Numerical Simulation for Distribution and Mass Transfer of Nutrient Substance within Microbeads in Different Culture Systems

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Abstract. Bioreactor is a good device for biological and chemical reactions. Microbeads have good application value in many fields because of their good permeability. This research was aimed to simulate the mass transfer process of glucose via microbeads in different culture systems including T-flasks, rotating cell culture systems and novel rotary perfusion bioreactor with Fluent software. The initial conditions are as follows. The mass fraction of glucose inside and outside the ball was 0.0 and 4.5e⁻⁶. The quality source was 6.05e⁻⁸, and the time step was 1e⁻³. When the T-flask was at the 10th turn and the time was at 45s, the concentration of glucose inside and outside the sphere would basically reach equilibrium. For the glucose concentration it would reach equilibrium at the 10th turn after 30s. It was confirmed that the mass transfer effect was better in Rotary cell culture system than that in other systems.

Introduction

Bioreactors are a kind of equipment which are used for biological or biochemical reactions, and can also monitor and control important parameters in culture conditions such as biomechanics, pressure, pH, temperature, metabolism of nutrients and wastes and mass transfer as well as 3D cultivation [¹⁻³]. 3D environment which is maximum close to the natural extracellular matrix can be created relying on bioreactor, so that we can culture specific types of cells in vitro [⁴]. Calcium alginate micro beads, as a carrier of immobilized cell technology, are commonly used for cell cultivation, cell protection and drug sustained-release. It has many properties such as good permeability, no obvious toxicity, metabolite biocompatible, simple operation and easily breaking the wall, small diffusion resistance of nutrient substance, mild conditions for immobilization and so on, some cells which are fragile and very sensitive to fluid shear stress, must be embedded in micro beads to protect from damage [⁵⁻⁹]. The research on nutrient distribution of different types of micro beads in bioreactors can make us understand mass characteristics and principles of transfer in micro beads, and explore whether encapsulated micro beads will affect the absorption of nutrients of internal cells [¹⁰⁻¹¹]. Simultaneously, it can also provide reference data for the optimization of bioreactors with culture conditions.

This study used Fluent software to simulate the glucose mass transfer process of microbeads in T-flask, rotary cell culture system as well as novel rotary perfused bioreactor, respectively. The experiment focused on numerical analysis for different flow fields inside different bioreactors. It provides a certain reference value for the subsequent cultured optimization of bioreactor.
Methods

Fluent Software Mathematics Model

ANSYS Fluent software (ANSYS, Canonsburg, PA, USA) was used to calculate fluid flow distribution, and heat and mass transfer in a complex geometric system. It provides ideal grid adaptability and can solve complex fluid problems easily by an irregular grid, which is provided by complex geometry.

Setting Conditions of Multiphase Simulation Reactors

The Physical Model of Reactors. As shown in Figure 1, these are diagrams of T-flask, rotary cell culture system devices and novel rotary perfused bioreactor, respectively.

![Figure 1](image1)

Figure 1. (A) T-flask; (B) Rotary cell culture system; (C) Novel rotary perfused bioreactor.

Gridding (Spatial Discretization). T-flask: Compared to the size of T-flask, the radius of microbeads was so small that the beads became a point. Mass transfer on the surface of the beads had a significant impact only on the flow field near the beads. If the border is relatively small, the model can be simplified into a two-dimensional plane. Parameters of the simplified two-dimensional model of the microbeads and relative boundary conditions were shown in Table 1.

Rotary cell culture system (RCCS): This system can also be simplified into a two-dimensional plane as the reason with above mentioned T-flask model. Parameters of the simplified two-dimensional simulation model of the microbeads and relative boundary conditions were also shown in Table 1. Four microbeads were set into the culture chamber, and they were relatively static with the bioreactor when it was rotating, 3D model of this system was shown in Figure 2.

Novel rotary perfused bioreactor (NRPB): The number of microbeads showed an effects on the mass transfer efficiency of this novel bioreactor. And the 3D grid model of NRPB was shown in Figure 2, four or five microbeads were arranged in a row into the culture chamber. During the rotation, they were kept relatively static with NRPB.

| Table 1. Parameters of the simplified two-dimensional model of the microbeads and Boundary conditions. |
|---|---|---|---|---|
| | Border | Ball radius (mm) | Globe distance (mm) | Model state | Initial conditions |
| | | | | | Glucose mass fraction i/o sphere | Quality of the source term | Time step |
| T-flask | 30x30 mm² | 1.1 | 10 | Stationary | 0.0/4.5e^6 | 6.05e^6 | 1e^3 |
| RCCS | Radius is 30 mm | 1.1 | 10 | Disk rotational speed is 20 rpm | 0.0/4.5e^6 | 6.05e^6 | 1e^3 |
| NRPB | Radius is 30 mm | 1.1 | 10 | Disk rotational speed is 10/12 rpm | 0.0/4.5e^6 | 6.05e^6 | 1e^3 |

![Figure 2](image2)

Figure 2. (A-B) 3D model of Rotary cell culture system; (C-D) 3D grid model of novel rotary perfused bioreactor.
**Conditions Setting.** (1) Microbeads with the same diameter had the same internal pore structure. (2) The main concentration outside the microbeads was uniform. (3) Microbeads with the same diameter had the same internal diffusion coefficient. (4) Solute concentration distribution was only along the radial direction of microbeads.

**Results**

**T-flask**

The distribution of mass fraction of glucose on the surface of microbeads in T-flasks were shown in Figure 3&4, it was found that the glucose concentration inside beads gradually increased with time in the mass transfer progress of microbeads, while the concentration outside decreased slightly. When the T-flask was at the 10th turn after 45s, the concentration of glucose inside and outside microbeads would basically reach an equilibrium. There was a very significant concentration gradient distribution from each of four corners toward the center. The thickness of surface mass was maximum in the center of the microbeads, and the thickness inside beads was greater than that of the outside. It was proved that glucose molecules could be driven uniformly into the microbeads interior by the concentration gradient.

![Figure 3](image1.png)

**Figure 3.** Distribution of mass fraction of glucose in the surface of sphere. (A) 15s; (B) 33s; (C) 36s; (D) 39s; (E) 45s.

![Figure 4](image2.png)

**Figure 4.** Distribution of mass fraction of glucose in the surface of sphere. (A) 42s; (B) 45s.

**Rotary Cell Culture System**

The distribution of mass fraction of glucose on the surface of microbeads in RCCS was shown in Figure 5. It was found that the glucose concentration inside the microbeads increased gradually with time while the outside concentration showed a slightly decreasing trend. The closer to the microbeads, the lower the concentration would be and the lowest concentration was located in the central position of four microbeads and the decreasing trend changed relatively uniform. When the reactor was at the 10th turn after 30s, the glucose concentration inside and outside the sphere would reach an equilibrium. It was proved that mass transfer effect was better in RCCS than that in T-flasks.
Figure 5. Distribution of mass fraction of glucose in the surface of sphere. (A) 0.5s, 1/6 cycle; (B) 18s, 6 cycle; (C) 21s, 7 cycle; (D) 30s, 10 cycle.

**Novel Rotary Perfused Bioreactor**

The distribution of mass fraction of glucose outside and inside the microbeads in a NRPB was shown in Figure 6&7. As shown in Figure 6, the concentration of glucose inside the microbeads increased gradually with time while the concentration of glucose outside the microbeads decreased slightly. When the reactor was at the 5th turn after 30s, the concentration of glucose inside and outside the microbeads would reach an equilibrium. Figure 6A&B showed that glucose concentration outside the beads showed a decreasing trend, the partial concentration reached a minimum value in the central portion. It was proved that the mass effects was better in the NRPB than that in T-flasks. The concentration of glucose in the two microbeads on the outside was the lowest, and the two in the middle was slightly higher. However, the gap of the concentration was not great. Because of the relatively lower concentration in the central portion, the concentration gradient was larger. The effect of rotational speed was not obvious in the two simulation process. However, under conditions of 12 rpm, the glucose mass fraction of surface microbeads was higher. In another word, due to high rotation speed, the microsphere mass transfer efficiency was higher, and then the equilibrium was reached faster. Presumably because the rotation speed was large, fluid shear stress increased, so that the process of glucose molecules into microbeads was accelerated.

Figure 6. Distribution of mass fraction of glucose outside and inside the sphere. (A) 4 microspheres, 10rpm, 40s, 4 cycle; (B) 4 microspheres, 12rpm, 40s, 3.3 cycle; (C) 5 microspheres, 10rpm, 18s, 3 cycle; (D) 5 microspheres, 10rpm, 30s, 5 cycle.

As shown in Figure 7, the concentration of glucose inside the microbeads increased gradually with time while the concentration of glucose outside the microbeads decreased slightly. When the reactor was at the 6th turn after 30s, the concentration of glucose inside and outside the sphere would reach an equilibrium and had a trend to stabilize. The number of micro plastic beads rotating has impact on efficiency of mass transfer in micro plastic beads. Five micro plastic beads were set in the reactor chamber as a line. During the rotation, the five gel beads remained relatively stationary when rotated in the perfusion bioreactor. From the simulation results, it was shown that the distribution of glucose concentration changes regularly at the beginning, but it had a tendency to converge in a very short period of time. It was proved that the material could be uniformly distributed in rotary perfusion reactor, and the initial concentration difference could be neglected.
Discussion & Conclusions

The distribution of nutrients in dynamic bioreactor was more uniform, which avoided the influence of low nutrients in the local area owing to the cell absorption within the microbeads. As the concentration of glucose around the beads was reduced, the concentration gradient would not be weakened, which ensured a higher mass transfer rate. It was proved that the mass transfer rate of microbeads was faster in a dynamic environment compared to static culture environment.

The flow status in culture chamber of NR PB was a comparatively standard laminar flow, and the microbeads had almost the same shear stress at the same position, so concentration gradient was the only factor which influenced mass transfer, which could explain the phenomenon shown in Figure 6D. Figure 7 proved that material could be uniformly distributed in NR PB, and the initial concentration difference could be neglected. The variation trend of concentration distribution shown in Figure 7C was different from that of four microbeads, which perhaps resulted from the factor of exit distribution of chamber, perfused material fluid aligned exactly with the middle microbeads, and then the distribution of glucose concentration was inconsistent in five microbeads.

By analyzing the two process simulations (10 rpm and 12 rpm), it was deduced that the trends in the two simulation processes were accordant. Although the difference of rotating speed was not obvious, the glucose mass fraction of surface microbeads was higher under conditions of 12 rpm. In another word, high rotating speed made great mass transfer efficiency and was easy to reach an equilibrium. Presumably the rotating speed was large and fluid shear stress increased, so that the process of glucose molecules into microbeads was therefore accelerated.

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