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Simulation of cell culture by a packed bed Bioreactor

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Abstract: Background

To monitor the data and parameters such as shear stress, hydrostatic pressure, temperature, humidity, and so on in a culture medium, numerical simulation can play an important role and help to design an optimum bioreactor.

Materials and methods

In the study, in the first step, the simulation was done by a homogenous porosity bed. Then for detection of the effectiveness on the temperature and humidity profile of the solid substrate, a heterogeneous porosity distribution was studied.

Results

The heterogeneous porosity could cause a variety of permeability and heterogeneous distribution of humidity. The normal distribution of cells was 0.6464. Finally, for uniform distribution of temperature in a designed bioreactor defined and simulated.

Conclusion

This heterogeneous porosity distribution led to a variable permeability distribution and also led to a heterogeneous humidity distribution in the system. The predicted temperature profile for the distribution of heterogeneous porosity was significantly different from that predicted one for the homogeneous bed. In a designed bioreactor, heterogeneity in each altitude not only could start heating but also reach the thermal equilibrium. This could also occur at the relevant altitude in a homogeneous bed.

Keywords: cell culture; bioreactor; porous environment; scaffolding.

1. Introduction

Cell culture is a process in which cells grow under controlled conditions and outside their natural environment [1]. One of the methods for cell culture is using a bioreactor or fermenter. A fermenter or bioreactor is a set of various equipment and components that provide controlled environmental conditions for the growth of microbes or the production of specific metabolites in a liquid or solid culture medium under sterile conditions. Various parameters such as the concentration of metabolites, fluid shear stress, temperature, and pressure inside a bioreactor are important and affect on cell culture [2]. The factors that influence a bioreactor design are hydrostatic pressure and shear stress. Various techniques have been used for cell culture in different bioreactors, but seed yields are not yet optimal. Less seed density affects the time and resources required to obtain ready-to-plant scaffolds. The most common seed method is static loading of cells on the scaffold, but the most important disadvantage of this method is the low seed efficiency and uneven cell distribution in the scaffold [2, 3]. Due to the small spaces in the biological reactors and the possibility of contamination, it will not be possible to measure all these parameters through the installation of measuring equipment. Therefore, numerical simulation can be used as a method to measure the effective parameters in different parts of a bioreactor. Although the results of numerical simulations will be approximate, they are very useful because it is more economical and time effectiveness. Using numerical simulation, the required parameters can be obtained at all points of the flow and the behavior of the fluid can be observed. By achieving these results, a bioreactor can be better designed and built at a lower cost.

In the present study, we used the commercial software CFD ANSYS FLUENT® 16.0, which tried to simulate and develop a mathematical model for heat and mass transfer in a packed bed bioreactor.

2- Materials and methods

Two geometric models were used for simulation. To simulate the flow and humidity changes of the substrate during the culture process, a multiphase model by Fluent software was used. Finally, according to the data obtained for humidity, the substrate temperature, and the changes of the culture material, a new geometry was created for the bioreactor. The reason for designing a new geometry was to create optimal conditions in the process of cell culture.

Dynamics examination, the Brinkman equation, and the nutrient transfer equation, as well as the equation of changes in cell density over time and space, were examined. These equations could show multi-physical problems.

The bioreactor for the dynamic state of cultivation consisted of two cylinders and the scaffolds were located in the annular region between the two cylinders. The inner cylinder was stationary. The gas exchange was allowed through the inner cylinder while the outer cylinder was impermeable and rotates in a controlled manner. This bioreactor had a dynamic culture medium, which had two useful factors: efficient mass transfer rate and low shear stress. In general, the tensile force and centrifugal force played an important role in keeping scaffolds in balance (fig 1).

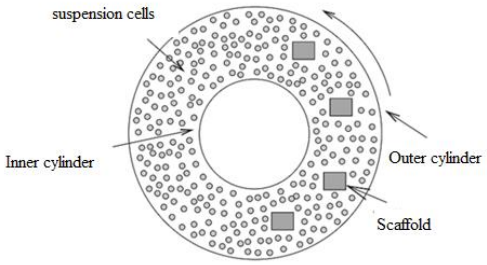

Figure 1. Rotating Bioreactor tube wall

To calculate fluid flow so many equations were used such as Brinkman equation; Nutrient transfer equation; cell growth equation; Navier stocks equations; continuity equation; Momentum equation; Semi-elliptic Navier-Stokes equations for steady, incompressible and two-dimensional flow; Dominant equations for turbulent flow; continuity equation for turbulent flow; Momentum equation for turbulent flow.

Simulation Overview

The first geometry simulation described the formation of a TME between solids and air during the autoclave of soybeans. The initial temperature was approximately 60°C then cooled during the experiment in the bioreactor. The second and third simulations were related to the experiment performed by Finkler in which filamentous fungi were grown on a mixture containing 27 kg of wheat bran (WB) and 3 kg of dry masses of sugarcane stalk (SCB). The second simulation described the TME of this fermentation during the first 20 minutes. In this period, the fungus was in its latency stage. Therefore, growth and metabolic activities were not included in the CFD model. The temperature of the substrate was increased from 25.6°C to 32°C by establishing a balance between the humidity of the bed and the gas phase. The third simulation described the growth of filamentous fungi after completion. The delay phase (which is about 10 hours after inoculation of the litter and loading of the litter in the bioreactor) was ended. During this stage, the fungus consumed the solid media and altered the porosity of the substrate, and affected the permeability of it. This fungus also produced heat and metabolic water, so that the slope of temperature and humidity was formed along the vertical axis. This experiment was specific and the growth phase took 16 hours. Under these conditions, the simulation was performed and presented for only 7-8 hours and only in 8.5 hours which the time growth was rapid. Between 8.5 and 8.7 hours, the simulation entered the divergence process. Therefore, the 8.5 hours would be considered as the end of the simulation.

The inner bed-chamber was rectangular. This base had 70 cm (x-axis), 60 cm (Y-axis), and a height of 50 cm (Z-axis). The outer shell was a cylinder that filled the space between the sides of the box and the cylinder with air. To avoid an excessive number of mesh cells, the central part considered only the inner chamber. The main space of the three-dimensional geometry was simplified and the thermocouple arrays were not included. However, the central rotation axis was considered. (6)

3. Simulation method

Wet solid substrate properties were calculated with the equations in table 1, the primary functions expressed as humidity. The operating pressure was set to 1.01 for both tests.

Table 1- Properties of wet particles of wheat bran (WB), sugarcane stalk (SCB) and soybean (SB) and the parameters characterizing the porous substrate as the yield of the initial moisture content (IMC)

Parameter	material	Equation/amount	Ref.
Solid particle density (kg m-3)	WB	0.974 + 0.226 IMC	[۴]
	SCB	0.578 + 0.00365 exp 0.149 IMC	[۵]
	SB	641.9	[۶]
Solid thermal conductivity (W m-1 °C- 1	WB	0.213 + 0.264 IMC	[۷]
	SCB	16.8 - 35.7 · 0.51 IMC 1	[۸]
	SB	0.3544 IMC + 0.86799	[۹]
Properties of solid mass in substrate	WB	0.91 - 0.016 exp (0IMC.30)	[۱۰]
	SCB	0.62 - 0.136 IMC	[۱۱]
	SB	0.4	[۱۲]

Substrate permeability and perforation parameters of the perforated plate flow were determined by Peso et al. In the same bioreactor and with the same amount of air flow, humidity and temperature was used as the Finkler test [13], the inertial resistance of the WB/SCB substrate was estimated to be C2 = 19.340 m^{-1}. The perforated plate was 0.002 m thick and was considered as a porous jump in the m^{-1} simulation region = 15 579/15 C^2. The specific heat capacity of the SCB/WB ratio, 9 to 1, was fully estimated by the heat capacity of WB. This approximation was reasonable given a large amount of wheat bran. The specific heat capacity of wheat bran, soybean, and liquid water was 1590, 1990, and 4184 J $kg^{-1}°C^{-1}$, respectively. The wet capacities of SCB and wet WB were calculated as the weighted average thermal capacity of dry solids and water, which were as follows:

$$C_p^{WB} = 1590 + 2594MC$$

$$C_p^{SB} = 1990 + 2194MC$$

Heat transfer coefficient (ha, $W°C^{-1}m^{-3}$) was estimated for soybean substrate.

$$ha = 44209.85\left[\frac{G(T_g + 273)}{0.0075P}\right]$$

Where G (kg-dry-air s^{-1}) was the velocity of the incoming air flow, T_g was the temperature of the gas phase and P (P_a) was the gas pressure. The selected T_g was the average between the inlet and outlet temperatures of the gas and was 43°C. This model has not assumed the solid and gaseous phases in moisture and thermal equilibrium; it was selected to simulate the "imbalance" option. Based on the analysis of Pessoa et al.[14], A time step of 0.01 seconds was used for the first stage of simulation of TME steps.

The second simulation required a smaller time step (0.001 seconds) to prevent numerical instability.

In the third simulation, the applied time step was 0.1 second due to the slow rate of change during the fermentation process.

4. Results

Geometry simulation

Air flow entered the system and the desired temperature specifications were applied. Initially, the lower thermocouples responded to airflow. Due to the large temperature difference between the bed and the air flow, the gas phase did not reach thermal equilibrium with it. Therefore, all heights were cooled by a specific speed simultaneously. No cooling air flow was observed to raise the bed at this speed. This speed could not be increased through the bed. At a height of 5 cm, the predicted temperature was in good agreement with the experimental specifications, but the difference between the experimental and predicted profiles was greater for other heights. Despite the differences, the simulation was behaved similarly to the experiment, because of the absence of visible cooling air flow.

In this geometry, a real SSF process, consisting of a substrate of 27 kg WB and 3 kg SCB (dry masses), was simulated for the initial TME phase. Initially, simulations were performed for a bed with homogeneous porosity. Then, to affect the temperature and humidity profile of solids in the bed, a heterogeneous porosity distribution was used. The initial porosity was randomly assigned to the cells based on the natural distribution with an average of 0.6464. This heterogeneous porosity distribution led to a variable permeability distribution. The flow lines that represent the air flow through the bed are shown in Figure 2. The flow velocity was higher at the air inlet and outlet and lower at the porous bed. The flow was disturbed inside the hollow chamber below the bed and in the laminar inside the pores of the bed. Temperature profiles were measured by Finkler for three-bed heights (5 cm, 18 cm, and 33 cm) during the initial heating of the bed. Experimentally, a heat transfer zone rose through the bed (Fig. 2). Initially, all altitudes were at room temperature (25.6°C). And the lowest height (5 cm) was the first case that heats up and raised the first set of four curves. The thermal equilibrium with the air phase (32°C) was reached by a sigmoid method. Then, the same behavior was repeated in a row, the next two sets of curves coming up at heights of 18 cm and 33 cm. As expected, for a heterogeneous bed at the same altitude, a heterogeneous permeability distribution led to a significant deviation (with a difference of up to 2.0°C) between four temperatures. These differences were occurred because of different porosities created by different permeability.

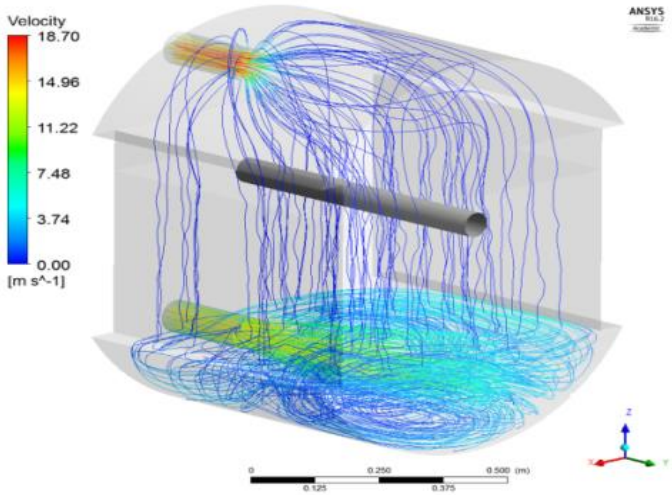

Figure 2- Speed of air flow through the substrate in the simulation

These phenomena were even more prevalent in experimental results, where temperature differences were observed up to 4.2°C at the same altitude. This showed that the real bed had a great variety of porosity. The predicted temperature profile for the distribution of heterogeneous porosity differed significantly from that predicted for the homogeneous one. The different heating rates seemed that to be effect on the experimental data qua for most thermocouple positions, the experimental temperature profile appeared before the corresponding profile predicted for the homogeneous substrate. However, only two laboratory profiles (one 18 cm height and one 33 cm height) were delayed in achieving thermal equilibrium with the incoming air. Considering all 12 thermocouples, the mean difference between the predicted temperatures for the bed was heterogeneous and the experimental temperature was 0.07°C. , Which was approximately 1% of the total temperature change during the experiment (from 6.4°C). This means that the model records the general trend for the bed temperature profile despite the relatively large standard deviation of 0.87°C. The variable porosity distributions led to a heterogeneous distribution of humidity in the bed.

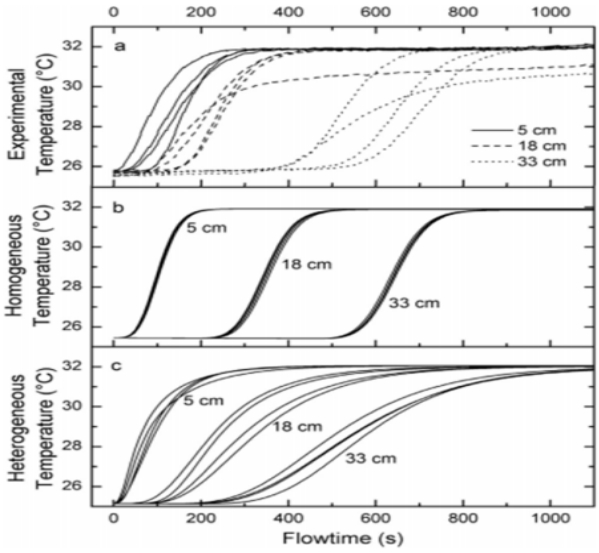

Figure 3- a) Temperature profile for initial heating of WB / SCB substrate in bioreactor b) Experimental data, thermocouple to thermocouple prediction of a CFD model for a substrate starting with homogeneous porosity distribution c) and prediction of a CFD model for a substrate with a heterogeneous porosity distribution To begin with, the lower altitudes are heated first, so that the curves increase by z = 5 cm, then z = 18 cm, and finally z = 33, respectively. Each curve represents the reading of a thermocouple. Thus four curves are created for each height (a total of 12 curves). The last one is related to thermocouples at a height of 33 cm.

The gradient amount of humidity was changed due to temperature variation over time in different parts of the system (Fig 4-7).

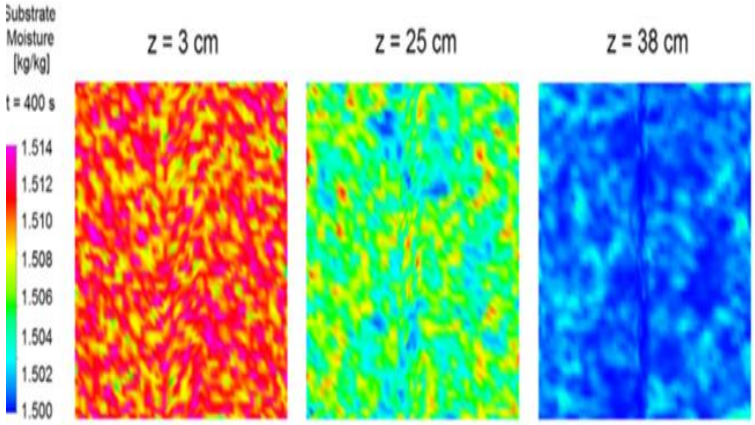

Figure 4- Enclosure for solids temperature, on plate XZ in 400 seconds, for a WB / SCB substrate starting with a heterogeneous porosity distribution. The white circle was the cross section of the central axis of the bioreactor

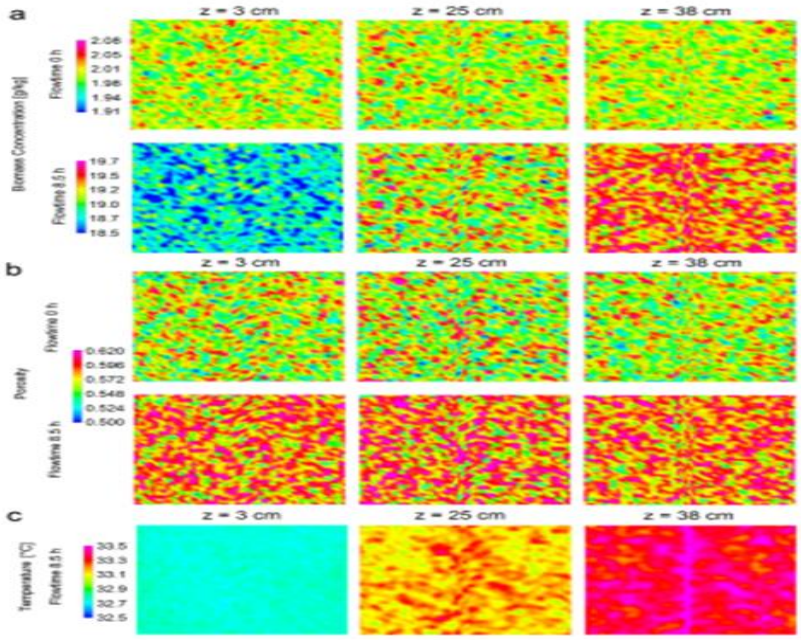

Figure 5- the third simulation predictions, comparing the initial (0 h) and final (8.5 h) values of the biomass concentration (a) and porosity (b) the predicted area, plus the temperature lines at 8.5 Hours (c).

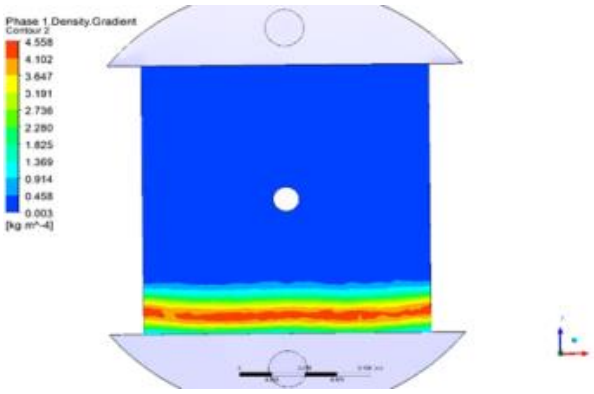

Figure 6- Density gradient of the first phase (air)

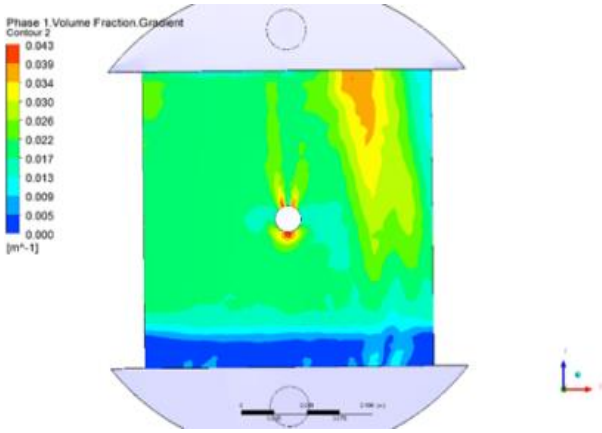

Figure 7- Volumetric fraction gradient of the first phase (air)

The temperature of the fluid could be increased in high porous contours in which the cultivation process was taking place (the data was not shown).

The vapor phase in the above contours could be concluded with increasing time. During the time, due to the passage of the system temperature from the saturation temperature of the liquid phase, it began to evaporate and caused an increasing concentration of this phase in the system.

According to the studies performed on the designed bioreactor and its simulation, the relevant model was validated. By designing a new system with optimized parameters such as humidity and temperature in the system, we would optimize the geometry of the relevant bioreactor. The obtained results from this simulation were showed that to check the number of elements and the accuracy of the mesh, the independence of the network in this system ought to be checked.

Based on the diagram, the cultured cells in the newly designed bioreactor were increased due to the presence of higher oxygen concentration and homogeneous distribution of nutrients in the scaffold (chart 1).

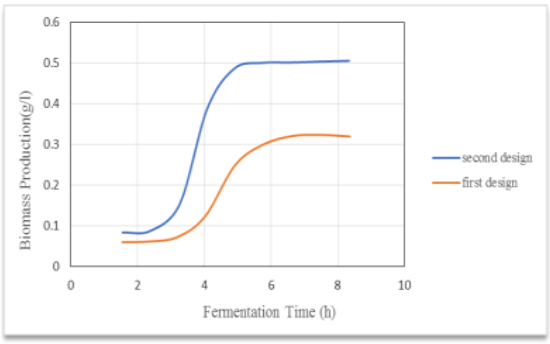

Chart 1- A comparison between the numbers of cells produced in the new and previous designed bioreactor

Network independence was checked out to ensure the accuracy of the simulation and computation results. In the model, the humidity rate in the system could be observed according to various elements. The percentage of difference of the 340,000-elements to one-million-elements was a maximum of 5 percent which was an acceptable result. Here the most important parameter for the cultivation process was the percentage of humidity according to various numbers of elements.

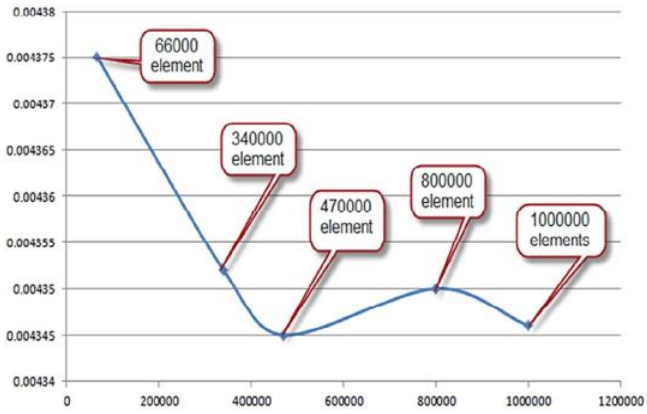

Chart 2- Graph of network independence (humidity in terms of number of elements)

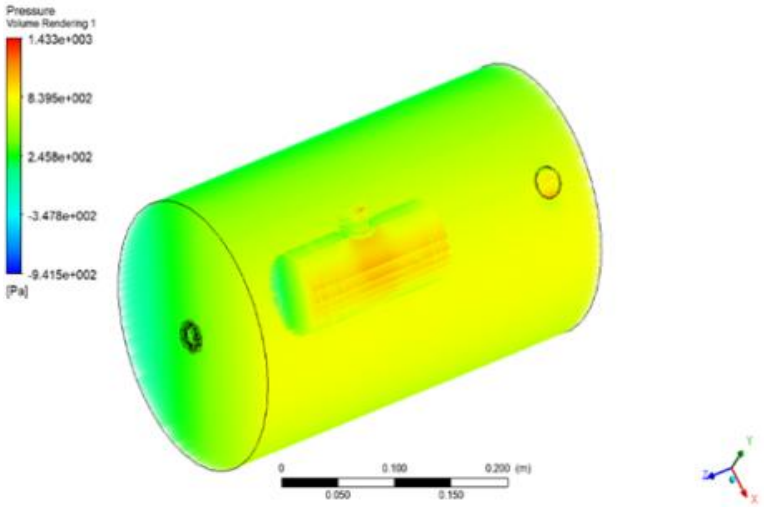

Figure 8- Volumetric pressure contour

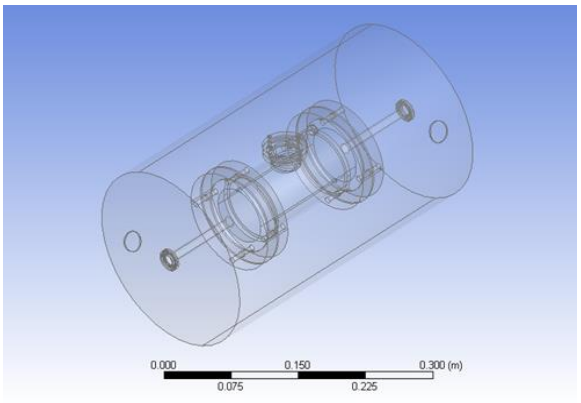

Figure 9- Designed geometry

According to the results obtained above, the rotation speed observed in the system was according to these contours. The homogeneity of the mixing rate in the system could be observed for creating a suitable environment for cultivation.

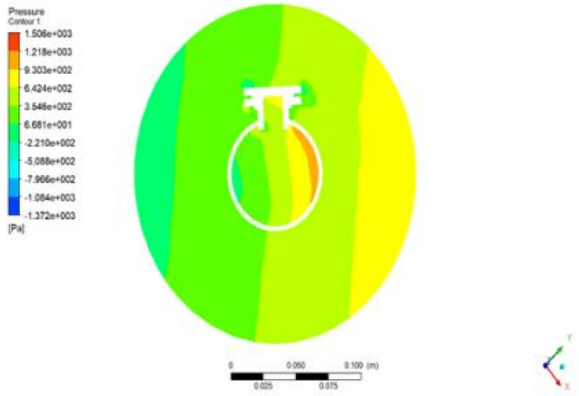

Figure 10- Pressure contour

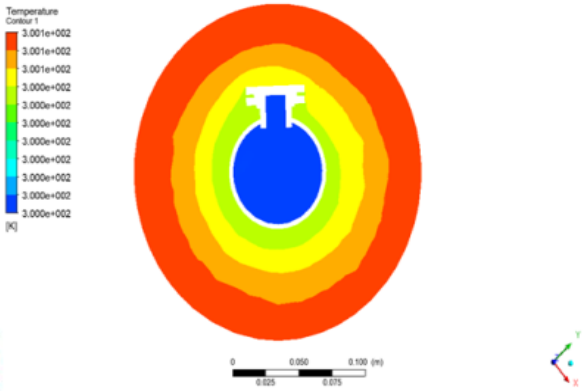

Figure 11- Temperature contour

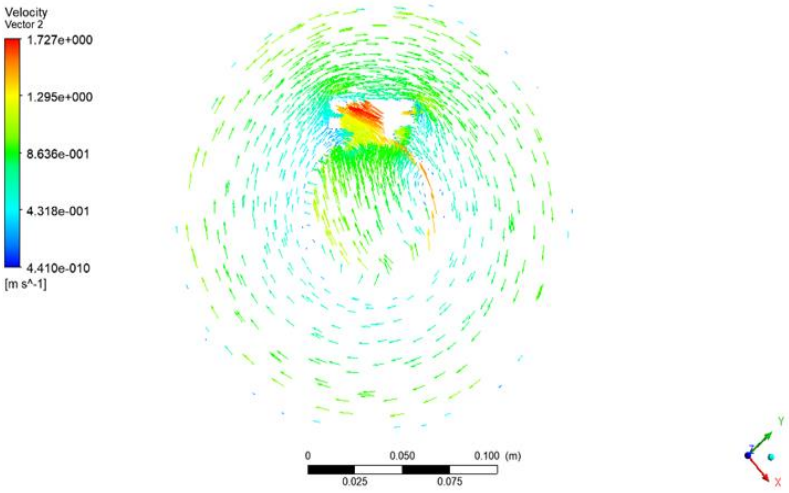

Figure 12- Speed vector

5. conclusion

Firstly, the model proposed by the article simulated. Then, with the help of optimal parameters for this model, a new design for the bioreactor system was done. Due to the existing geometric properties, this system had advantages such as uniform temperature distribution, uniform flow distribution, controlled temperature, and important parameters such as humidity.

This study showed that the parameters such as shear stress, hydrostatic pressure, scaffold temperature, and humidity were the most important factors in the cell culture. Simulations were performed for a bed with homogeneous porosity. In order to influence the temperature and humidity profile of solids in the bed, a heterogeneous porosity distribution was used. The initial porosity was randomly assigned to the cells based on the natural distribution with an average of 0.6464. This heterogeneous porosity distribution led to a variable permeability distribution and also led to a heterogeneous humidity distribution in the system. The predicted temperature profile for the distribution of heterogeneous porosity was significantly different from that predicted one for the homogeneous bed. Heterogeneity in each altitude not only could start heating sooner but also reach the thermal equilibrium. This could also occur at the relevant altitude in a homogeneous bed.

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