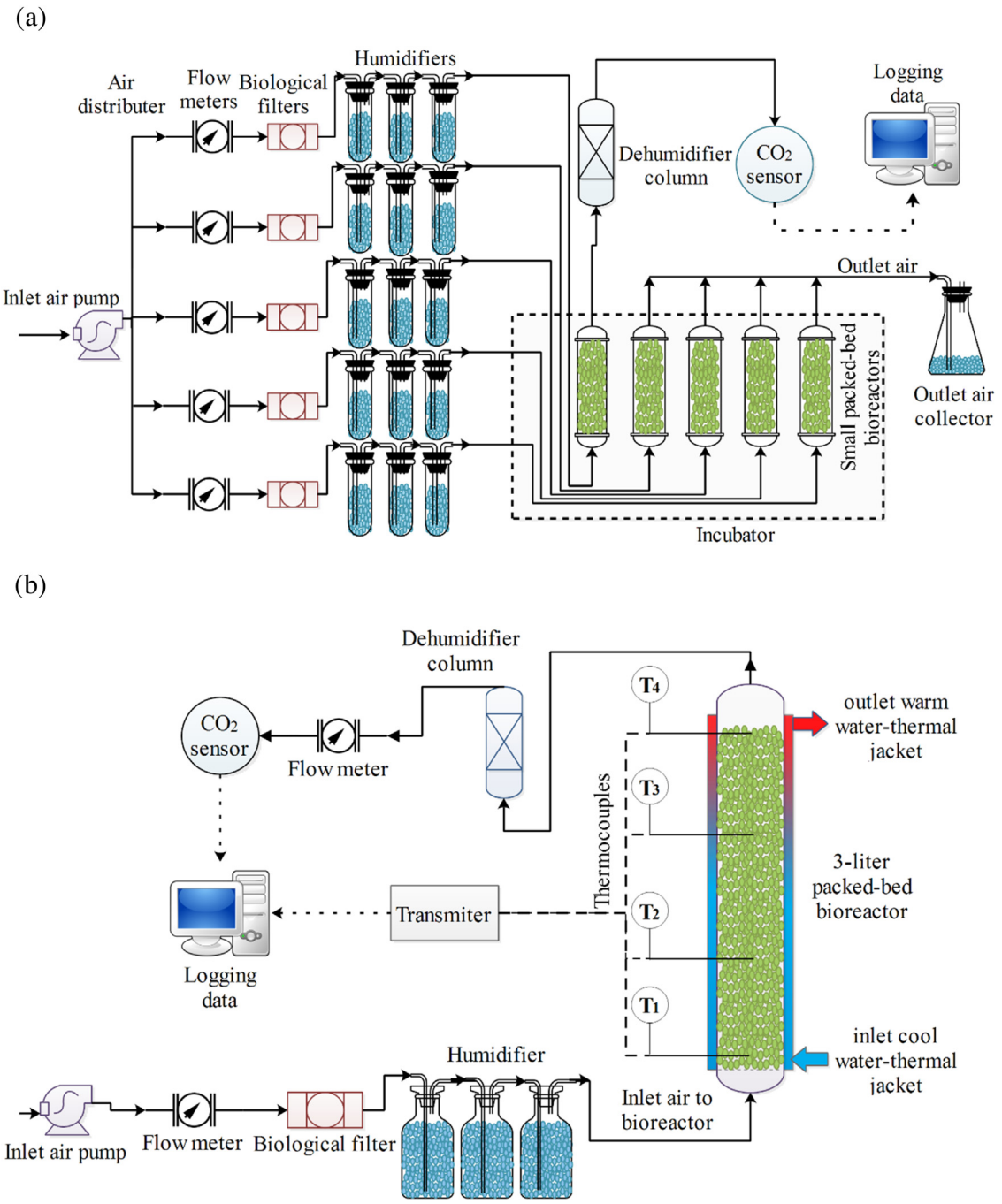

Fig. 1 – Schematic diagrams of packed-bed bioreactors set-ups. (a) Small packed-bed bioreactors set up; (b) 3-liter packed-bed bioreactor set up.

2.4.2. *3-liter packed-bed bioreactor*

Fig. 1(b) shows the schematic diagram of 3-liter glass packed-bed bioreactor set up. The bioreactor was an autoclavable glass cylindrical column with the height of 60 cm and the internal diameter of 6 cm. The column was surrounded by a thermal jacket. The water inside the jacket was circulated by a pump and a water bath (not shown) regulated the thermal jacket temperature. Thermocouples (DAIHAN, South Korea) were placed at 5, 20, 45 and 60 cm heights and in the center (radius equal to zero) of bed to sense the temperatures during the fermentation. The results of thermocouples were transmitted by a data transmitter and sent to a computer for logging. The filtered and humidified air with the rate of 0.5 L h^{-1} g IDS was blown to the bottom of the bioreactor and then, dried through passing the dehumidifier column. The similar CO_2 sensor mentioned above, used in the same way to measure CO_2 production every hour. The column sterilization was performed using autoclave steam with a pressure of 1.2 bar, at 100 °C and for 20 min. The duration of the fermentation process was 120 h and the bioreactor contained 0.34 kg IDS. Fermentation process was carried out 2-times and the mean of the results with corresponding standard deviations were reported.

2.5. *Analytical and measurement methods*

2.5.1. *Sample treatment*

For sampling, the contents of each small packed-bed bioreactors were mixed well and uniformed. For 3-liter packed-bed bioreactor, samples were taken from the corresponding

heights of 5, 20, 45 and 60 cm of fermented contents. The taken samples were used to determine the products formation analysis. The moisture content of the fermented bed was determined by weight loss after drying the specimen at 80 °C after 1 day (Khanahmadi et al., 2006).

For measuring metabolic water production in small packed-bed bioreactors, the following assumptions are considered:

1 It is assumed that in small packed-bed bioreactors, due to the small size of the bioreactors and the saturation of inlet air, temperature gradient and therefor evaporation are insignificant.
2 Consumption of water by extracellular hydrolysis reactions is negligible (Mitchell and Krieger, 2006b).

In additions, the control sample bioreactor (mentioned in Section 2.4.1) was used to ensure that initial moisture content has no changes due to inlet saturated air during fermentation. Hence, metabolic water production could be determined by measuring changes in moisture content of fermented bed.

2.5.2. *Biosurfactant analytical procedure*

For rhamnolipid extraction, a few grams of fermented substrate were added to 100 mL distilled water in an erlenmeyer and shaken intensively. The prepared suspension was filtered and squeezed out manually and the permeation was centrifuged at 9000 × *g* for 20 min. The pH of the supernatant solution was adjusted to 2 using HCl (1 N). Under this condition, rhamnolipid precipitated. The precipitated rhamnolipid was centrifuged at 9000 × *g* for 20 min. The supernatant was discarded and extraction operation were done 3 times by 4.5 mL of chloroform–methanol (2:1, v/v). The organic phase were concentrated at vacuum condition at 45 °C. Finally, the remained material was extracted crude rhamnolipid and the amount of rhamnolipid was determined by measuring rhamnose using phenol-sulfuric acid method (DuBois et al., 1956). The amounts of rhamnolipid were reported based on initial dry substrate weight (IDS).

2.6. *Kinetic models*

The equations used to determine the kinetic parameters are presented in the following. In this study, logistic growth model was used to describe the growth of *P. aeruginosa* (Eq. 1).

$$\frac{dX}{dt} = \mu_m X\left(1 - \frac{X}{X_m}\right) \tag{1}$$

This model represents the effect of biomass on growth rate (Tsoularis and Wallace, 2002). Integration of Eq. (1) at initial time ($t = 0$) while $X = X_0$, gives:

$$X = \frac{X_m}{1 + \left(\left(\frac{X_m}{X_0}\right) - 1\right) e^{-\mu_m t}} \tag{2}$$

Luedeking–Piret equation was used to describe the product formation rate (PR) (Eq. 3) (Luedeking and Piret, 1959).

$$PR = \frac{dP}{dt} = Y_{P/X}\frac{dX}{dt} + m_P X \tag{3}$$

where product formation rate is dependent upon growth rate ($\frac{dX}{dt}$) and biomass (X) (Mazaheri and Shojaosadati, 2013).

CO_2 is a product rate (CPR) of the microorganism, so the CO_2 production rate (CPR) could be written in the form of Eq. (4) by substituting Eqs. (1) and (2) into Eq. (3) (Lareo et al., 2006).

$$CPR = \frac{dCCP}{dt} = \left\{\frac{\mu_m}{Y_{X/CO_2}}\left(1 - \frac{1}{\left(\left(\frac{X_m}{X_0} - 1\right)e^{-\mu_m t} + 1\right)}\right) + m_{CO_2}\right\} \times \left(\frac{X_m}{\left(\left(\frac{X_m}{X_0} - 1\right)e^{-\mu_m t} + 1\right)}\right) \tag{4}$$

Integration of Eq. (4) over time gives cumulative CO_2 production (CCP) (Eq. 5).

$$CCP = CCP_0 + X_m\left\{\frac{1}{Y_{X/CO_2}\left(\left(\frac{X_m}{X_0} - 1\right)e^{-\mu_m t} + 1\right)} - \frac{1}{Y_{X/CO_2}\left(\frac{X_m}{X_0}\right)} + \frac{m_{CO_2}}{\mu_m} Ln\left(\frac{\left(\left(\frac{X_m}{X_0} - 1\right)e^{-\mu_m t} + 1\right)}{\left(\frac{X_m}{X_0}\right)e^{-\mu_m t}}\right)\right\} \tag{5}$$

In the same way, as said for CO_2, Eqs. (6) and (7) give rhamnolipid and metabolic water production, respectively.

$$RL = RL_0 + X_m\left\{\frac{Y_{RL/X}}{\left(\left(\frac{X_m}{X_0} - 1\right)e^{-\mu_m t} + 1\right)} - \frac{Y_{RL/X}}{\left(\frac{X_m}{X_0}\right)} + \frac{m_{RL}}{\mu_m} Ln\left(\frac{\left(\left(\frac{X_m}{X_0} - 1\right)e^{-\mu_m t} + 1\right)}{\left(\frac{X_m}{X_0}\right)e^{-\mu_m t}}\right)\right\} \tag{6}$$

$$W = W_0 + X_m\left\{\frac{1}{Y_{X/W}\left(\left(\frac{X_m}{X_0} - 1\right)e^{-\mu_m t} + 1\right)} - \frac{1}{Y_{X/W}\left(\frac{X_m}{X_0}\right)} + \frac{m_W}{\mu_m} Ln\left(\frac{\left(\left(\frac{X_m}{X_0} - 1\right)e^{-\mu_m t} + 1\right)}{\left(\frac{X_m}{X_0}\right)e^{-\mu_m t}}\right)\right\} \tag{7}$$

In the present study, using Hamidi-Esfahani et al. equation (Hamidi-Esfahani et al., 2004) that were proposed for *A. niger* and modifying it, an equation for describing kinetic parameters dependence on bed temperatures and moisture contents was presented. Hamidi-Esfahani et al. model is presented by Eq. (8). Where μ is specific growth rate parameter that is function of T and Y. The equation has two parts. The first part is Ratkowsky equation (Ratkowsky et al., 1983) and the second part is a polynomial equation that describe temperature and moisture content dependence, respectively.

$$\mu = [a_1(T - T_{min})(1 - \exp(a_2(T - T_{max})))]^2 \times \left(b_0 + b_1 Y + b_2 Y^2\right) \tag{8}$$

In order to better fitting of this equation to the experimental results of the applied bacteria, in this study a modified form of the equation was presented (Eq. 9).

$$KP(T, Y) = a_1(T - T_{min})[1 - \exp(a_2(T - T_{max}))]^2 \times \left(b_0 + b_1 Y + b_2 Y^2\right) \tag{9}$$

where $KP(T, Y)$ is a kinetic parameter such as μ and X_m that is function of T and Y.

2.7. Modeling of 3-liter packed-bed bioreactor

In order to validate the kinetic parameters obtained from small packed-bed bioreactors in a larger scale, a 3-liter bioreactor was modeled.

Assumptions of modeling are as follows:

1 The system of the bioreactor is pseudo-homogeneous. It means the physical properties of the air and the bed are the same and constant at all points of the bioreactor. In addition, substrate properties do not change with temperature change or substrate consumption and biomass production (Fanaei and Vaziri, 2009; Sangsurasak and Mitchell, 1998; Saucedo-Castañeda et al., 1990).
2 Flow of air is only in axial direction and the variations in air velocity within bed are neglected (Ashley et al., 1999).
3 O_2 and glucose do not inhibit the growth of microorganism (Zolfaghari-Esmaeelabadi and Hejazi, 2018).
4 The air flow is always saturated with water vapor.
5 As the air temperature changes, the water-carrying capacity of air changes.
6 Cell death is neglected during the fermentation process.
7 Heat resistance of bioreactor wall is neglected.
8 The growth of microorganism, products formation and environmental conditions (bed temperature and moisture content) are influenced by each other.

According to the above assumptions, necessary equations for modeling are presented as follows:

2.7.1. Evaporation rate

According to the assumption of a pseudo-homogeneous bed, the Eq. (10) describes evaporation rate (Mitchell et al., 2006a).

$$R_{evap} = \rho_{air} V_z \frac{dH_{sat}}{dT} \times \frac{dT}{dz} \times V \tag{10}$$

Water-carrying capacity of air is related to temperature. Hence, with a change in temperature, the water-carrying capacity of air changes. This causes changes in evaporation rate of water from bed. Eq. (11) presents water-carrying capacity of air (Mitchell et al., 2006b).

$$\frac{dH_{sat}}{dT} = \frac{0.62413\, bp}{(T+c)^2 \left(\frac{p}{d \exp\left(a - \frac{b}{T+c}\right)} - 1 \right)^2 d. \exp\left(a - \frac{b}{T+c}\right)} \tag{11}$$

2.7.2. Water balance for bed

Eq. (12) represents a mass balance on the water in the bed (Mitchell et al., 2006a). This equation says that the rate of change in the mass of water in the bed (BW) depends on the rate of metabolic water production by the microorganisms and the evaporation rate.

$$\frac{dBW}{dt} = \frac{dW}{dt} - \left(\frac{1}{IDS}\right) R_{evap} \tag{12}$$

Eq. (13) was used in order to convert BW to Y (bed moisture content (wet basis)) (Mitchell and Krieger, 2006a).

$$Y = \frac{BW}{1 + BW} \tag{13}$$

2.7.3. Energy balance for bed

The 2-dimensional heat transfer model presented in this study covers all the phenomena of heat transfer occurring in the bed (Eq. 14) (Sangsurasak and Mitchell, 1998). This model, from left to right, consists of the terms of accumulation of heat in the bed, the production of heat from the metabolic activity of microorganism, the transfer of heat from the forced air flow through the bed in axial direction (axial convection), the heat removal by water evaporation, thermal influx in axial direction, and conductive heat transfer is in radial direction, respectively.

$$\rho_b C_{pb} \left(\frac{\partial T}{\partial t}\right) = \rho_s (1-\varepsilon) Y_Q \left(\frac{dX}{dt}\right) - \rho_a C_{pa} V_z \left(\frac{\partial T}{\partial z}\right) - \rho_a \lambda V_z \left(\frac{dH_{sat}}{dT}\right)\left(\frac{\partial T}{\partial z}\right) + K_b \left(\frac{\partial^2 T}{\partial z^2}\right) + \frac{K_b}{r}\frac{\partial T}{\partial r} + K_b \left(\frac{\partial^2 T}{\partial r^2}\right) \tag{14}$$

In Eqs. (15)–(17), values of bed density, thermal conductivity and heat capacity of bed are presented as the average of the properties of the substrate and the air (Sangsurasak and Mitchell, 1998).

$$\rho_b = \varepsilon \rho_a + (1-\varepsilon)\rho_s \tag{15}$$

$$K_b = \varepsilon K_a + (1-\varepsilon) K_s \tag{16}$$

$$C_{pb} = \frac{\left[\varepsilon \rho_a \left(C_{pa} + \lambda \left(\frac{dH_{sat}}{dT}\right) + (1-\varepsilon)\rho_s C_{ps}\right)\right]}{\rho_b} \tag{17}$$

2.7.4. Initial and boundary conditions

In order to solve the 2-dimensional heat transfer equation, initial condition in Eq. (18) and boundary conditions in Eqs. (19)–(22) are presented, respectively. In the boundary conditions equations, the effect of inlet air flux to the bioreactor on the bed temperature and the thermal jacket were applied.

$$at\ t = 0 \quad T = 27^{\circ}C \tag{18}$$

$$at\ z = 0 \quad T = T_a \tag{19}$$

$$at\ z = Z \quad \frac{\partial T}{\partial z} = 0 \tag{20}$$

$$at\ r = 0 \quad \frac{\partial T}{\partial z} = 0 \tag{21}$$

$$at\ r = R \quad -K_b \frac{\partial T}{\partial r} = h(T - T_J) \tag{22}$$

2.8. Curve fitting and numerical solution

Toolbox of MATLAB® 7 (R2011b) software were used for nonlinear curve fitting of kinetic models to experimental results of small packed-bed bioreactor.

In order to model the 3-liter bioreactor, all the kinetic Eqs. (1), (2), (5)–(7) and (9)–(22) were solved simultaneously for every element of the bed. According to this, all these equations were coupled to each other. Hence, the growth of microorganism affected the temperature and moisture content of the bed, and on the other hand, growth depended upon the environmental conditions (bed temperature and moisture content). In addition, products formation was dependent upon the growth

Table 1 – Parameters values for 3-liter bioreactor modeling.

Symbol	Parameters values	References
a, b, c, d[a]	18.30, 3816.44, 227.02, 133.322	(Perry et al., 1984)
BW	g water kg^{-1} IDS	Eq. (12)
CCP	g CO_2 kg^{-1} IDS	Eq. (5)
CPR	g CO_2 kg^{-1} IDS h^{-1}	Eq. (4)
C_{pa}	1180 J kg^{-1} °C^{-1}	(Himmelblau, 1982)
C_{pb}	J kg^{-1} °C^{-1}	Eq. (17)
C_{ps}	2500 J kg^{-1} °C^{-1}	(Kustermann et al., 1981)
$\frac{dH_{sat}}{dT}$	g water kg^{-1} air °C^{-1}	Eq. (11)
h	J m^{-2} s^{-1} °C^{-1}	Eq. (22)
IDS	0.34 kg	Measured
K_a	74.16 J h^{-1} m^{-1} °C^{-1}	(Himmelblau, 1982)
K_b	J h^{-1} m^{-1} °C^{-1}	Eq. (16)
K_s	1440 J h^{-1} m^{-1} °C^{-1}	(Kustermann et al., 1981)
p	101325 Pa	Measured
R	0.03 m	Measured
R_{evap}	g water h^{-1}	Eq. (10)
RL	g rhamnolipid kg^{-1} IDS	Eq. (6)
T	°C	Eq. (14)
T_0	27 °C	Measured
T_J	30 °C	Measured
T_a	30 °C	Measured
V_z	60 m h^{-1}	Measured
W	g water kg^{-1} IDS	Eq. (7)
W_0	2.33×10^3 g water kg^{-1} IDS	Measured
X	g biomass kg^{-1} IDS	Eq. (2)
X_0	2.1 g biomass kg^{-1} IDS	Measured
Y	g water kg^{-1} IWS	Eq. (13)
Y_0	700 g water kg^{-1} IWS	Measured
Y_Q	8.4×10^3 J g^{-1} biomass	(Sivaprakasam et al., 2008)
Z	0.6 m	Measured
ε[b]	0.38	Calculated
λ	2414.3 J g^{-1} water	(Himmelblau, 1982)
ρ_a	1.14 kg m^{-3}	(Perry et al., 1984)
ρ_b	kg m^{-3}	Eq. (15)
ρ_s	750 kg m^{-3}	Measured

[a] Dimensionless.
[b] $1 - \frac{\rho_b}{\rho_s}$ (Mitchell et al., 2006b).

of microorganism and the kinetic parameters of the products that both of them depended upon the environmental conditions.

To solve the heat transfer equation in 3-liter bioreactor, by finite difference method, the partial differential equation was converted to ordinary differential equation by using central difference approximation. To solve the set of ordinary differential equations, Runge–Kutta method was used defined by the function ODE 45 in MATLAB®. Levenberg–Marquardt algorithm was used to estimate convective heat transfer coefficient (h) (Kanzow et al., 2004). Needed parameters values are presented in Table 1. The initial values for cumulative CO_2 (CCP_0) and rhamnolipid (RL_0) were assumed zero.

3. Results and discussion

3.1. *Experimental results in small packed-bed bioreactors*

3.1.1. *Preliminary experiments*

To determine the appropriate range of temperatures and moisture contents for kinetic studies, the microorganism were first cultivated in upper and lower levels of the temperatures and moisture contents presented in Section 2.4.1. Results showed that significant growth was not observed at the initial bed moisture contents lower than 65% and upper than 75%. The growth at temperatures of 45 and 20 °C was insignificant and at upper and lower temperatures no growth was observed. According to this, kinetic studies were carried out at ranges of 25–40 °C and 65–75% for bed temperatures and initial moisture contents, respectively.

3.1.2. *Effect of temperature and moisture content on respiration and rhamnolipid production*

Fig. 2 represents the effect of both bed temperature and initial moisture content on profiles of CO_2 production rate and rhamnolipid production.

Fig. 2(a–c) represents the profiles of CO_2 production rate (*CPR*) at different temperatures and moisture contents. To prevent from illegible profiles, the error bars have not been shown in the data profiles. According to the *CPR* profiles, it is observed that after the lag phase, a peak in *CPR* represents logarithmic phase. The landing point represents the departure of the logarithmic phase and the start of the stationary phase (Rodriguez-Duran et al., 2011).

According to Fig. 2(a–c), in general, with increasing in temperature from 25 to 30 °C and higher temperatures, the lag phase is reduced. This point is noticeable with respect to the results shown at initial moisture content of 65%. The lag phase is about 17 h at 25 °C, which decreases for up to 5 h at higher temperatures. A similar result is observed for other initial moisture contents. Therefore, the fermentation temperature is an effective factor in reducing the lag phase of *P. aeruginosa* growth. At 75% initial moisture content (Fig. 2c), the *CPR* profiles at temperatures of 30 °C and 35 °C are different from the results in other moisture contents for the mentioned temperatures. This difference is at the time the microorganism leaves the logarithmic phase. At this condition of temperatures and moisture content, the growth of the microorganism enters the stationary phase at about 40 h of fermentation process. However, this happens sooner for other conditions of temperatures and moisture contents. According to Fig. 2(a–c), in general, with increasing the temperature from 25 to 30 °C and 35 °C, a significant increase in the maximum values of CO_2 production rate is observed. It can be concluded that at temperatures of 30 and 35 °C the microorganism is at the optimal temperature for the growth. It is also observed that the maximum CO_2 production rates at 25 and 40 °C do not vary at the different moisture contents and these values are about 4 g CO_2 kg^{-1} IDS h^{-1}. However, the effect of moisture content for the temperatures of 30 and 35 °C is different. At 65 and 75% moisture contents, the maximum CO_2 production rates are 6 and 5 g CO_2 kg^{-1} IDS h^{-1}, respectively, and at moisture content of 70% is equal to 9.5 g CO_2 kg^{-1} IDS h^{-1}.

Fig. 2(d–f) shows the rhamnolipid production profiles at different temperatures and initial moisture contents. In general, the rhamnolipid production profiles for a specified temper-

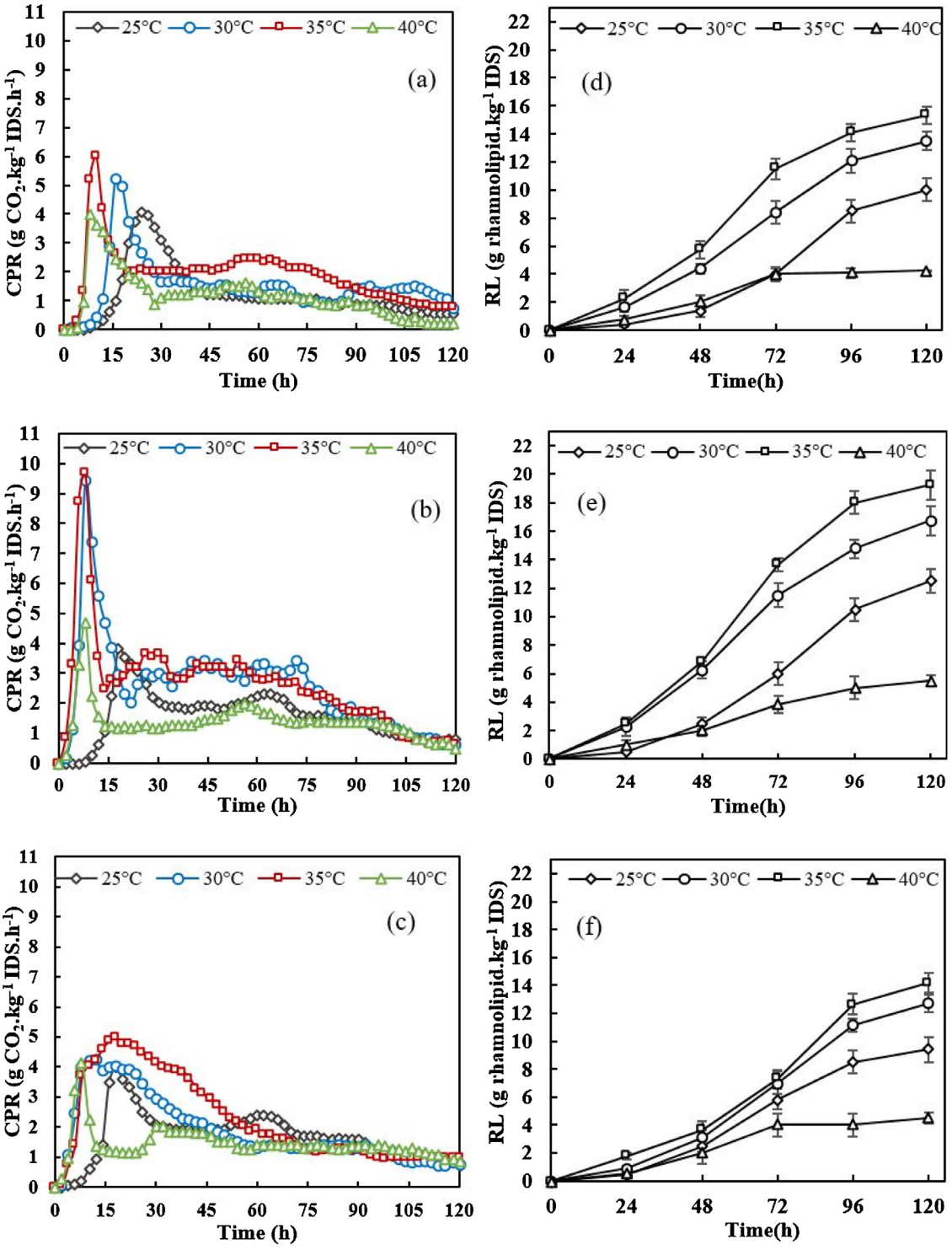

Fig. 2 – Effect of bed temperature and initial moisture content on profiles of CO_2 production rate (left side) and rhamnolipid production (right side). CO_2 production rate profiles at different temperatures for (a) 65, (b) 70 and (c) 75% of initial bed moisture contents. Rhamnolipid production profiles at different temperatures for (d) 65, (e) 70 and (f) 75% of initial bed moisture content.

ature at different moisture contents are similar in terms of trends. Rhamnolipid production is observed at the end of the first day of fermentation process. However, for all moisture contents, at the temperature of 25 °C due to the long lag phase, rhamnolipid production is observed on the second day. In addition, at this temperature, the highest change in slope of rhamnolipid production profiles has occurred at the time of 72–96 h. However, for other temperatures at different moisture contents, the highest change in slope of rhamnolipid production profiles is observed at 48–72 h. Also at 96–120 h, less variation in the amount of rhamnolipid production is observed. The study of rhamnolipid production profiles at moisture content of 75% and temperatures of 30 and 35 °C (Fig. 2f) shows that the slope of the profiles for rhamnolipid in the logarithmic phase (0–40 h) is less than the stationary phase (41–120 h). According to Fig. 2(d–f), at certain initial moisture content, by increasing the temperature from 25 to 30 and 35 °C, the increase in the amount of rhamnolipid production occurs at any time during the fermentation process. However, the production of rhamnolipid at 40 °C is greatly reduced. The

results show the temperatures of 30 and 35 °C have the highest amount of rhamnolipid at the end of fermentation at all the moisture contents. Regarding the effect of moisture content on the production of rhamnolipid, as seen previously for the kinetics of CO_2 production, at the moisture content of 70% that the microorganism benefited from better conditions for growth, more rhamnolipid was produced than other moisture contents at the end of the process (19.5 g rhamnolipid kg^{-1} IDS).

Rhamnolipid production profiles indicate that there is a relationship between the production of rhamnolipid and the growth of microorganism. However, the highest rate (profiles slope changes) of rhamnolipid production is observed in the stationary phase. Some references have reported different profiles for the production of rhamnolipid by *P. aeruginosa*, that some of them are growth-dependent and some non-growth-related products (Camilios Neto et al., 2008; Silva et al., 2010; Zhu et al., 2012).

According to the results mentioned in above this section, the *CPR* profiles could be used as a quick tool to estimate the time of entering the stationary phase that the main production of rhamnolipid by the bacteria will occur at this period of fermentation.

Neto et al. used sugar cane and corn bran with 3% glycerol for growth of *P. aeruginosa* in SSF. The maximum production was reported 28 g rhamnolipid · kg^{-1} IDS. Also they concluded the rhamnolipid production kinetic was linear and rhamnolipid production began from the beginning of the process (Camilios Neto et al., 2008).

3.2. *Determination of kinetic parameters*

3.2.1. *Growth and CO_2 kinetic parameters*

As discussed in the introduction section, one of the methods for determining the kinetic parameters of microorganism's growth in SSF is to evaluate the CO_2 production results during fermentation. In order to determine the kinetic parameters of growth and CO_2, Eq. (5) was fitted to the experimental results of cumulative producing CO_2 at different initial bed moisture contents (Fig. 3 for 75% initial bed moisture). The fitting results at different temperatures and moisture contents are presented in Table 2 and coefficient of determination (R^2) has a values greater than 0.99.

Table 2 shows at a certain moisture content, for temperatures of 30, 35 and 40 °C, the maximum specific growth rate (μ_m) is at its maximum values. However, it is considerable that at 40 °C, although μ_m is high, the maximum biomass concentration (X_m) is low. The reason may be explained by the

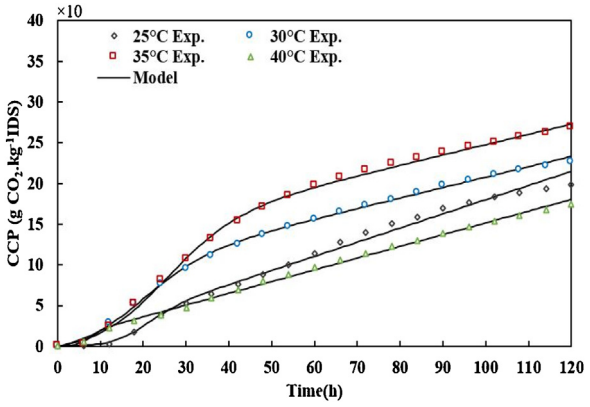

Fig. 3 – Cumulative CO_2 profiles for different temperatures at the initial bed moisture content of 75%. Symbols represent experimental data. The lines represent the Eq. (5) fitted to the cumulative CO_2 data.

fact that due to high temperature, the rate of enzymatic reactions of microorganism increases, but on the other hand, this high temperature leads to inactivation of microbial enzymes. Additionally, the initial substrate moisture content or more precise form known as water activity (a_w) is an important factor in the growth of microorganisms. High amounts of moisture contents results in reduction of oxygen diffusion within bed particles. However, a small amount of moisture can make nutrients less available to microorganisms. As shown in Table 2, at the moisture content of 70%, μ_m and X_m are higher for temperatures of 30 and 35 °C than other moisture contents. Thus, the microorganism is at optimal level of a_w. The results of the table show that μ_m, X_m and Y_{X/CO_2} have different values at different conditions, and are influenced by bed temperature and moisture content. However, m_{CO_2} at different conditions of the bed has approximately the same values.

In different references, a variety of values of μ_m for *P. aeruginosa* has been reported. In a SmF study, the parameter μ_m was reported 0.55–0.7 h^{-1} (Wei et al., 2005). In another study, the μ_m in a chemostat bioreactor and at 25 °C was expressed as 0.29 h^{-1} (Beyenal et al., 2003). Also, the results of this parameter in a batch bioreactor of SmF system, for the temperatures of 30, 35 and 42 °C are 0.31, 0.6 and 0.55 h^{-1}, respectively (Chen et al., 2007). In a recently published research in SSF, μ_m was determined using cumulative CO_2 at 35 °C based on different models of substrate-limited growth kinetics and it had the values ranging 0.25–0.49 h^{-1} (Zolfaghari-Esmaeelabadi and Hejazi, 2018). Variation in the values of the obtained parameters in the articles is attributable mainly to difference in

Table 2 – Obtained parameters from the CO_2 profiles for different temperatures and initial bed moisture contents by using Eq. (5) fitted to the cumulative CO_2 profiles.

Initial bed moisture content (%)	Bed temperature (°C)	μ_m	X_m	Y_{X/CO_2}	m_{CO_2}	R^2
65	25	0.11	81	1.84	0.006	0.993
	30	0.20	120	2.38	0.005	0.997
	35	0.26	131	2.42	0.005	0.997
	40	0.24	72	1.8	0.006	0.995
70	25	0.16	102	1.76	0.006	0.993
	30	0.31	170	2.52	0.005	0.998
	35	0.33	184	2.60	0.005	0.996
	40	0.27	63	1.93	0.005	0.994
75	25	0.13	71	1.68	0.006	0.991
	30	0.23	103	2.29	0.005	0.997
	35	0.25	119	2.31	0.006	0.997
	40	0.22	42	1.84	0.006	0.995

Table 3 – Estimated parameters from the rhamnolipid profiles for different temperatures and initial bed moisture contents.

Initial bed moisture content (%)	Bed temperature (°C)	$Y_{RL/X}$	m_{RL}	R^2
65	25	0.006	0.003	0.984
	30	0.010	0.002	0.985
	35	0.012	0.002	0.986
	40	0.002	0.002	0.982
70	25	0.009	0.002	0.982
	30	0.013	0.003	0.989
	35	0.016	0.003	0.991
	40	0.003	0.002	0.982
75	25	0.005	0.002	0.988
	30	0.007	0.002	0.984
	35	0.009	0.003	0.990
	40	0.002	0.002	0.980

method of growth parameters estimation, operating conditions and the applied substrate.

3.2.2. *Rhamnolipid kinetic parameters*

In order to determine $Y_{RL/X}$ and m_p, the equation of rhamnolipid production (Eq. 6) was fitted to rhamnolipid production profiles (Fig. 2d–f). The parameters μ_m and X_m required for fitting were used from Table 2. The results of fitting for each rhamnolipid production profile are presented in Table 3. The R^2 for these results has values of 0.98–0.99. The results show that for temperatures of 30 and 35 °C at all moisture contents, $Y_{RL/X}$ has higher values than the temperatures of 25 and 40 °C. Similar to the results concluded for growth kinetics in the previous section, the highest value of $Y_{RL/X}$ is observed at moisture content of 70% and the temperature of 35 °C. According to Table 3, $Y_{RL/X}$ has the values 0.002–0.016 g rhamnolipid g^{-1} biomass and for m_p values are about 0.003 g rhamnolipid g^{-1} biomass h^{-1}. Thus, $Y_{RL/X}$ is influenced by temperature and moisture contents.

In a research, the values of $Y_{RL/X}$ and m_p for *P. aeruginosa* in SmF were reported 0.177 and 0.013, respectively (Zhu et al., 2012). In another research in SSF, $Y_{P/X}$ for biopigment production, the values of 0.003–0.017 were reported (de Carvalho et al., 2006).

3.2.3. *Metabolic water kinetic parameters*

Metabolic water is considered as a microorganism product that its parameters could be determined in the way as mentioned for CO_2 and rhamnolipid production. Thus, in this study, the effects of temperature and initial bed moisture content on metabolic water production were investigated. Eventually, the results for different initial bed moisture contents were largely similar. The results for metabolic water production at moisture content of 70% and temperature of 25 °C are shown in Fig. 4. As seen, metabolic water production is observed in the end of first 24 h growth and it continues until the end of fermentation. In the final days of fermentation, the variation in the amount of produced metabolic water is less than first days of fermentation.

By fitting Eq. (7) to metabolic water profiles for different temperatures, kinetic parameters of metabolic water were determined. Obtained values for $Y_{X/W}$ and m_W range from 1.52 to 1.54 g biomass g^{-1} water and 0.017–0.019 g water g^{-1} biomass h^{-1}, respectively. The presented values of μ_m and X_m at different temperatures in Table 2 were used for the curve fitting. Fig. 4 shows the experimental data and model for metabolic water production and R^2 is 0.988. According to

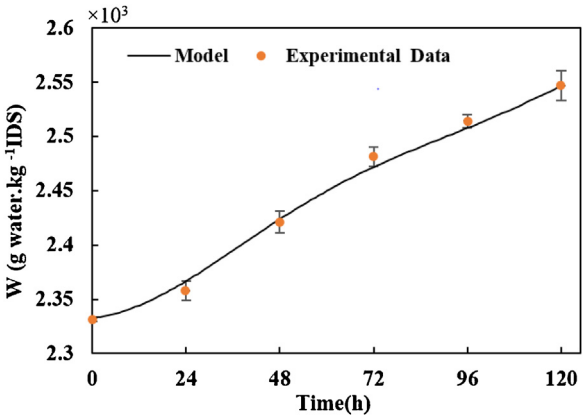

Fig. 4 – Experimental data and model for metabolic water production. Points are experimental data for T = 25 °C and Y = 70%.

the obtained results, it seems that the kinetic parameters of metabolic water production are not influenced by bed temperature and moisture content.

As comparison, the results of other microorganisms, $Y_{X/W}$ and m_W for fungus *Rhizopus oligosporus* are 3.3 g biomass g^{-1} water and 0.01 g water g^{-1} biomass h^{-1}, respectively (Sargantanis et al., 1993).

3.2.4. *Dependence of kinetic parameters on temperature and moisture content*

In order to determine the dependence of kinetic parameters μ_m, X_m, Y_{X/CO_2} and $Y_{RL/X}$ on temperature and bed moisture content, both models of Eqs. (8) and (9) were fitted to the values of these parameters that have been presented in Tables 2 and 3. As mentioned in Section 3.1.1, the temperatures 18 and 45 °C are determined as T_{min} and T_{max}. The results of 3-dimensional curve fittings are presented in Table 4. In this table, fit parameters for the equations that describe the dependence of kinetic parameters on temperature and moisture content are presented. Results showed R^2 for Hamidi-Esfahani et al. model (Eq. 8) and modified model (Eq. 9) has values 0.908–0.920 and 0.974–0.980, respectively. Hence, the modified model showed better fitting to experimental data of kinetic parameters. Fig. 5 represents the curve-fitting surface for $Y_{RL/X}$ using modified model.

According to Table 4, each kinetic parameter was described as function of environmental conditions by correspond obtained fit parameters. The presented functions could be used for modeling the growth of *P. aeruginosa* and products formation in SSF bioreactors. In most researches, these

Table 4 – Obtained fit parameters describing dependence of μ_m, X_m, Y_{X/CO_2} and $Y_{RL/X}$ on temperature and bed moisture content for two models of Eqs. (8) and (9).

Kinetic parameters	Models	Fit parameters					
		a_1	a_2	$-b_0$	b_1	$-b_2$	R^2
μ_m	Eq. (8)	2.981	0.201	0.431	2.010	0.528	0.919
	Eq. (9)	2.189	0.284	0.418	1.220	0.867	0.974
X_m	Eq. (8)	1.002	0.138	0.355	1.652	0.532	0.908
	Eq. (9)	1.130	0.125	0.404	1.217	0.882	0.978
Y_{X/CO_2}	Eq. (8)	1.522	0.009	1.681	0.005	0.0001	0.920
	Eq. (9)	2.082	0.016	1.385	0.043	0.0003	0.980
$Y_{RL/X}$	Eq. (8)	0.296	0.120	0.419	0.008	0.0001	0.914
	Eq. (9)	0.221	0.095	0.487	0.014	0.0001	0.974

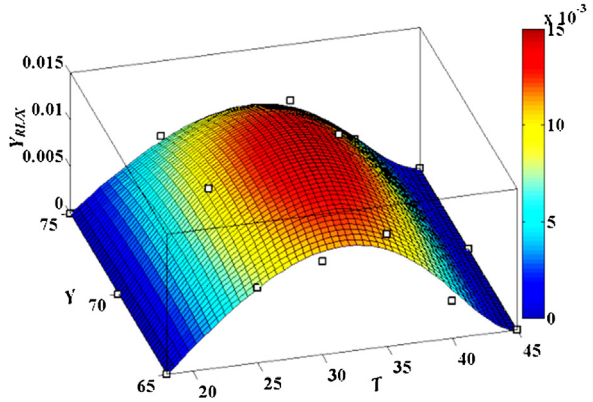

Fig. 5 – 3-Dimensional curve fitting for $Y_{RL/X}$ using modified model. The surface is model prediction and points are values of $Y_{RL/X}$ at different temperatures and bed moisture contents.

parameters are considered constant and determined at optimal condition. However, as we showed, the kinetic parameters are dependent upon environmental conditions that mostly vary during a SSF process.

3.3. *Validation of 3-liter packed-bed bioreactor modeling results*

In Fig. 6, the results of the modeling and the experimental data obtained from the 3-liter bioreactor are presented. In order to model the 3-liter bioreactor, the obtained parameters of previous sections were used. Hence, The values for m_{CO_2}, m_{RL}, m_w and $Y_{X/W}$ were considered 0.005, 0.002, 0.018 and 1.53, respectively. Also, the presented fit parameters (a_1, a_2, b_0, b_1, b_2) in Table 4 were used in Eq. (9) to describe effect of environmental conditions on X_m, μ_m, $Y_{RL/X}$ and Y_{X/CO_2}.

In Fig. 6(a), the model predicts bed temperature variations for different heights within 48 hours of fermentation, which is the peak of microorganism activity. In this figure, it is seen that by beginning the logarithmic phase, the temperature of the bed increases rapidly and at 15 h of fermentation, reaches 43 °C for the height of 60 cm. With the exit of microorganisms from the logarithmic phase and the heat removal from the bed, the different points of the bed are in thermal balance with the inlet air and R^2 for the predicted temperature by the model has values of 0.950–0.986. Since the bed temperature and microorganism growth are two variables that are

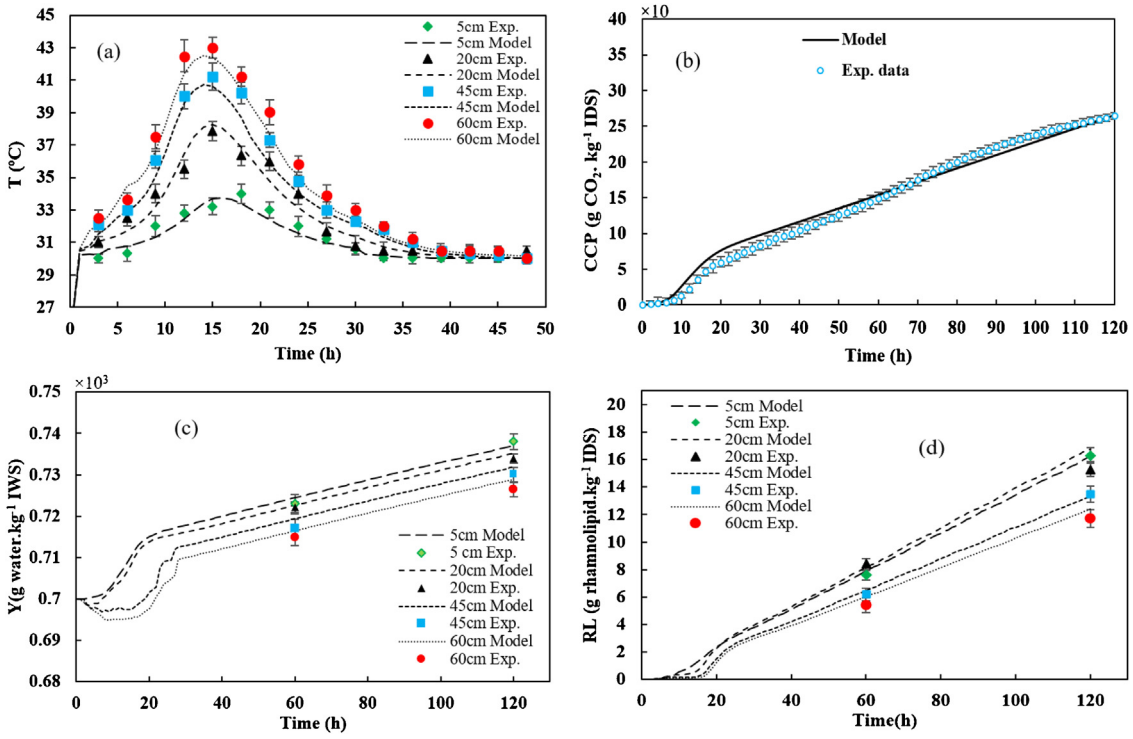

Fig. 6 – Modeling and experimental results in 3-liter bioreactor for different heights of the bioreactor: (a) bed temperature (T); (b) cumulative CO_2 production (CCP); (c) bed moisture content (Y); (d) rhamnolipid production (RL).

highly dependent upon each other, the matching of the results of the modeled bed temperature on its experimental results indicates the validity of the models and growth parameters presented in the small packed-bed bioreactor.

Fig. 6(b) represents the experimental data and model results for cumulative CO_2. Model results are presented as the average of predicted values of cumulative CO_2 for different points of 3-liter packed-bed bioreactor. The model could well predict the maximum and the trend of cumulative CO_2 during the fermentation. The value of R^2 for this prediction is 0.982.

Fig. 6(c) and (d) shows the experimental results and models predictions for bed moisture content and rhamnolipid production for various heights of the 3-liter packed-bed bioreactor. It is seen that the models predictions are satisfactory for prediction of values of bed moisture content and rhamnolipid production at 60 and 120 h of fermentation. In Fig. 6(c), the model predicts a decrease in bed moisture content for 45 and 60 cm due to the increase in temperature caused by the logarithmic phase and the evaporation of the bed moisture content. By the exit of the microorganism from the logarithmic phase and lowering the bed temperature, the evaporation and metabolic water production lead to a continuous increase for bed moisture to the end of the process. Fig. 6(d) depicts a delay in the production of rhamnolipid at the beginning of fermentation, especially for the higher points of the bed (heights of 45 and 60 cm). This is due to the negative effect of the temperature peak in the bed (in the logarithmic phase) on the kinetic parameters of growth (μ_m, X_m) and the yield coefficient for rhamnolipid production ($Y_{RL/X}$). By exit of the microorganism from logarithmic phase, the production of rhamnolipid begins at the higher points of the bed (45 cm and higher heights). However, it is seen that the initial delay in the production of rhamnolipid results a difference in its final values for the high points and lower points of the bioreactor.

According to the above, the presented models described well the effect of environmental conditions on the growth kinetic of the bacteria and the effect of growth on environmental conditions vice versa.

4. Conclusions

The profiles of *P. aeruginosa* respiration and rhamnolipid production indicated that initial moisture content 70% and $T = 35\,^{\circ}C$ were the optimal conditions for growth and rhamnolipid production. The results showed that temperature and moisture content are two significant variables. These environmental conditions could be effective for the growth and products formation of the bacteria, probably by affecting enzymatic reactions and facilitating nutrients and oxygen availability for the microorganism.

The kinetic parameters of the bacteria were investigated and determined at different environmental conditions in SSF. Some parameters were presented as functions of the bed temperature and moisture content by using the modified model. The presented modified model had a good fitting to experimental data in comparison to proposed model by Hamidi-Esfahani et al. (Hamidi-Esfahani et al., 2004) for *A. niger*. The obtained kinetic parameters and functions could be used for modeling growth and products formation of *P. aeruginosa* in SSF bioreactors.

The kinetic parameters and functions were evaluated in a 2-dimensional modeling of a 3-liter packed bed bioreactor. The results of the experimental data and models yielded a good agreement. In addition, results showed that coupling transport phenomena to kinetic models that were functions of environmental conditions led to accurate predictions of SSF bioreactors.

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