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SIMULATION OF POLY-(HYDROXYBUTYRATE) FROM METHANE IN VERTICAL LOOP BIOREACTOR

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ABSTRACT

Bio-plastics are eco-friendly biopolymer finding tremendous application in food and pharmaceutical industries. Bio-plastics have suitable physicochemical, mechanical properties, and does not cause any type of hazardous pollution upon disposal but have high production cost. This can be minimized by screening potential bio-polymers producing strains, selecting inexpensive raw material, simulation and optimization of cultivation condition. In this study, simulation of bacterial production of poly-β-hydroxybutyrate from methane in vertical loop fermentor was carried out by Comsol 5.2 software in 3-dimensional mode. Mass transfer in the process of bacterial growth was investigated via the feed of methane substrate. The graphs of cell density and growth confirmed the results of the simulation according to time. Meshing and independence analysis of the mesh carried out. The initial concentration of microorganism was 0.001 g/L than in the optimal condition and different duration of time was reached 50% of methane and 50% of gas in the reactor that was the highest value of growth microorganism. The results of the simulation were confirmed to experimental results with less than 5% error.

1.Introduction

One of the most important poly-hydroxy-alkanoates is poly-hydroxybutyrate (PHB), which is formed as intracellular granules in different microstructures. Three unique characteristics of PHB are thermoplasticity, water resistance and biodegradability Lee (1996); Booma, et al. (1994); Golzar, et al.

(2020); Malmir, et al. (2020). However, their world wide application is still limited due to the high production cost. PHB total cost depends on some parameters e.g. the substrate, microorganism, cultivation system, and down-stream processing. The utilization of cheap substrates Khosravi-Darani and Bucci (2015); Darani, et al. (2006); Khosravi-Darani, et al. (2019); Ghoddosi, et al. (2019); Mokhtari-Hosseini, et al. (2009); Mokhtari-Hosseini, et al. (2009); Bozorg, et al. (2015); Koller, et al. (2017); Khosravi-Darani, et al. (2013), model-ing Shahhosseini, et al. (2003), suitable bioreactor and design of experiments Khosravi Darani, et al. (2003); Vasheghani Farahani, et al. (2004), as well as recovery method Khosravi-Darani and Vasheghani-Farahani (2005); Khosravi Darani, et al. (2003); Vasheghani Farahani, et al. (2004). These factors would not only decrease the production cost of PHB but also help in increasing productivity Khanna and Srivastava (2005).

One way to increase PHB production from natural gas is to use new bacterial species in bio-fermenters with appropriate hydrodynamics Khanna and Srivastava (2005); Rashidi, et al. (2020); Reddy, et al. (2003). Among the carbon sources available, methane is the most suitable substrate for PHB production, which is both readily available and inexpensive. It is also necessary to design a proper fermenter for fermentation with sufficient efficiency. For biomass production from methane, the fermenter must be designed to allow adequate mass transfer from air to liquid flow, CO_2 removal, mixing and oxygen supply Shah, et al. (2008); Okan, et al. (2019); S.R. Mofradnia, et al. (2018); Mofradnia, et al. (2019); Rahnama, et al. (2012); Moradi, et al. (2019). Mixing with blades is not suitable for biomass production in large fermenters due to high-energy consumption. In addition, high energy is needed for running these kind of fermenters. Also, it is possible that a contaminating micro-particle enters the fermenter during the blade's design and installation Rahnama, et al. (2012); Zhang, et al. (2008); Moradi, et al. (2019); Khosravi Darani, et al. (2018); Rezapour, et al. (2019). In a loop fermenter, mixing and aeration performs well through the circulation of the medium. These fermenters perform best in heat transfer, mass transfer, aeration and substrate mixing and require local sampling and analysis. These fermenters are very high. In addition to these benefits, the energy required to transfer each kg of oxygen is low Yazdian, et al. (2009); Wendlandt, et al. (2005); Memari, et al. (2020).

According to the above, in this study, the simulation of a vertical loop bioreactor, a type of airlift bioreactor, to produce PHB was investigated by COMSOL software.

2. Materials and methods

2.1. Simulation

Loop bioreactor simulation was performed by COMSOL Multi-physics simulation software as a set of simulations that can solve the differential equations of nonlinear systems by partial derivative with finite element method in 1, 2 and 3D spaces. Also in this software, the problem can be presented I the form of a mathematical formula (in the form of equations) and a physical one (selection of the physical model, such as the transfer of dilute components in solution) Soheil Rezazadeh Mofradnia, et al. (2018); Mofradnia, et al. (2019); Multiphysics (2012).

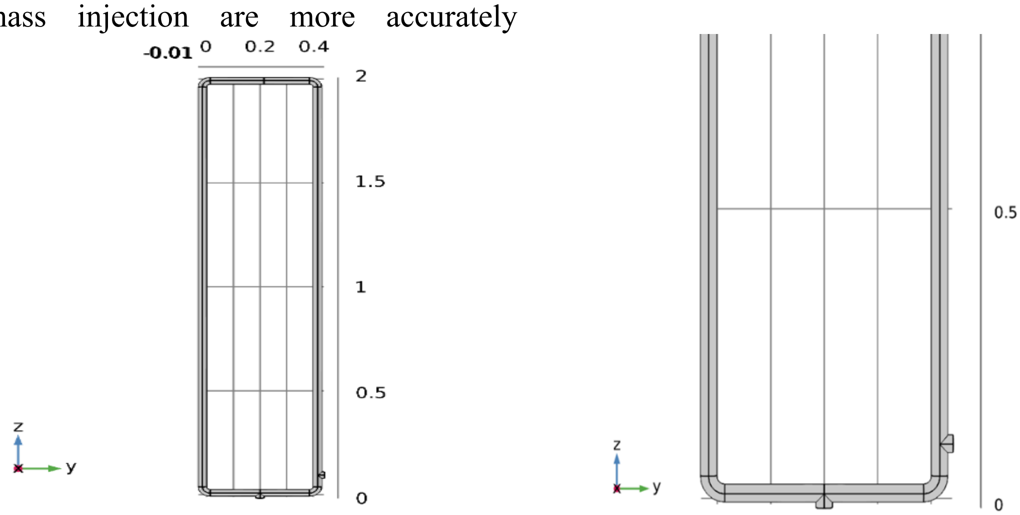
For the present simulation, the dilute component transfer module is used. In order to define the desired module, the properties and constants available in the COMSOL software are used for the simulation module. COMSOL software has a library of inform-ation on material properties and constants. However, if the property of the material in question is not present in the software library, its value can be directly defined in the module used as a parameter Mousavi, et al. (2010). The dilute compon-ent transfer module is used for both the liquid and gas phases. As in the gas phase, only the mass transfer from the gas bulb to the gas- liquid boundary occurs, and in the liquid phase, dissolved gases are transferred to the liquid bulk and the biomass production reaction takes place in the liquid phase. Since the momentum equilibrium is not in the bioreactor, the velocity of the liquid phase and the gas phase in the equations are considered constant. Average velocity of the gas phase and the liquid phase in the bioreact-or were 0.035 and 0.35 m/s.

2.2. The geometry of the bioreactor

The bioreactor's dimensions including length, height, diameters as well as diameter of gas and biomass injection port were 0.45, 2.00, 0.03 and 0.03 m, respectively. Figure 1 shows the overall scheme of the loop bioreac-tor (Figure 1a) and the ports of gas and biomass injection are more accurately specified (Figure 1b). As shown in table 1, for the bioreactor walls the non-slip boundary condition means zero velocity and fixed boundary of the system. Also the condition of no flux, i.e. n. Ni=0, means that none of the system's components are going out of the system. The gas output condition is also given by n. Di∇Ci =0, which means the components of the system are leaving through the fluid. In the initial condition of this model, the concentration of component in the liquid and gas phases at t=0 are considered to be 0. Initial and boundary conditions are presented in table 1,

2.3. Meshing

Meshing for the present 3D geometry is done through finite number of elements. The meshing is considered as triangular elements. In figure 2a, an overview of the meshed bioreactor is shown. Also in figure 2b, this meshing is partially displayed at the injection site.

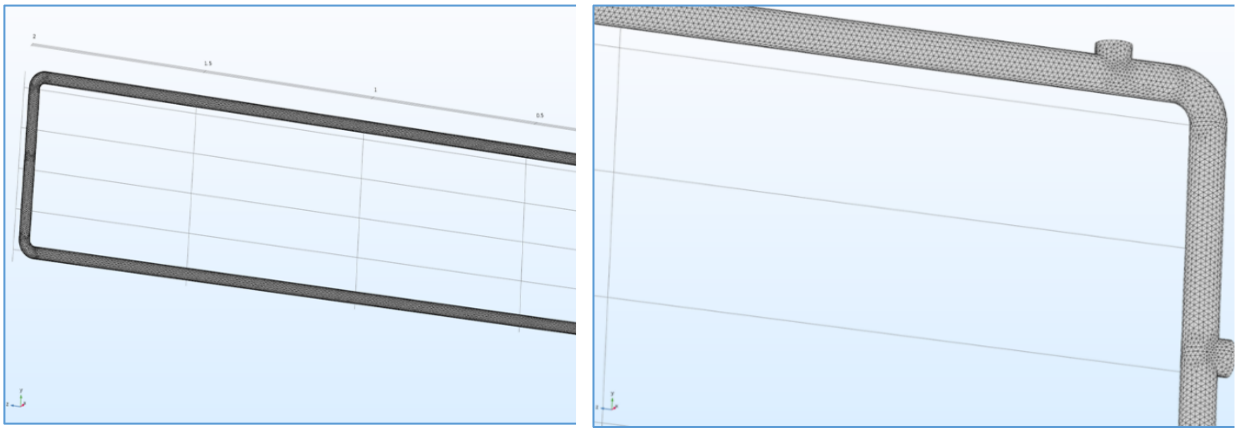


(a) (b)

Figure 1. The overall scheme of the bioreactor (a) and location of gas and biomass injection (b).

Table 1. The boundary conditions used in the simulation.

Boundary	Value	Explanations
Gas injection	CH_4=20.1 mol/m^3 O_2=4.221 mol/m^3	Dynamically (time dependent)
Biomass injection	Cbio=0.0088 mol/m^3	Zero moment injection
Walls	No flux condition through the wall	
Gas outlet	Outflow condition	



(a) **(b)**

Figure 2. An overview of the meshed bioreactor with triangular elements (a), Close view of the bioreactor meshed by gas and biomass injection sites (b).

3.Results and discussions

In this study, the performance of Hirsutas methylocystis for the production of PHB was simulated. According to the equations and information, simulating the bioreactor in the COMSOL software, the mean change of the concentration of the components in the liquid phase in different ratios of methane to air, 0.2/0.8 (methane to air), 0.5/0.5 (methane to air) and 0.8/0.2 (methane to air) were calculated by time.

3.1. Changes in the concentration of methane and oxygen substrates over time

As can be seen in figure 3 (a and b), the concentration of both components initially increases, because the gas mass reaches liquid-gas contact surface. In the study of Aghamiri et al. (2012), the kinetics of protozoan production of natural gas by Methylomonas microstructure in the gas phase with an equal volume of air and natural gas was studied experimentally and by mathematical modeling Wu, et al. (2012). Different models of Mund, Moser, Tseir, Iba, Andrews and Novak have been used to describe the kinetics of cell growth. Methane and Oxygen as growth restrictive substrates, were considered in the concentration range of 2 to 10 mg/l were used to establish the phases of inactivity and death using a time-dependent death coefficient. The results of this study showed that mathematical models can express well the cell mass production's dynamic in the growth till death phases. The kinetic parameters of each model were extracted by it's comparison with the experimental data. The results also showed that Mund and Moser models more accurately describe cell mass production than other models in this study.

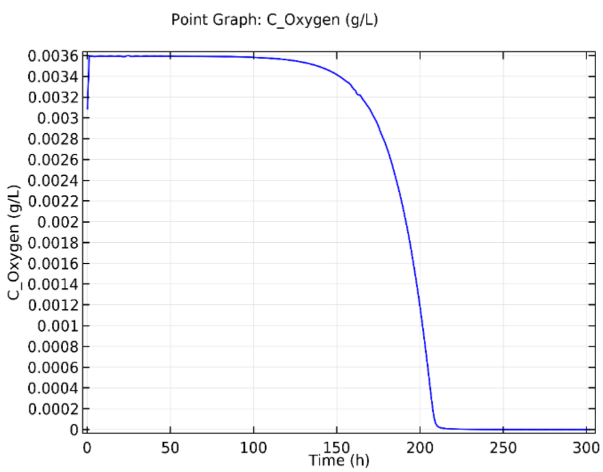
It also showed that the amount of methane and dissolved oxygen in the cell culture medium decreased with increasing cell mass production rate. In other words, when cell mass growth is low, the amount of methane and oxygen in reach of the cell is high and as cell growth increases, the amount of methane and dissolved oxygen consumption increases Yazdian, et al. (2010).

Figure 4 (a & b) show the increase in methane and oxygen concentration in the gas-liquid interface more precisely. According to the figures, it can be seen that after the injection of methane and air into the bioreactor, their concentration stabilized after 1.5 h, indicating that the two components reach saturation. Then, the reaction of these two components with biomass begins over time and their concentration decreases (Figure 4 c & d). This process is the same for all of the methane/ air ratios.

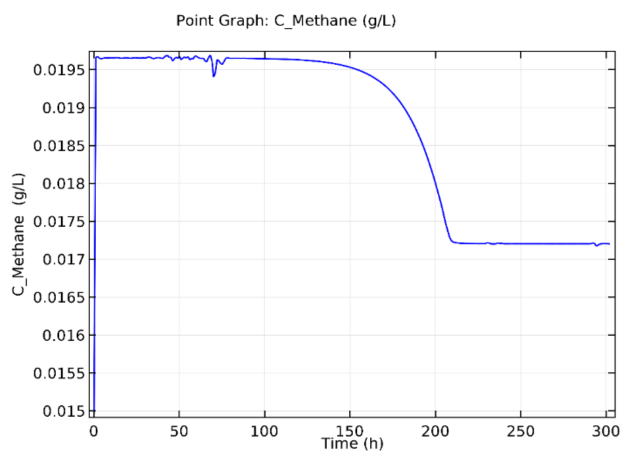
3.2. Changes in methane and oxygen substrate's concentrations over time

Figure 4 shows the change in biomass growth rate and the biomass concentration in the ratio of 50% methane and 50% air. According to the biomass growth diagram in Fig. 4, initially the mass concentration increases and then with the constant growth rate, reaches it's maximum over time, these findings are in good agreement with the obtained results by Bagde, et al. (2014).

According to the Fig 4., the biomass growth rate is initially controlled by the concentration of methane and air. Given the constant μ dependence on the concentration of these two components, the growth rate of the mass is fixed after some time (Eq. 3.4). In other words, μ reaches it's maximum value and becomes independent of the concentration of the components.

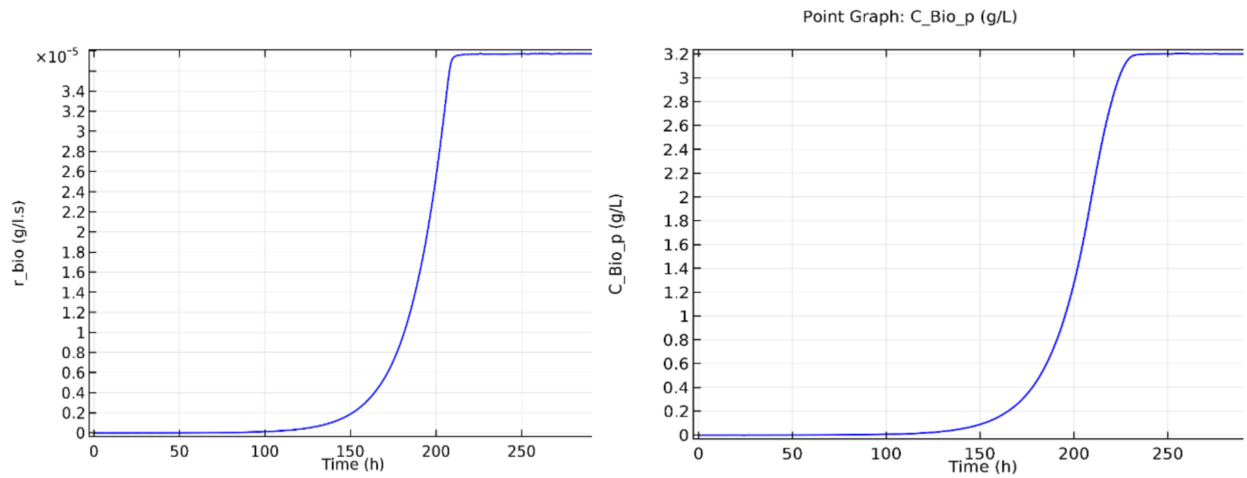


(a)



(b)

Figure 3. Profile of O_2 (a) and CH_4 concentration over time (b).



(c) (d)

Figure 4. Changes in oxygen (a) and methan (b) concentration as well as biomass growth rate (c) and biomass concentration (d) over time.

Over time, the biomass growth rate becomes dependent on the mass concentration. This has led to another increase in the biomass growth rate, with the concentration of air and methane eventually falling. As the result, the μ also decreases and the biomass growth rate remains constant over time.

In 2012, mathematical modeling of loop bioreactor to produce protozoan protein from methane by aerobic microstructure of Methylomonas species was simulated by Bagde, et al. (2014). The mass equilibrium equation was written for each part separately and according to the available assumptions, and then the resulting ordinary and partial differential equations were solved simultaneously. The mathematical model used for the upstream, downstream and horizontal flow sections is the axial dispersion model and for the pump, liquid- gas separator and static mixer sections is the model of a series stirred tank. The mass transfer between the phases and the reaction kinetics is considered. Model sensitivity analysis was also performed for gas and liquid velocity variations as well as CH_4 to total inlet air ratio and the optimum operating conditions were evaluated. For the initial conditions assumed, the substrates in the gas and liquid phases and the cells have uniform dispersions through all sections from the outset, and also oxygen and methane in the liquid and gas phases have reached equilibrium state. The initial concentration of the cell is 1 g/L. Excessive oxygen in the environment causes a side reaction and has an inhibitory effect on cell growth. The effect of increasing and decreasing the inlet oxygen component on the rate of cell growth can be observed by changing the ratio of inlet air to inlet methane. As the volume of air increases from 0.5 to 0.6, the cell growth increases, further increase in air volume results in decreased cell growth due to oxygen inhibition or methane restriction. The best condition for the produc-tion of biomass by the microorganisms in the given operating conditions are gas flow rate of 1.5 L/min, a liquid flow rate of 15 L/min and the ratio of 0.6 for methane to total inlet gas Rahnama, et al. (2012); Ghoddosi, et al. (2019).

3.3. Mesh- independent result*s*

In order to investigate the independence of the results of the simulation with respect to mesh, meshing with different numbers must be performed. By decreasing the mesh size to the point that the difference between the obtained results are less than 0.5%, the solution is independent of the computational mesh. The number of elements was considered as 6770 to 810707. In table 2, the biomass concentration for the number of elements is examined. Considering the aver-age biomass concentration obtained in the bioreactor, the concentration of this component was slightly changed and the error percentage was less than 0.5%. So, the responses obtained are independent of the mesh and the number of 261128 elements is considered as the number of appropriate elements.

Table 2. The results with respect to the number of elements.

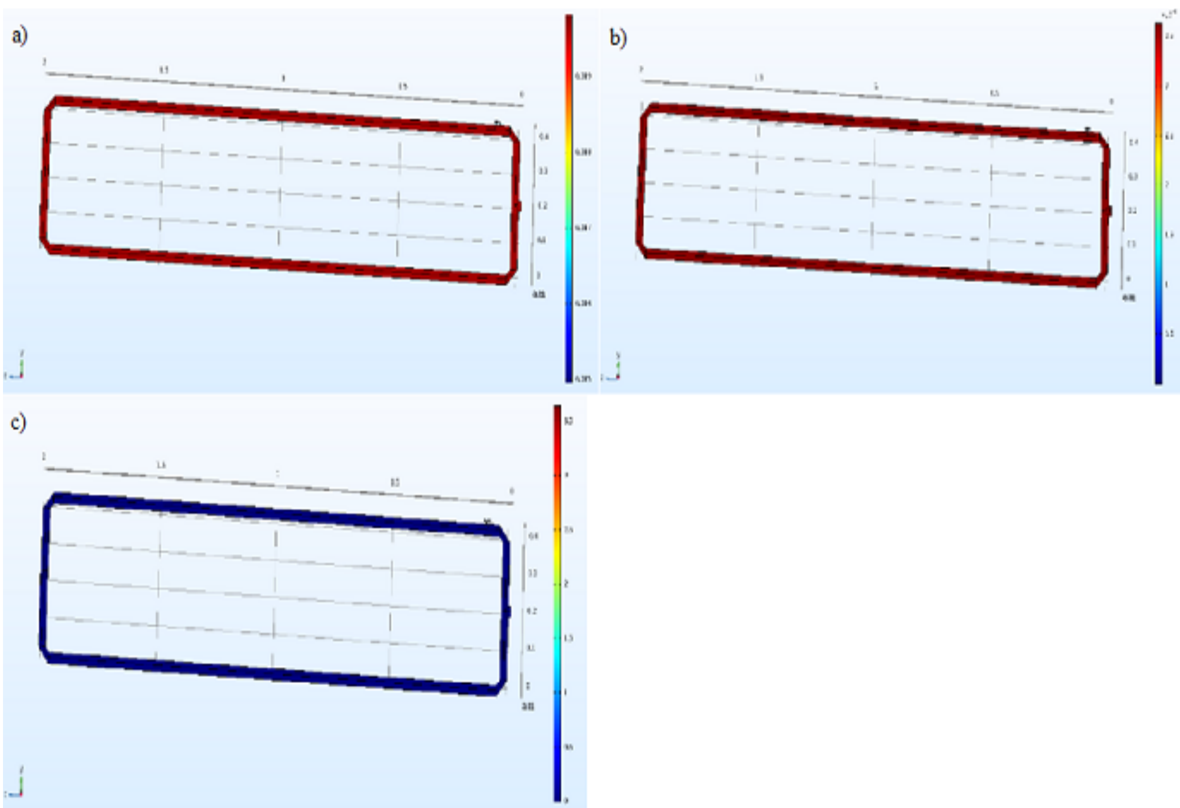
Element's number	6770	62811	261128	810707
Biomass concentration (mol/m^2)	0.052147	0.064958	0.065955	0.065956

3.4. Investigation of methane and air concentration contours

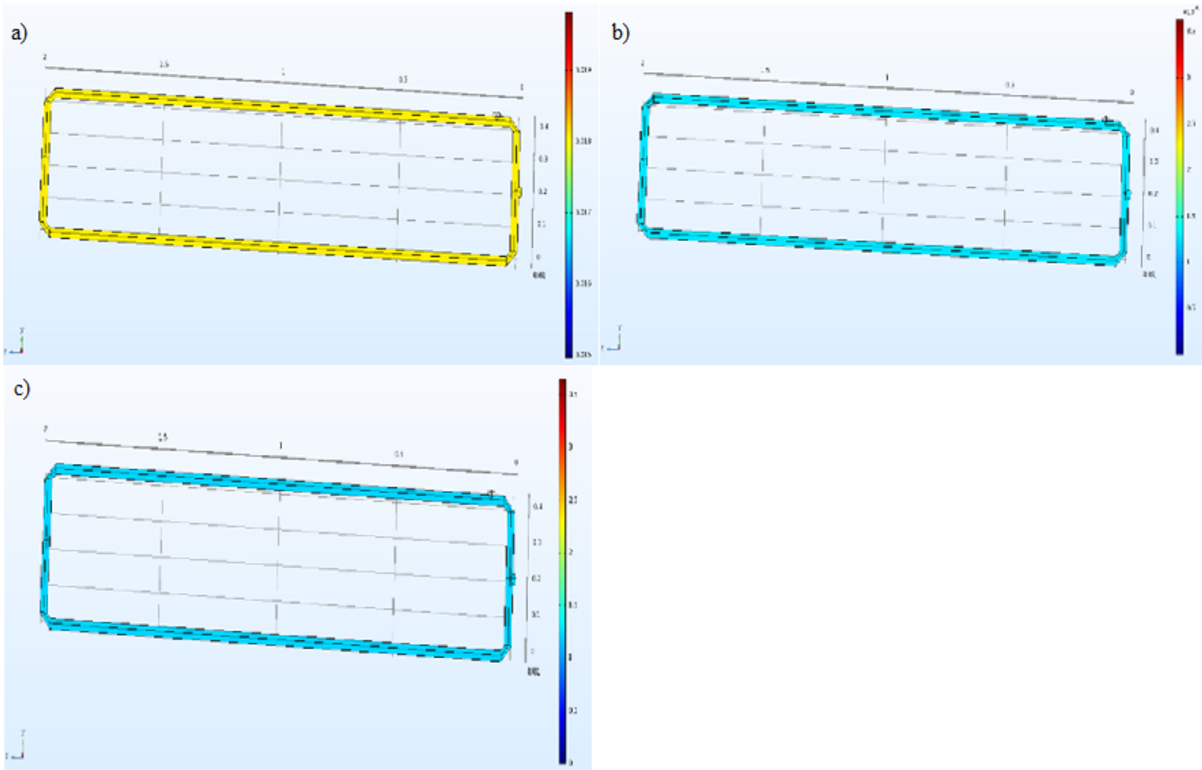
3.4.1. Methane and air concentrations in the ratio of 50% methane and 50% air

Figure 5 (I) (a-c) shows the contours of methane, air and biomass concentration in g/L, in the ratio of 50% methane and 50% air at 100 hours, respectively. In figure 5 (II) (a-c) the contours of CH_4, air and biomass concentration in g/L, in the ratio of 50% CH_4 and 50% air at 200 h are shown respectively. Also figure 5 (III) (a-c) shows the contours of methane, air and biomass concentration in g/L, in the ratio of 50% methane and 50% air at 250 hours, respectively.

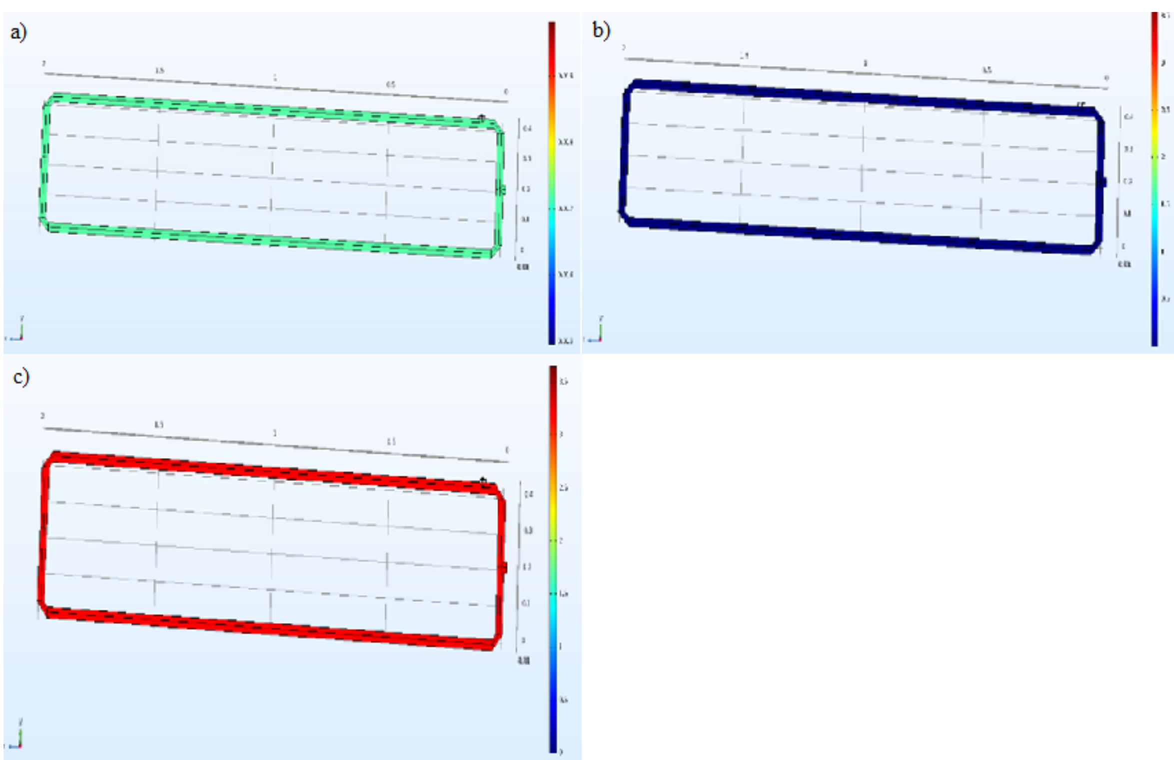
5 (I)



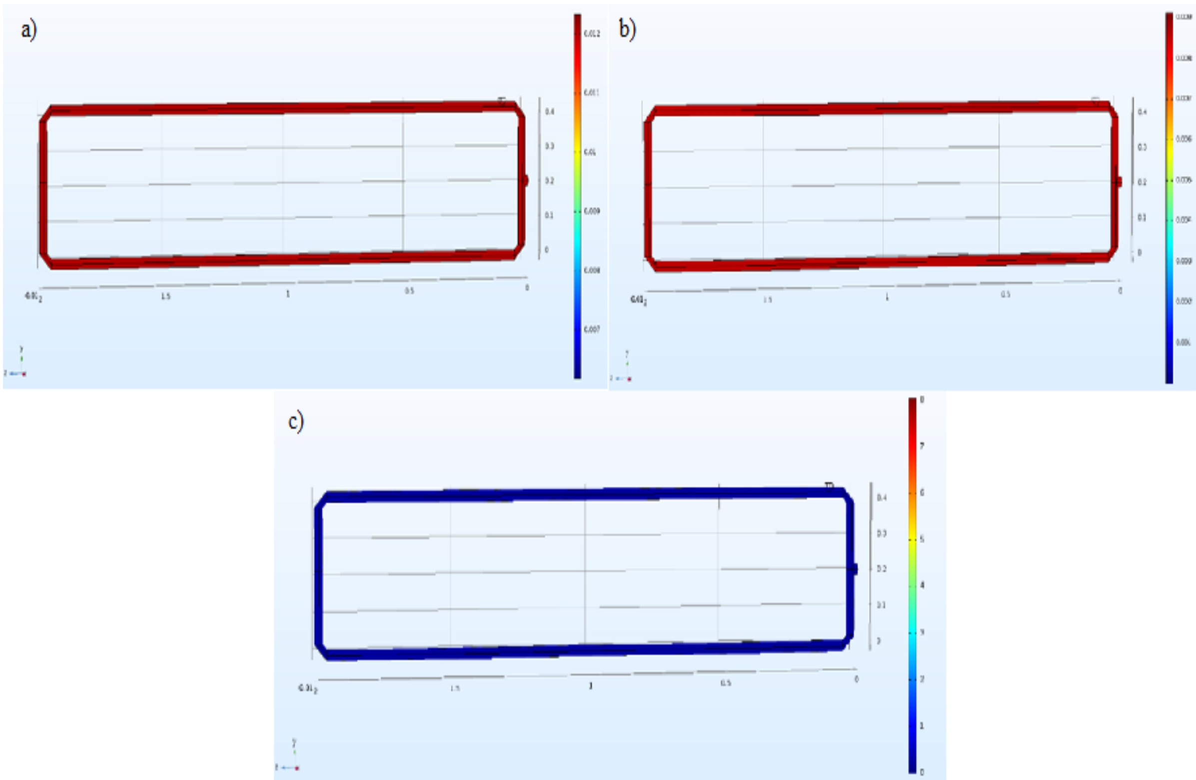
5 (II)



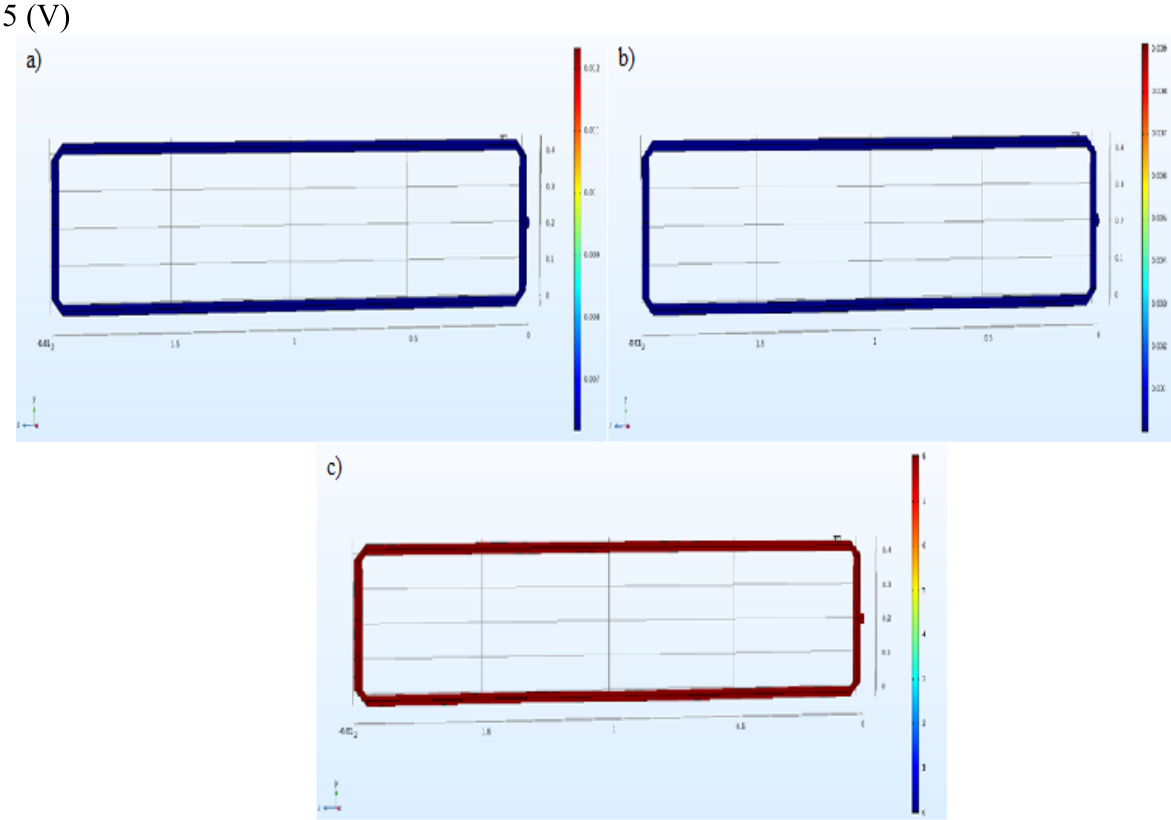
5 (III)



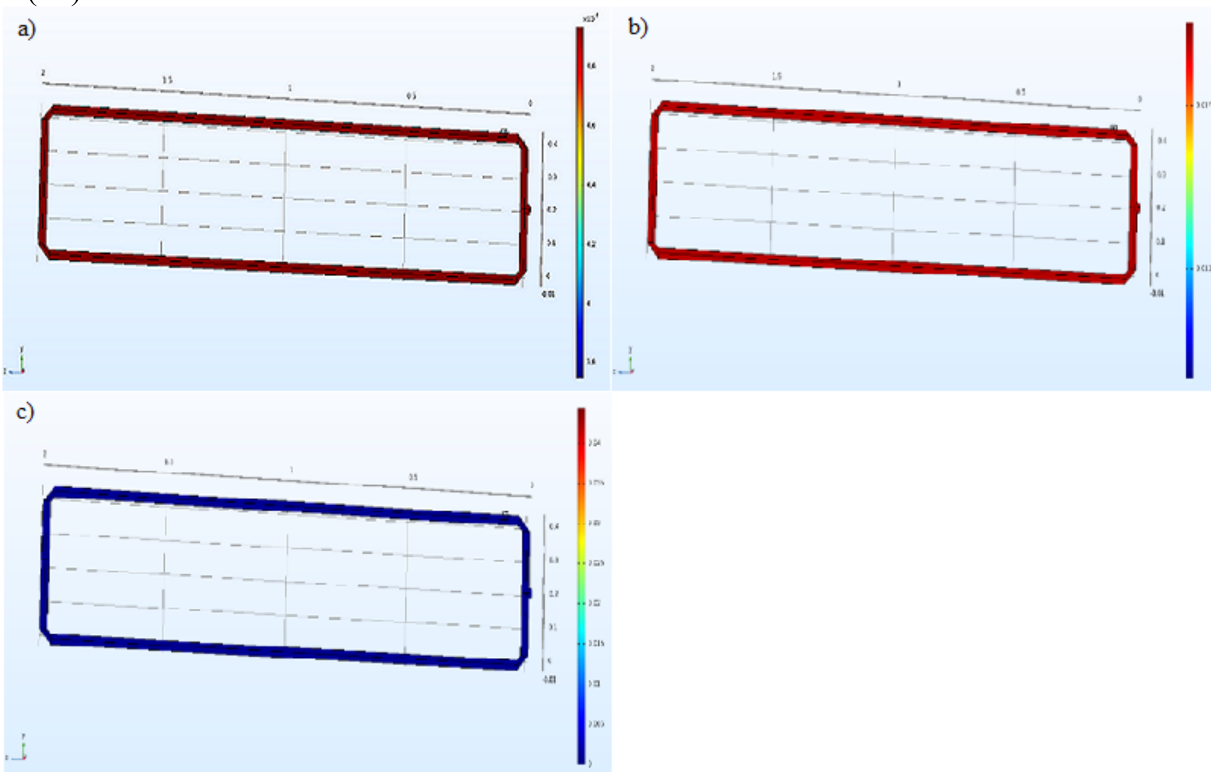
5 (IV)



5 (V)



5 (VI)



5 (VII)

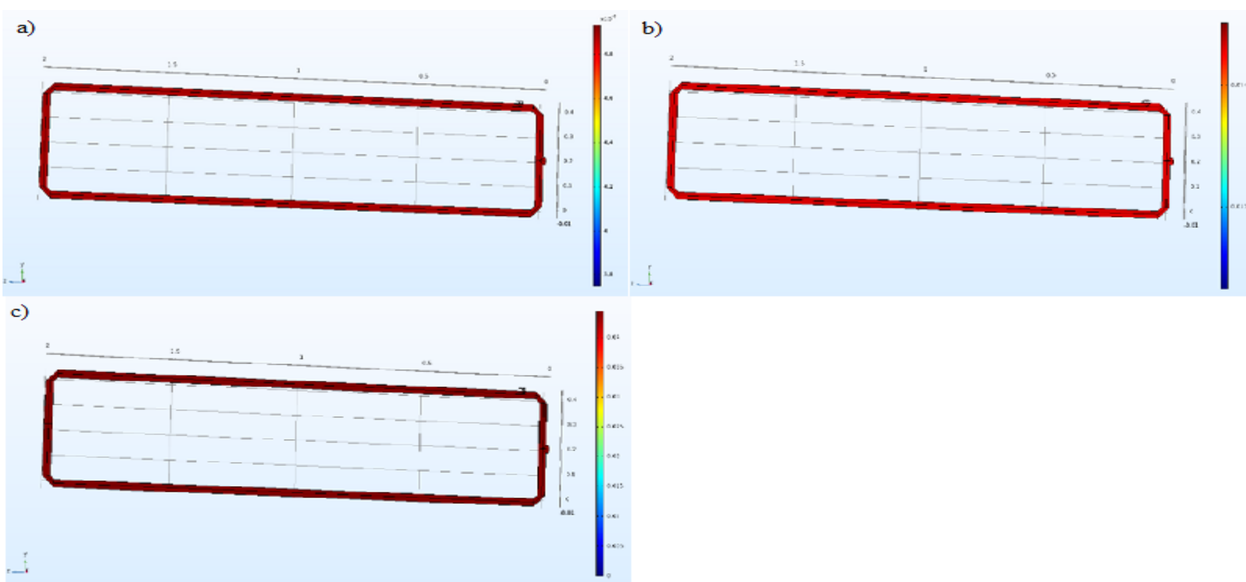


Figure 5. The concentration contours (g/L) in 50% methane and 50% air at 100 (I), 200 (II), 250 (III) hours for methane (a), air (b) and biomass (c). The concentration contours (g/L) in 80% methane and 20% air at 250 (IV), 400 (V) hours for methane (a), air (b) and biomass (c). The concentration contours (g/L) in 20% methane and 80% air at 250 (VI), 400 (VII) hours for methane (a), air (b) and biomass (c).

Table 3. Average change in component's concentration over time at different air and methane. ratios (g/L)

CH4 / air	t = 100 hr.	t = 200 hr.	t = 250 hr.	t = 400 hr.
0.8 / 0.2	CH4 = 0.0123(g/l) O2 = 0.009(g/l) Biomass = 8.66e-4(g/l)	CH4 = 0.0122(g/l) O2 = 0.00899(g/l) Biomass = 0.039(g/l)	CH4 = 0.0108(g/l) O2 = 0.0067(g/l) Biomass =1.41(g/l)	CH4 = 0.0062(g/l) O2 = 4.98e-6(g/l) Biomass =2.51(g/l)
0.5 / 0.5	CH4 = 0.01964 (g/l) O2 = 0.00355 (g/l) Biomass = 0.00632 (g/l)	CH4 = 0.0181(g/l) O2 = 0.00115(g/l) Biomass =1.28(g/l)	CH4 = 0.017267(g/l) O2 = 7.765e-7(g/l) Biomass =3.243(g/l)	CH4 = 0.01724 (g/l) O2 = 3.72e-7(g/l) Biomass =8.245(g/l)
0.2 / 0.8	CH4 = 0.00495(g/l) O2 = 0.01445(g/l) Biomass =7e-5(g/l)	CH4 = 0.00494(g/l) O2 = 0.01442(g/l) Biomass =3.82e-4(g/l)	CH4 = 0.00493(g/l) O2 = 0.01441(g/l) Biomass =0.0021(g/l)	CH4 = 0.0049(g/l) O2 = 0.01440(g/l) Biomass =0.044 (g/l)

3.4.2. Methane and air concentration in 80% methane and 20% air ratio

In figure 5 (IV) (a-c) the contours of methane, air and biomass concentration (g/L) in the ratio of methane/air 80/20 at 250 and 400 (V) h are shown respectively.

3.4.3 Methane and air concentration in 20% methane and 80% air

In figure 5 (VI) (a-c) the contours of methane, air and biomass concentration in g/L, in the ratio of 20% methane and 80% air at 250 hours are shown respectively. Figure 5 (VII) (a-c) shows the contours of methane, air and biomass concentration in g/L, in the ratio of 20% methane and 80% air at 400 hours, respectively.

In 2010, Yazdian et al. designed a horizontal loop bioreactor for biomass production using CFD software. In this study, parameters affecting bacterial growth rate such as inlet air velocity, inlet fluid velocity, bubble diameter and viscosity were investigated. The contours of methane and air concentration changes as well as biomass concentration change were studied at 0.5, 1, 1.5 and 4 s. In optimum conditions, the highest growth rate for the bacteria was obtained with 50% methane to 50% air ratio. It was also observed that in the horizontal sections of the bioreactor, the rate of biomass production was much higher than that in the vertical sections Yazdian, et al. (2010).

Finally, table 3 presents the variation in the concentration of methane and air components along with the biomass concentration in the liquid phase in g/L at different times in different ratios of methane and air. The biomass concentration increased over time in each ratio of methane to air, but at the same time, the air and methane concentrations showed a decreasing trend in the liquid phase. In addition, as the concentration of inlet air increases, the biomass production rate decreases.

4. Conclusions

Despite wide application in different fields, the high cost of PHB production has made it economic unprofitable comparing to synthetic polymers. Separation cost, carbon source used, fermentation process and carbon substrate efficiency are some of the factors affecting PHB production cost. Production of Polyhydroxy Butyrate is usually increased under nutrient deficiency conditions. Therefore, after reaching a high cell density, it's production should be increased by eliminating or reducing a nutrient such as nitrogen in the bacterium. In this study, a vertical loop bioreactor was simulated by COMSOL software to produce biomass from methanotroph methanocystis hirsutas bacterium purchased from Microbial Bank of Iran Scientific and Industrial Research Organization using natural gas. The Mund's kinetic model is considered for the kinetics of cell mass growth. The simulated environment is a laboratory-scale 3-L bioreactor. Reactor simulation was performed in 3D by determining the reactor dimensions, along with flow parameters, growth kinetics constants and mass transfer coefficient in the liquid phase. The amount of PHB produced from methane (municipal gas) by the bacterium Methylocystis hirsute was 3 g/L using a loop air system at optimum conditions for 250 hours of fermentation. By comparing these results with other studies of PHB production from methane, this bacterial species introduced as a novel and efficient type. According to the relationship of growth rate for the biomass, the process of changing the concentration of the components is as follows,

The upward trend of increasing methane and air concentrations in the liquid phase due to their transfer from the gas phase to the liquid-gas interface. Constant concentration of these components due to equilibrium concentration with liquid phase. Decreasing trend for both components and increasing biomass concentration due to biomass growth response. The highest biomass growth was observed for the ratio of 50% methane and 50% air, which was 3 g/L at 250 h with 0.5% error reported in the thesis.

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