Pollution, 5(4): 709-719, Autumn 2019
DOI: 10.22059/poll.2019.276270.585
Print ISSN: 2383-451X Online ISSN: 2383-4501
Web Page: https://jpoll.ut.ac.ir, Email: jpoll@ut.ac.ir

Bioenergetic Aspects of Dibenzothiophene Desulfurization by Growing Cells of *Ralstonia eutropha*

Dejaloud, A.[1], Habibi, A.[2*], Vahabzadeh, F.[1] and Akbari, E.[1]

1. Department of Chemical Engineering, Amirkabir University of Technology (Tehran Polytechnic), Tehran, Iran
2. Department of Chemical Engineering, Faculty of Engineering, Razi University, Kermanshah, Iran

Received: 17.02.2019 Accepted: 23.05.2019

ABSTRACT: The present study focuses on effects of initial pH on dibenzothiophene (DBT) desulfurization via 4S pathway by growing cells of *Ralstonia eutropha*. For so doing, temporal changes of biomass concentration, glucose as a sole carbon source, pH value, and 2-hydroxybiphenyl (2-HBP) formation have been monitored during the bioprocess. The biomass concentration has been modeled by the logistic equation and results show that the values of maximum specific growth rate (μ_{max}) and maximum cell concentration (X_{max}) have increased in line with the rise of initial pH from 6 to 9. This confirms the effect of pH on the energetics of cell growth via altering the proton gradient and manipulating ATP-related metabolic pathways. By considering the Pirt's maintenance concept, the bioenergetic aspects of DBT desulfurization process are affected by changes in pH, where the maximum specific DBT conversion rate (0.0014 $mmol/g_{cell}.h$) has been obtained at initial pH of 8. Additionally, the kinetic modeling of the 2-HBP formation through the Luedeking-Piret model indicates that the DBT desulfurization rate is linearly related to the cell growth rate, instead of biomass concentration. The growth associated and non-growth associated 2-HBP formation constants have been obtained 3.82 $mg_{2\text{-}HBP}/g_{cell}$ and 0.06 $mg_{2\text{-}HBP}/g_{cell}.h$, respectively at an initial pH of 8.

Keywords: pH effect, logistic equation, Luedeking-Piret model, Pirt concept.

INTRODUCTION

Emission of sulfur oxides from fossil fuel combustion has brought about serious environmental consequences, resulting in human health problems, during the last decades (Mohebali & Ball, 2016). Since 2010 strict regulations have been imposed to cut down the sulfur content in diesel to 10 mg/L (Kilbane & Stark, 2016; Debabov, 2010). The hydrodesulfurization (HDS) is one of the common processes for sulfur removal from petrochemical compounds; however, its poor performance in removing refractory compounds limits its practical application in ultra-deep desulfurization (Carvajal et al., 2017; Martinez et al., 2017). Within the last years, biodesulfurization (BDS), an environmentally friendly technology, has demonstrated high potentials to solve the problem under mild operational conditions (Kilbane & Stark, 2016).

Dibenzothiophene (DBT), which is a model sulfur compound, could be biologically removed in a sulfur-specific

* Corresponding Author, Email: a.habibi@razi.ac.ir

route, called 4S pathway, where the carbon skeleton of DBT is released intact as 2-hydroxybiphenyl (2-HBP). Through this pathway DBT is metabolized to 2-HBP and sulfite as the final products (Soleimani et al., 2007; Martinez et al., 2015). Thanks to its need for cofactors (NADH and $FMNH_2$), this pathway has been identified as an energy-intensive process to support the three-step oxidation of DBT to 2-HBP (Martinez et al., 2015; Boniek et al., 2015). Intracellular NADH, produced from glycolysis, fatty acid oxidation, and the citric acid cycle, can be converted to NAD^+ under aerobic growth (i.e., electron transfer chain) as well as anaerobic growth (i.e., fermentative pathways), and is also oxidized directly by NADH oxidase in other metabolic pathways (Zhou et al., 2009). Hence, oxidation and regeneration of NADH plays an important role in manipulation of the intracellular ATP content. The DBT uptake into cells is driven by ATP hydrolysis (Wang et al., 2011; Martinez et al., 2015), enabling BDS process to be intensely affected by ATP availability. Previous work by *Ralstonia eutropha* for DBT desulfurization showed that the BDS process was efficiently performed in energy-sufficient culture media, where the carbon source (glucose) was in excess (Dejaloud et al., 2017). Although some studies have dealt on kinetics of DBT desulfurization (Caro et al., 2008; Boltes et al., 2013), there are scarce details for bioenergetic aspects of the BDS process, with regard to the changes in operational conditions and/or growth media specification (Martin et al., 2005; del Olmo et al., 2005).

The present study studies the effect of pH on BDS ability of *R. eutropha* under energy-sufficient culture medium. As a product formation model associated with the logistic growth equation, Luedeking-Piret Equation has been applied in order to predict the desulfurizing capability of the growing cells. In addition, the cell growth yield and maintenance coefficient in the energy-sufficient culture have also been evaluated by Pirt's maintenance concept at different pH values.

MATERIALS AND METHODS

The microorganism, used in this study, was *R. eutropha* (PTCC 1615), purchased from the Persian Type Culture Collection (Iranian Research Organization for Science and Technology "IROST"). The growth medium and cultivation conditions were selected on the basis of previous works (Dejaloud et al., 2017). In each DBT desulfurization experiment, 90 mL of a sulfur-free mineral solution (SFMS) contained 7.53 mM of KH_2PO_4, 7.54 mM of K_2HPO_4, 14.96 mM of NH_4Cl, and 0.20 mM of $MgCl_2.6H_2O$, and got transferred to a 250-mL conical flask. Glucose and DBT were added at concentrations of 111 and 0.05 mM to the SFMS solution as the carbon and sulfur source, respectively. DBT was initially dissolved in dimethylformamide (54.27 mM), then to be added to the medium. Prior to sterilization, initial pH of the SFMF medium was set on a desirable level (pH= 6, 7, 8, and 9), using 2 N solutions of NaOH and HCl. Inoculation was carried out as described below (Dejaloud et al., 2017):

As much as 10 mL of the grown cultures on GYP medium got centrifuged at 8000×g for 15 min (Heraeus Biofuge Stratos, Germany), and washed twice with SFMS solution, then to be transferred to the DBT desulfurization medium. The initial biomass concentration was 0.24 ± 0.03 g/L. The culture was incubated in a rotary shaker at 150 rpm and 30 °C for 24 h. All experiments were performed in duplicate and the error bars, as shown in the figures, represent the standard deviations of the measured values.

The cell growth was spectrophotometrically determined at 600 nm (JASCO V-550, Japan). The optical density (OD_{600}) values were converted to the

dry cell weight concentration (X), according to the following calibration equation:

$$X\ (\text{g/L}) = 0.4967 \times \text{OD}_{600} \quad (1)$$

The glucose concentration was measured with a glucose assay kit (ZiestChem Diagnostics, Iran), with the glucose uptake rate ($-\frac{dS}{dt}$) estimated from the slope of the glucose consumption versus time plot, and the following equation was used to determine the specific glucose uptake rate (Q_{Glucose}) (Millard et al., 2017):

$$Q_{Glucose} = -\frac{1}{X}\frac{dS}{dt} \quad (2)$$

Where S and t stand for glucose concentration and the time of the DBT biodesulfurization process, respectively.

The 2-HBP, produced in the BDS process, was extracted with the same volume of ethyl acetate after getting acidified to a pH rate of 2 with 1 N HCl. A portion of the ethyl acetate layer (10 µL) was used for GC analysis (Agilent 7890B), using an Agilent J&W DB-5 capillary column (30 m×0.32 mm×0.25 µm, 19091J-413) along with a Flame Ionization Detector (FID). The N_2 carrier gas flow rate was maintained at 5 mL/min and the column temperature was programmed to ascend from 120° C to 245° C at a rate of 30° C/min. The injector and detector temperatures were set at 250° and 300° C, respectively. The specific DBT conversion rate (Q_{DBT}) was determined via the following expression:

$$Q_{DBT}\ (mmol_{DBT}\, g_{cell}^{-1} h^{-1}) = \frac{C_{2-HBP}}{X \times t} \quad (3)$$

Where $C_{2\text{-HBP,t}}$ is the 2-HBP concentration.

In order to estimate the kinetic constants of the DBT desulfurization process, the Levenberg-Marquardt algorithm was applied as a nonlinear regression technique (SigmaPlot version 12.3; Systat Software Inc.) to fit the experimental data. The kinetic parameters were estimated by finding the minimum sum of squares of differences between the measured values (Y_{exp}) and the values, predicted by the model ($\hat{Y}$), with the standard estimation error ($S_{\text{y.x}}$), taken to show the model adequacy:

$$S_{y.x} = \sqrt{\frac{\sum \left(Y_{\text{exp}} - \hat{Y}\right)^2}{df}} \quad (4)$$

Where, df represents the degree of freedom (the number of data minus the number of fitting parameters). The t-test was also used to statistically assess the validity of the fitted models, and the significance level was set to a P-value below 0.05 (95% confidence interval) (Lapin, 1997).

RESULTS AND DISCUSSION

Fig. 1 shows the experimental results, obtained from biological DBT desulfurization process by *R. eutropha* at different initial pH values. In all initial pH values, the growth of *R. eutropha* cells was immediately initiated at the beginning of the process without a noticeable lag time, then to arrive at a stationary phase after 12 h. Fig. 1 also shows that glucose was not completely depleted from the medium at the beginning of stationary phase, which confirms that the bacterial growth cessation in the energy-sufficient medium mainly occurred due to consumption of other essential growth factors such as amino acids or vitamins. The reduction of pH was detected during the cell growth in all experiments and was maintained almost constant afterwards, after entering the stationary phase. In fact, the excess protons, generated from substrate oxidation in the cells, were secreted into the medium so as to regulate the internal pH and maintain energy homeostasis in the cell. These excess protons along with acidic-byproducts helped acidifying the culture medium. Similar observations were also reported in *E. coli* growth on excess glucose-containing medium under aerobic condition due to the accumulation of acetate as an acidic by-

product (Srinivasan & Mahadevan, 2010). Fig. 1 presents the 2-HBP concentration as the final metabolite of DBT desulfurization in the 4S pathway. According to the data, the concentration of 2-HBP increased only at the growth phase, confirming that *R. eutropha* was unable to desulfurize DBT during the stationary phase.

DBT biodesulfurization as an energy-consuming process has an influence on the cellular energy balance. Fig. 2 shows that 4 moles of NADH are required per one mole of DBT, desulfurized by bacterial cells, while 3 moles of ATP are produced per one mole of NADH, oxidized in the aerobic respiration of glucose (Zubay, 1998; Kilbane & Stark, 2016). The glucose metabolism can be regulated through the activity of the key glycolytic enzymes such as phosphofructokinase (PFK), glyceraldehyde 3-phosphate dehydrogenase (GAPDH), phosphoglycerate kinase (PGK), and pyruvate kinase (PK) (Tsai & Lee, 1990). The phosphorylation reaction of fructose 6-phosphate to fructose 1,6-diphosphate, catalyzed by PFK, is identified as a key regulatory step in glycolysis. The PFK in the most bacteria is allosterically activated by ADP and inhibited by phosphoenolpyruvate, showing no response to ATP and citrate (Tsai & Lee, 1990). Glucose transport and energy production by bacterial cells under aerobic condition are considered in terms of the active transport system, which uses cellular energy in the form of ATP to move the substrate across the cell membrane against the concentration gradient. The membrane-bound ATPase is responsible for providing the required energy of the active transport system where the proton-motive force is created by the aerobic electron transportation (Doelle et al., 1981).

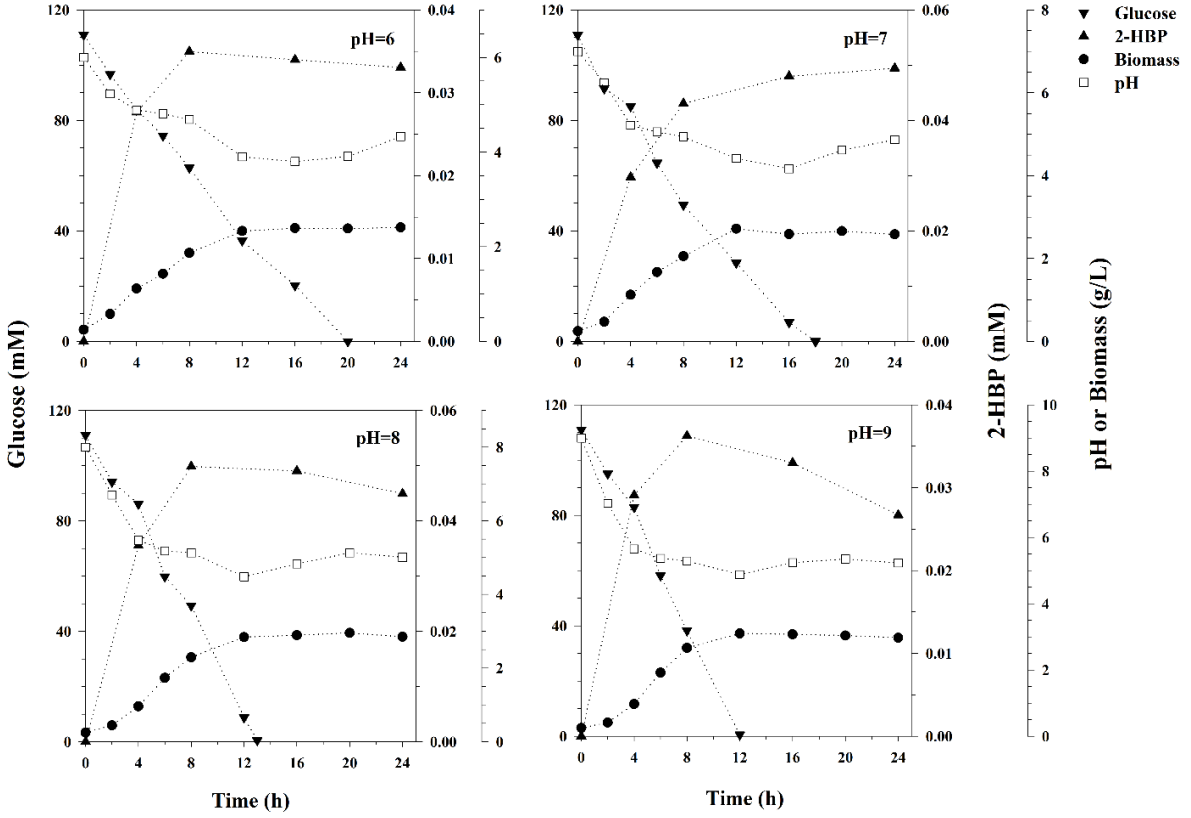

Fig. 1. Time course of glucose concentration, pH of the medium, biomass, and 2-HBP formation, during the DBT biodesulfurization process by *R. eutropha* at different initial pH values

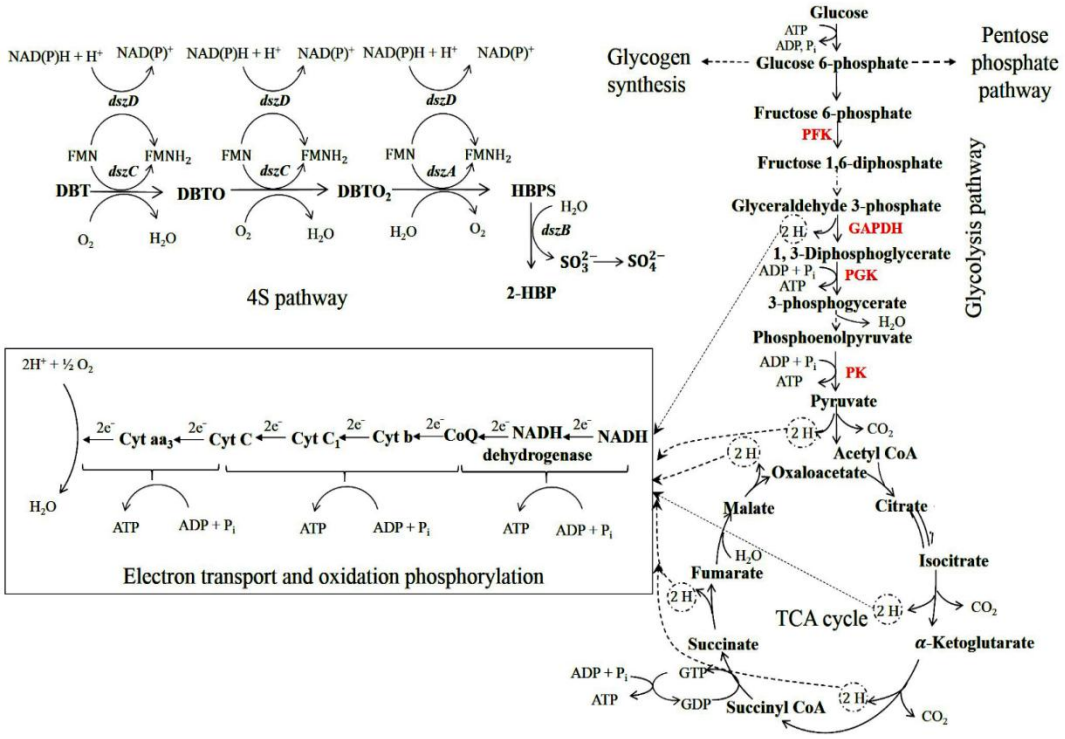

Fig. 2. Schematic representation of metabolic pathways, involved in DBT desulfurization process. The enzymes indicated in red are the main control points in allosteric regulation of glucose metabolism in the glycolytic pathway (Zubay, 1998; Kilbane & Stark, 2016). DBTO, DBT sulfoxide; $DBTO_2$, DBT sulfone; HPBS, hydroxyphenylbenzene sulfonate; *dszC*, dibenzothiophene monooxygenase; *dszA*, dibenzothiophene sulfone monooxygenase; *dszB*, HPBS desulfinase; *dszD*, and NADH/FMN oxidoreductase.

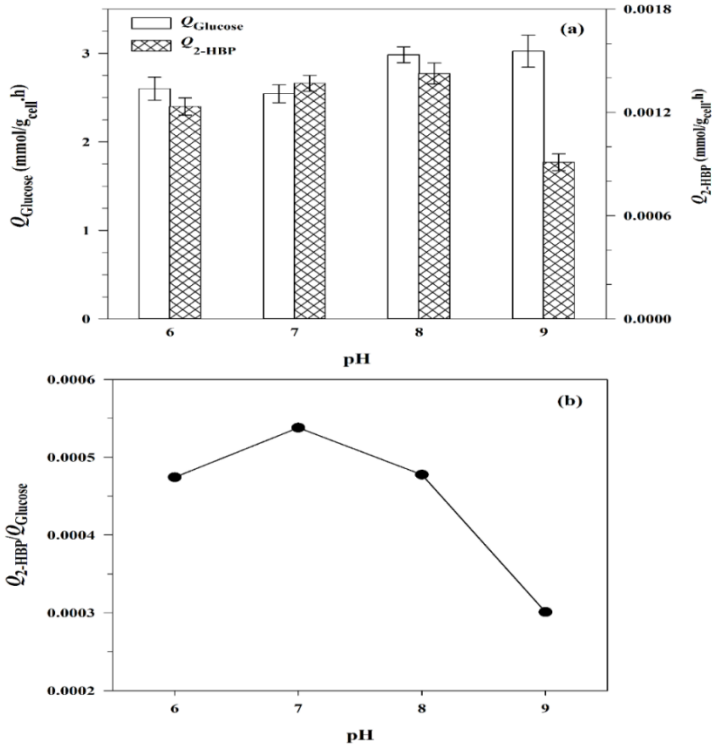

Fig. 3. Dependency of the specific glucose uptake rate ($Q_{Glucose}$) and the specific DBT conversion rate (Q_{DBT}) on initial pH (experimental data of growth phase was used)

Fig. 3a represents the results of the specific glucose uptake ($Q_{Glucose}$) and specific DBT conversion rate (Q_{DBT}) at different initial pH values. As expected, in the presence of glucose as an electron donor, the medium with higher initial pH efficiently causes the protons to sink, which in turn enhances the rate of glycolysis. Here, the Q_{DBT} was increased by increasing pH from 0.0012 mmol/g_{cell}.h at pH 6 to 0.0014 mmol/g_{cell}.h at pH 8 whereas further increase in pH resulted to a decrement in the Q_{DBT} (0.0009 mmol/g_{cell}.h at pH 9). The ratio of Q_{DBT} to $Q_{Glucose}$ was also compared in the experiments to clarify the role of initial pH in DBT desulfurization process (Fig. 3b), with maximum value of $Q_{DBT}/Q_{Glucose}$ ratio, obtained at initial pH value of 7.0.

The logistic equation with the following expression includes the carrying capacity of the environment to present a growth model where resources availability is limited for the cell and the environment could not support any further population growth:

$$\frac{dX}{dt} = \mu_{max} X(1 - \frac{X}{X_{max}}) \quad (5)$$

Where μ_{max} and X_{max} are the maximum specific growth rate (1/h) and maximum cell concentration (g/L), respectively.

Considering X_0 as the initial biomass concentration, the integrated Eq. (5) provides a relation to predict temporal change in biomass concentration during DBT desulfurization process.

$$X = \frac{X_0 e^{\mu_{max} t}}{1 - \frac{X_0}{X_{max}}(1 - e^{\mu_{max} t})} \quad (6)$$

In the present study, Eq. (6) did predict biomass concentration, the results of which can be seen in Fig. 4a. Furthermore, Table 1 lists the relevant constants of the logistic model for each initial pH. The independency of the constants was evaluated through calculation of t-value (defined as the ratio of the coefficient to the relevant standard error). Small values of *Sy.x* in Table 1 confirm the adequacy of the developed model. According to the results, increasing initial pH from 6 to 9, led to a rise in both specific growth rate (μ_{max}) and maximum carrying capacity (X_{max}). It also increased the specific glucose uptake rate in the experiments. The positive linear relation between the specific substrate uptake rate and specific growth rate has been postulated by the Pirt's maintenance model (Pirt, 1965; 1982; Tsai & Lee, 1990). The values of μ_{max} and X_{max} rose as a result of pH increase in Table 1. In fact, the regulation of internal pH in the cells through exporting protons affects the biomass yield in the microorganisms (Repaske & Adler, 1981).

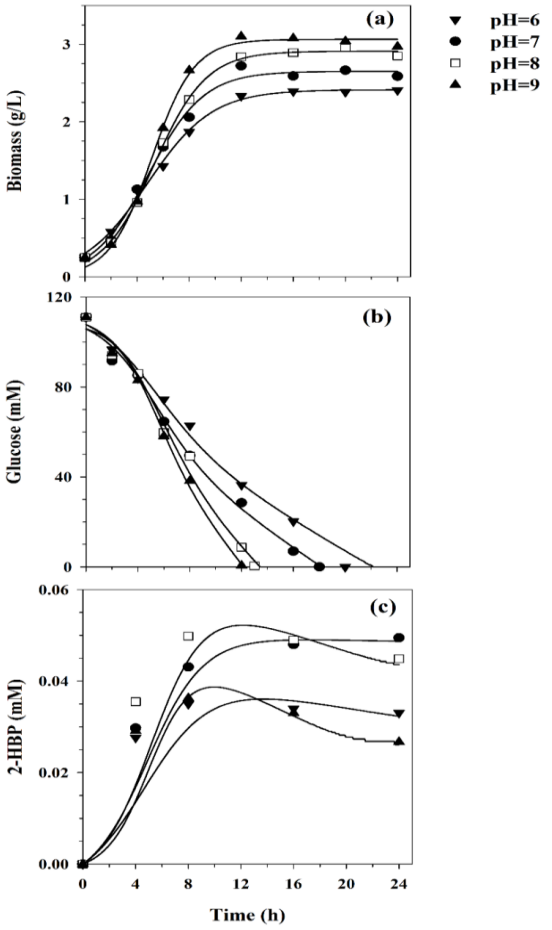

Fig. 4. Comparison of dynamic modeling of DBT desulfurization process with the experimental data in terms of (a) biomass, (b) glucose, (c) and 2-HBP formation

Table 1. Estimated values of maximum specific growth rate (μ_{max}) and maximum cell concentration (X_{max}), obtained by means of fitting the experimental data to the logistic equation (Eq. 6)

Initial pH	μ_{max} (h^{-1})		X_{max} (g/L)		$S_{y.x}$
	Value	*t*-test	Value	*t*-test	
6.0	0.40 ± 0.06	15.50	2.41 ± 0.08	71.77	0.06
7.0	0.47 ± 0.10	10.96	2.65 ± 0.12	53.01	0.09
8.0	0.50 ± 0.05	23.88	2.91 ± 0.06	117.30	0.05
9.0	0.62 ± 0.10	14.45	3.06 ± 0.10	76.48	0.08

Once the energy of growth (energy for formation of new cells) and maintenance energy (the energy required for cellular functions) are distinguished, glucose consumption rate could be determined via the Pirt Model (Neijssel & Tempest, 1976; Pirt, 1982; Razvi et al., 2008):

$$(\frac{dS}{dt})_T = -\frac{1}{Y_G}\frac{dX}{dt} - mX \quad (7)$$

Where, Y_G is the growth yield ($g_{cell}/g_{glucose}$) and "*m*" is the maintenance coefficient ($g_{glucose}/g_{cell}.h$). Dividing Eq. (7) with Eq. (5) and integrating the obtained equation between X_0 and X, provides the following expression to describe the substrate concentration as a function of biomass concentration during the process:

$$S = S_0 - \frac{1}{Y_G}(X - X_0) + \frac{mX_{max}}{\mu_{max}}\ln(\frac{X_{max} - X}{X_{max} - X_0}) \quad (8)$$

Fig. 4b demonstrates the results of simulation by means of Eq. (8). Also, Table 2 lists the relevant values of Y_G and "*m"* at different initial pH values. The *Syx* and *t*-values, presented in Table 2, indicate the prediction capacity of Eq. (8) when describing the bioenergetics aspects of DBT desulfurization process. Results of Table 2 show that the increase of initial pH led to an increase of "*m*", while a noticeable effect of pH on Y_G was not observed (0.24 to 0.28 $g_{cell}/g_{glucose}$). Respiration-driven transport was coupled with the NADH-linked or flavin-linked substrate oxidation, in which 3 or 2 moles of ATP got produced per one mole of oxidized $NADH/FADH_2$. Additionally, hydrogen ions are needed to drive ATP synthesis via ATPase (Fig. 2) (Doelle et al., 1981). Considering the DBT desulfurization as an energy-intensive process, the excess protons produced in cellular respiration system should be directed towards the BDS process. The proton-motive force reversed the ATPase catalysis from ATP hydrolysis to ATP synthesis and was expectedly coupled with a net proton uptake. Thus, higher values of "*m*" for high initial pH indicate the cells' effort to regulate the internal pH by importing protons via formation of 2-HBP as a predominant product in this process. However, the excess energy in the culture media with ammonium sulfate is generally dispersed in energy spilling mechanisms such as decreasing the efficiency of ATP generation, membrane potential dissipation, ATP hydrolysis, and futile cycles, characterized as non-growth functions (van Bodegom, 2007; Dejaloud et al., 2017). In bioenergetic studies of energy-sufficient culture media without major product formation, the parameters of Y_G and "*m*" in Eq. (8) are considered as apparent values of maximum growth yield and maintenance coefficient, no longer having ant physical meaning proposed by Pirt (1965). The apparent values of Y_G and "*m*" assess the effects of glucose uptake regulation and energy spilling activity (Tsai & Lee, 1990).

Table 2. Values of maintenance coefficient (m) and theoretical growth yield (Y_G), obtained by fitting the experimental data to Eq. (8)

Initial pH	m ($g_{glucose}/g_{cell}.h$)		Y_G ($g_{cell}/g_{glucose}$)		$S_{y.x}$
	Value	*t*-test	Value	*t*-test	
6	0.26 ± 0.08	7.72	0.24 ± 0.09	6.36	0.86
7	0.27 ± 0.17	4.37	0.25 ± 0.12	5.82	0.81
8	0.36 ± 0.32	3.57	0.24 ± 0.13	5.92	0.65
9	0.41 ± 0.31	4.25	0.28 ± 0.16	5.42	0.80

Fig. 4c shows the concentration of 2-HBP produced, during DBT desulfurization by *R. eutropha*, whose capability in DBT desulfurization at pH = 7 and 8 was higher than other pH values. These results are in agreement with the ones, presented by Kim et al., (2004), who reported the optimal pH ranging between 7 and 8 for DBT desulfurization by growing cells of *Gordonia* sp. CYKS1. It can be also observed in Fig. 4c that 2-HBP concentration reached its maximum values after 8 h of cultivation, corresponding to the cessation of exponential growth phase.

The kinetics of 2-HBP production can be described by Luedeking-Piret model, taking into account both biomass concentration (X) and growth rate ($\frac{dX}{dt}$) (Luedeking & Piret, 1959):

$$\frac{dP}{dt} = \alpha \frac{dX}{dt} + \beta X \qquad (9)$$

Where, α ($mg_{2\text{-HBP}}/g_{cell}$) and β ($mg_{2\text{-HBP}}/g_{cell}.h$) are growth- and non-growth-associated product formation parameters, respectively.

Dividing Eq. (9) by Eq. (5) yields:

$$\frac{dP}{dX} = \alpha + \frac{\beta X_{max}}{\mu_{maz}(X_{max} - X)} \qquad (10)$$

While integration of Eq. (10) between X_0 and X, while considering the initial product concentration as P_0, yields the following expression:

$$P = P_0 + \alpha(X - X_0) + \frac{\beta X_{max}}{\mu_{max}} \ln\left(\frac{X_{max} - X}{X_{max} - X_0}\right) \qquad (11)$$

Fig. 4c compares between the experimental data and predicted 2-HBP concentration by Eq. (11). Also, Table 3 summarizes the values of α and β for different initial pH values. The value of α was increased from 3.37 $mg_{2\text{-HBP}}/g_{cell}$ at pH = 6 to 3.82 $mg_{2\text{-HBP}}/g_{cell}$ at pH = 8, while no noticeable change in the values of β was observed. Kinetic studies showed that α values were significantly higher than the β ones for all experiments, which confirmed the fact that 2-HBP is a growth-associated product in the desulfurization process. Table 4 compares the values of α and β, obtained in the present work, with the ones, determined in previous works. The results confirmed that the growing cells of *R. eutropha* had a relatively high BDS capability, as compared with the performance of other reported bacteria. For instance, Del Olmo et al., (2005) studied the effects of operational conditions, such as temperature and pH on desulfurization efficiency of *R. erythropolis* IGTS8, and found an optimum desulfurizing capability at initial pH of 6.7. The maximum values of α and β in Table 4 have been reported by Del Olmo et al., (2005) when pH was not altered freely and controlled at 6.7 through addition of NaOH and Tris-HCl solutions.

Table 3. Values of (α) growth-associated and (β) non-growth associated product formation, obtained by fitting the experimental data to Eq. (11)

Initial pH	α ($mg_{2\text{-}HBP}/g_{cell}$)		β ($mg_{2\text{-}HBP}/g_{cell}.h$)		$S_{y.x}$
	Value	*t*-test	Value	*t*-test	
6	3.37 ± 0.45	23.61	0.04 ± 0.03	3.89	0.20
7	3.53 ± 0.58	19.36	0	-	0.33
8	3.82 ± 0.57	21.79	0.06 ± 0.04	4.57	0.33
9	2.80 ± 0.27	33.03	0.08 ± 0.02	11.33	0.17

Table 4. Comparison of the present work with the results, previously reported on DBT desulfurization

Microorganism	Carbon source	Initial pH	α ($mg_{2\text{-}HBP}/g_{cell}$)	B ($mg_{2\text{-}HBP}/g_{cell}.h$)	Operational mode	Ref.
Microbacterium sp. strain ZD-M2	54.29 mM of glycerol	7	0.01	0	Growing cells	(Chen et al., 2008)
Rhodococcus erythropolis IGTS8	111 mM of glucose	6.7 (control)*	5.43	0.12	Resting cells	(del Olmo et al., 2005)
Pseudomonas putida CECT5279	136 mM of glutamic acid	7 (control)*	0.80	0.02	Resting cells	(Martin et al., 2005)
		7	1.43	0.08		
	111 mM of glucose	7 (control)*	0.21	0.02		
		7	0.17	0.17		
Ralstonia eutropha	111 mM of glucose	7	3.53	0	Growing cells	This work
		8	3.82	0.06		

* The pH was maintained to the initial value during the process

CONCLUSIONS

The present study focused on the impact of initial pH in biological DBT desulfurization by growing *R. eutropha* cells. Results showed that the pH played a significant role in controlling the yield as well as specific rate of DBT desulfurization. The best desulfurization efficiency, i.e., up to 98%, was observed at initial pH of 7 for an initial DBT concentration of 0.05 mM after 24 hours of cultivation. A combined model of the logistic, Pirt's maintenance, and Luedeking-Piret equations was employed to mathematically describe the temporal changes of cell growth, glucose utilization, and 2-HBP production during the DBT desulfurization. The specific glucose uptake rate increased at an alkaline pH along with enhancement of activities of several key glycolytic enzymes, particularly PFK. The effects of pH on both maintenance coefficient and growth yield showed the different strategies, applied by growing cells to manipulate the NADH availability due to intracellular ATP content adjustment along with obtaining the highest cell concentration, the highest growth yield, and the increased BDS efficiency, as a ATP-based bioprocess. Thus, manipulation of ATP supply and demand could enhance the ability of microorganism to resist environmental stress due to the high ATP demand of cells in DBT desulfurization process.

REFERENCES

Boltes, K., del Aguila, R. A. and García-Calvo, E. (2013). Effect of mass transfer on biodesulfurization kinetics of alkylated forms of dibenzothiophene by *Pseudomonas putida* CECT5279. J. Chem. Technol. Biotechnol., 88(3); 422-431.

Boniek, D., Figueiredo, D., dos Santos, A. F. B. and de Resende Stoianoff, M. A. (2015). Biodesulfurization: a mini review about the immediate search for the future technology. Clean Technol. Environ. Policy, 17(1); 29-37.

Caro, A., Boltes, K., Leton, P. and Garcia-Calvo, E. (2008). Description of by-product inhibition effects on biodesulfurization of dibenzothiophene in biphasic media. Biodegradation, 19(4); 599-611.

Carvajal, P., Dinamarca, M. A., Baeza, P., Camu, E. and Ojeda, J. (2017). Removal of sulfur-containing organic molecules adsorbed on inorganic supports by *Rhodococcus Rhodochrous* spp. Biotechnol. Lett., 39; 241-245.

Chen, H., Zhang, W.J., Cai, Y. B., Zhang, Y. and Li, W. (2008). Elucidation of 2-hydroxybiphenyl effect on dibenzothiophene desulfurization by *Microbacterium* sp. strain ZD-M2. Bioresour. Technol., 99(15); 6928-6933.

Debabov, V. G. (2010). Microbial desulfurization of motor fuel. Appl. Biochem. Microbiol., 46; 733-738.

Dejaloud, A., Vahabzadeh, F. and Habibi, A. (2017). *Ralstonia eutropha* as a biocatalyst for desulfurization of dibenzothiophene. Bioprocess. Biosyst. Eng., 40(7); 969-980.

del Olmo, C. H., Santos, V. E., Alcon, A. and Garcia-Ochoa, F. (2005). Production of a *Rhodococcus erythropolis* IGTS8 biocatalyst for DBT biodesulfurization: influence of operational conditions. Biochem. Eng. J., 22(3); 229-237.

Doelle, H. W., Ewings, K. N. and Hollywood, N. W. (1981). Regulation of glucose metabolism in bacterial systems. Adv. Biochem. Eng./Biotechnol., 23; 1-36.

Kilbane, J. J. and Stark, B. (2016). Biodesulfurization: a model system for microbial physiology research. World J. Microbiol. Biotechnol., 32(8); 137.

Kim, Y. J., Chang, J. H., Cho, K. S., Ryu, H. W. and Chang, Y. K. (2004). A physiological study on growth and dibenzothiophene (DBT) desulfurization characteristics of *Gordonia* sp. CYKS1. Korean J. Chem. Eng., 21(2); 436-441.

Lapin, L. L. (1997). Modern Engineering Statistics. (Belmont, CA: Duxbury Press)

Luedeking, R. and Piret, E. L. (1959). A Kinetic study of the lactic acid fermentation. Batch process at controlled pH. J. Biochem. Microbiol. Technol. Eng., 1(4); 393-412.

Martin, A. B., Alcon, A., Santos, V. E. and Garcia-Ochoa, F. (2005). Production of a biocatalyst of *Pseudomonas putida* CECT5279 for DBT biodesulfurization: Influence of the operational conditions. Energy Fuels, 19; 775-782.

Martinez, I., Mohamed, M. E., Santos, V. E., Garcia, J. L., Garcia-Ochoa, F. and Diaz, E. (2017). Metabolic and process engineering for biodesulfurization in Gram-negative bacteria. J. Biotechnol., 262; 47-55.

Martinez, I., Santos, V. E., Alcon, A. and Garcia-Ochoa, F. (2015). Enhancement of the biodesulfurization capacity of *Pseudomonas putida* CECT5279 by co-substrate addition. Process Biochem., 50(1); 119-124.

Millard, P., Smallbone, K. and Mendes, P. (2017). Metabolic regulation is sufficient for global and robust coordination of glucose uptake, catabolism, energy production and growth in *Escherichia coli*. PLOS Comput. Biol., 13(2); 1-24.

Mohebali, G. and Ball, A. S. (2016). Biodesulfurization of diesel fuels-Past, present and future perspectives. Int. Biodeterior. Biodegrad. 110; 163-180.

Neijssel, O. M. and Tempest, D. W. (1976). Bioenergetic aspects of aerobic growth of *Klebsiella aerogenes* NCTC 418 in carbon-limited and carbon-sufficient chemostat culture. Arch. Microbiol., 107(2); 215-221.

Pirt, S. J. (1965). The maintenance energy of bacteria in growing cultures. Proc. R. Soc. London, Ser. B, 163(991); 224-231.

Pirt, S. J. (1982). Maintenance energy: a general model for energy-limited and energy-sufficient growth. Arch. Microbiol., 133(4); 300-302.

Razvi, A., Zhang, Z. and Lan, C. Q. (2008). Effects of glucose and nitrogen source concentration on batch fermentation kinetics of *Lactococcus lactis* under hemin-stimulated respirative condition. Biotechnol. Prog., 24(4); 852-858.

Repaske, D. R. and Adler, J. (1981). Change in intracellularr pH of *Escherichia coli* mediates the chemotactic response to certain attractants and repellents. J. Bacteriol., 145(3); 1196-1208.

Soleimani, M., Bassi, A. and Margaritis, A. (2007). Biodesulfurization of refractory organic sulfur compounds in fossil fuels. Biotechnol. Adv., 25(6); 570-596.

Srinivasan, K. and Mahadevan, R. (2010). Characterization of proton production and consumption associated with microbial metabolism. BMC Biotechnol., 10(2); 1-10.

Tsai, S. P. and Lee, Y. H. (1990). A model for energy-sufficient culture growth. Biotechnol. Bioeng., 35(2); 138-145.

van Bodegom, P. (2007). Microbial maintenance: A critical review on its quantification. Microb. Ecol., 53(4); 513-523.

Wang, Z. L., Wang, D., Li, Q., Li, W. L., Tang, H. and

Xing, J. M. (2011). Enhanced biodesulfurization by expression of dibenzothiophene uptake genes in *Rhodococcus erythropolis.* World J. Microbiol. Biotechnol., 27(9); 1965-1970.

Zhou, J., Liu, L., Shi, Z., Du, G. and Chen, J. (2009). ATP in current biotechnology: Regulation, applications and perspectives. Biotechnol. Adv., 27(1); 94-101.

Zubay, G. L. (1998). Biochemistry. (Dubuque, Iowa: William C Brown Pub)