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Numerical investigation of understandings
and optimization on hydrodynamic
characteristics of bioreactors for
iPS cell mass cultivation

Liya WANG

SEPTEMBER 2021

Numerical investigation of understandings
and optimization on hydrodynamic
characteristics of bioreactors for
iPS cell mass cultivation

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ABSTRACT

Induced pluripotent stem (iPS) cells have shown great potential in many applications owing to their special characteristics of unlimited self-renewal and differentiation ability. However, low yield of cells due to suboptimal culture environment and improper culture method has become a limiting factor for the development of the applications since most applications require massive iPS cells that would be difficult to achieve using traditional culture processes and bioreactors which can be applied for single cell suspension culture because of technical limitations. Therefore, the objective of this study is to investigate the dynamic characteristics of the bioreactor with different geometries and operating manners using numerical simulations. Moreover, the Bayesian optimization is employed to obtain an optimal control parameter in a new bioreactor.

Firstly, the cells need to be distributed uniformly in static culture to produce high-quality cells. The cell behavior in a rocking bioreactor under different shaking conditions is therefore numerically investigated. The shear stress acting on the cells and characteristics of cell aggregate dispersion are quantitatively evaluated. The simulation results show that the shear stress increases monotonically with the increment of shaking velocity and inclination angle. Meanwhile, the cells are dispersed efficiently in the whole tank with a large angle. As a consequence, considering these effects and factors, qualitative relation combined drag force, gravitational force and Reynolds number is concluded based on a specific analysis of the control parameters.

Secondly, because the cell production is dependent on the area of the rocking bioreactor in static culture, a tentative study on suspension culture in a cylindrical orbitally shaken bioreactor is conducted. A novel shaking technology that takes into consideration of the periodic alternate rotation is developed, which could improve the suspension ability of cells in the same range of shear damage compared with the classical shaking manner, meanwhile efficiently reducing cell agglomeration in the center part at the bottom wall. More importantly, a further investigation of fluid flow characteristics with the new shaking method is carried out for various operating conditions.

Finally, an orbitally shaken bioreactor equipped with a vaulted bump at the bottom for suspension culture with conventional shaking manner is developed due to the high energy consumption of the novel shaking method. The newly designed bioreactor not only greatly improves the oxygen mass transfer and suspension quality without the increment of shear stress, but also efficiently reduces the cell aggregation in the center region at the bottom wall. Furthermore, the Bayesian optimization for seeking an optimal working parameter owing to high-accuracy prediction is employed, which significantly reduces the computational cost. Simulation results indicate that the optimized bioreactor is much more preferable for iPS cell cultivation than the original bump bioreactor owing to its suitable oxygen concentration and suspension quality.

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CHAPTER 1

INTRODUCTION

1.1 Background

According to the report from the world health organization (WHO), the biggest killer of people life is heart disease, responsible for 16% of the world's total deaths in 2020 [1]. Patients recover from heart transplant is gradually available with the development of pharmacological therapy and surgical interventions, especially the emergence of regenerative medicine, which provide a new treatment strategy to the heart disease.

1.1.1 Regenerative medicine

Regenerative medicine is a field that involves the repair and replacement of human cells, tissues or organs damaged by age, disease or trauma to establish, restore and enhance normal function [2–4]. It is an area with great promise that targets at facilitating the availability of safe and effective treatments. The broad scope of regenerative medicine products includes cell therapies, therapeutic tissue engineering products, and human cells [5, 6]. The beneficial effect of regenerative medicine ultimately depends on the number of administered cells reaching the target tissue, their viability, and their promotion of tissue regeneration. It is inevitably admitted that there are still numerous limitations on the clinical utility with regard to the efficiency of generation rates and heterogeneity [7]. The present therapy of transplantation of intact organs and tissues to treat organ and tissue failures and loss suffers from limited donor supply and often severe immune complications, but these obstacles may potentially be avoided through the usage of regenerative medicine strategies [8].

In addition, the rapid advancement of regenerative medicine is the progression of human stem cells into clinical trials. The delivery of therapeutic cells that directly contribute to the structure and function of newly reprogrammed tissues or organs is a principle procedure of regenerative medicine [9]. The cells applied in the therapy are either autologous or allogeneic, and are typically

differentiated cells that still maintain sufficiently proliferative capacity. Stem cells such as induced pluripotent stem (iPS), embryonic stem (ES), neural stem (NS) and hematopoietic stem (HS) cells, therefore, have been investigated for understanding the properties. It is surprisingly found that iPS cells have the remarkable capability of unlimited self-renew and differentiation, which have shown great potential in a vast range of biomedical applications, including regenerative medicine, disease modelling, and drug testing [10, 11].

1.1.2 iPS cells

As some stem cell generation requires the destruction of human embryo's raising controversy and ethical concern, alternative sources of iPS cells without challenges of ethical and immunological issues have since been developed because they are generated from the patients' own somatic cells by the induction of defined transcription factors [12–14]. These iPS cells have certain special characteristics including unlimited self-renewal and differentiation ability. Therefore, in terms of simplicity of the technology and circumvention of ethical concerns, iPS cells are considered far more useful than other stem cells, and offer great potential for advances in basic biomedical research and generation of therapeutic cell types. Besides, the first clinical application of iPS cells has been successfully finished in 2014. A patient's skin-derived iPS cells were differentiated into retinal pigment epithelium, which was then transplanted into the eye of the same patient, and there was no evidence of immune rejection or adverse unexpected proliferation issues after 18 months [15]. This achievement has propelled the discipline of iPS cells to greater heights.

Despite of the above numerous advances and successful applications of iPS cells in biomedical technology, the cell culture manner have raised concerns. In recent years, the selection and optimization of cultivation method for iPS cells gradually catches researchers' attention. In fact, both iPS cells generation and proliferation require a series of artificial operations, which significantly increases the difficulties of cell cultivation. Moreover, the classical culture method is much labor- and cost-intensive, and it is highly susceptible to the technician's skill in the terms of cell quality and quantity. These demerits urge researchers to develop an automatic and effective method for

cell cultivation.

1.2 iPS cell culture methods

Various culture methods have been developed to achieve the survival of hepatocytes in regenerative medicine [16]. All of these methods aim to improve the mass transfer and viability from the surface of the culture medium into the inner volume. Currently, the employed conventional methods for cell cultivation can be divided into various types, as shown in Figure 1.1. The main objective of this study is to investigate and optimize the culture environment for suspension culture and preprocessing stage of static culture.

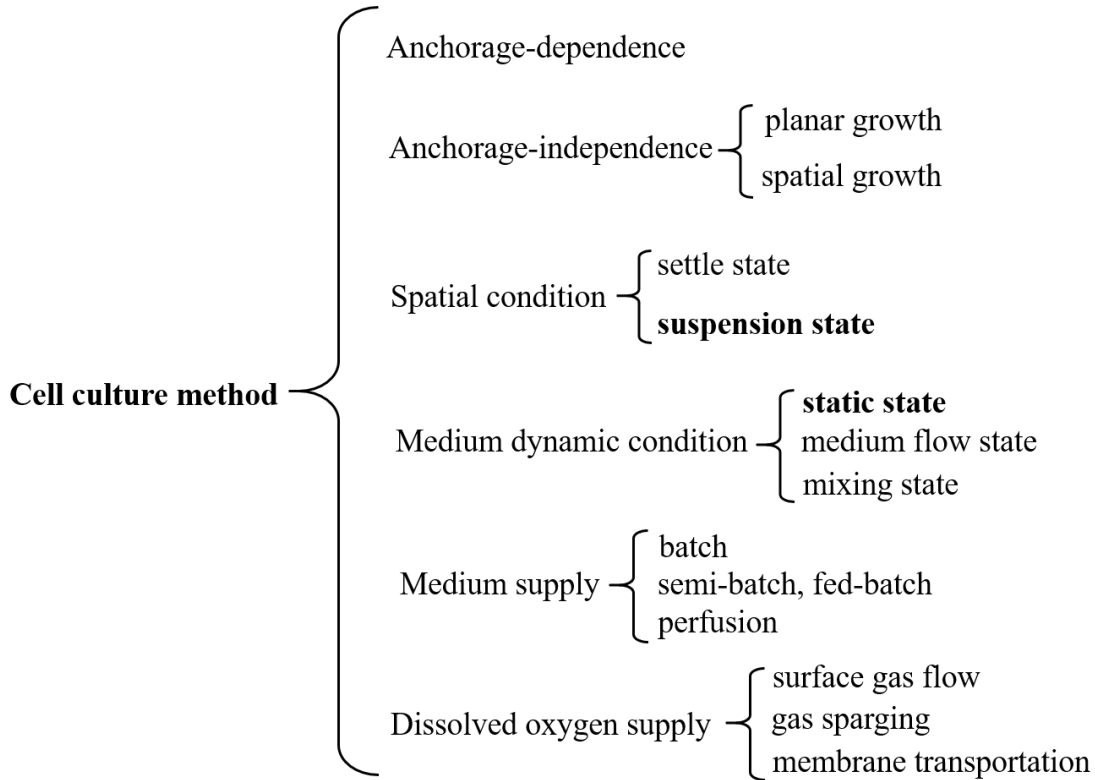


Figure 1.1 Sketch of cell culture methods.

1.2.1 Static culture method

Static culture of iPS cell aggregates is carried out by seeding single cell to produce the uniform aggregates. The specific process of the static culture method is shown in Figure 1.2. It is possible

to change the size of cell colonies in static culture [17], and this process is merely realized using suspension culture method. Additionally, the shear force induced by flowing culture liquid acting on cells are greatly inhibited. For these reasons, the static culture method is widely used in scientific research and clinical trials for cells large scale production. Unfortunately, the tremendous alterations of culture medium by manual operation certainly increase working load, and it may destroy the cell tissues.

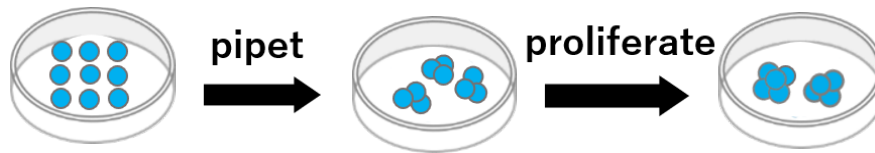


Figure 1.2 Sketch of the process for static culture method.

Before the operation of the static culture method, an important procedure is to distribute the cells uniformly in the bioreactor by various shaking behaviors. High-density distribution should be avoided in principle because it will increase the risks of cell death due to the insufficient oxygen supply [18]. The uniform cell distribution is highly expected to accelerate further cultivation, which is one of the objective in this study.

1.2.2 Suspension culture method

Suspension culture remains a popular manner, which operates mechanical culture conditions to maintain specialized features of cultured cells. The principle of the suspension culture method is to put cell colonies inside a container and keep the cells in a suspension state either by agitation or shaking the vessel [19]. In the agitation-based approach, the container can be gently stirred or shaken to make a sufficient number of cell suspended in the culture medium with the least shear damage, as illustrated in Figure 1.3. The behavior of continuous agitation will prevent cells from adhering to the bottom wall of the vessel [20]. Also, the strong stirred behavior should be bypassed since it could generate intensively local shear stress.

Suspension culture is regarded as a mature and old cell culture method, whereas the demerits could not be negligible. The limitation of suspension culture method is the lower suspension

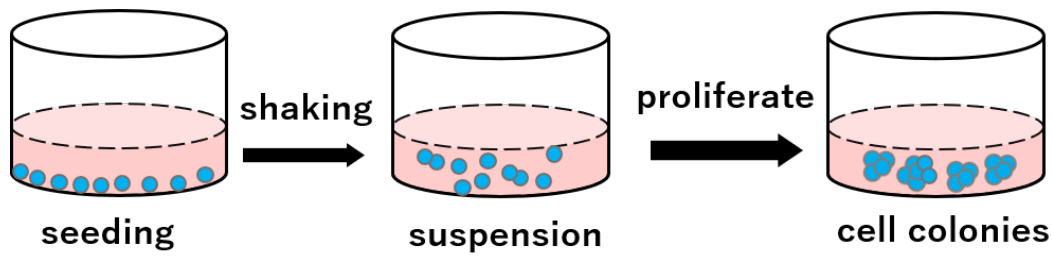


Figure 1.3 Sketch of the process for suspension culture method.

level of cells. Consequently, the cells tend to sink and accumulate at the bottom wall of the vessel [21]. Despite the present disadvantage on the ability to suspend cells, the great demand for suspension culture increases for both academic and industrial applications. The advantage of this culture method is the provision of large-scale production of many bioproducts in clinical trials, non-intensive and relatively homogeneous high cell volume density microenvironment which can be easily monitored.

1.3 Bioreactors

Cells are typically cultivated in flasks or dishes in incubators, which provide the cells with a suitable environment for growth such as temperature, humidity and pH. However, since oxygen and nutrition diffusion is restricted, these incubators are not preferable for culturing iPS cells. Hence, a bioreactor is specially defined as a device in which biological processes (cell expansion, differentiation or tissue formation on biomaterial scaffolds) occur in a strictly controlled environment, including the exchange of oxygen, nutrients and metabolites [22]. Nowadays, various types of shaking bioreactors of scalable production of cells have emerged for scientific research and clinical trials. These bioreactors aim to provide a suitable environment for cell proliferation such as efficient nutrition diffusion, an increase of cell suspension level, and relatively lower shear stress. The widely used shaking bioreactors for culturing iPS cells include reciprocal shaking bioreactor, rotating wall bioreactor, rocking bioreactor and orbitally shaken bioreactor. Therein, the investigation and optimization of rocking bioreactor and orbitally shaken bioreactor are the main objective in this study.

1.3.1 Reciprocal shaking bioreactor

Reciprocal shaking bioreactor is shaken with reciprocal motion such as figure-“eight” motion and “up-down” motion [23, 24]. Recently, a novel reciprocal shaking bioreactor was firstly developed by Sekimoto et al. [25], which was shaken periodically in a horizontal plane with two shaking methods, namely, single reciprocal shaking (SRS) and multi-reciprocal shaking (MRS), as shown in Figure 1.4. The shaking trajectory is modelled as a cosine curve. The uniformity of cell dispersion is highly independent on the shaking velocity and shaking distance. Large shaking velocity could efficiently distribute the cells, and the strong shear stress is accordingly generated. Thus, further investigation and optimization on this type of bioreactor is necessary.

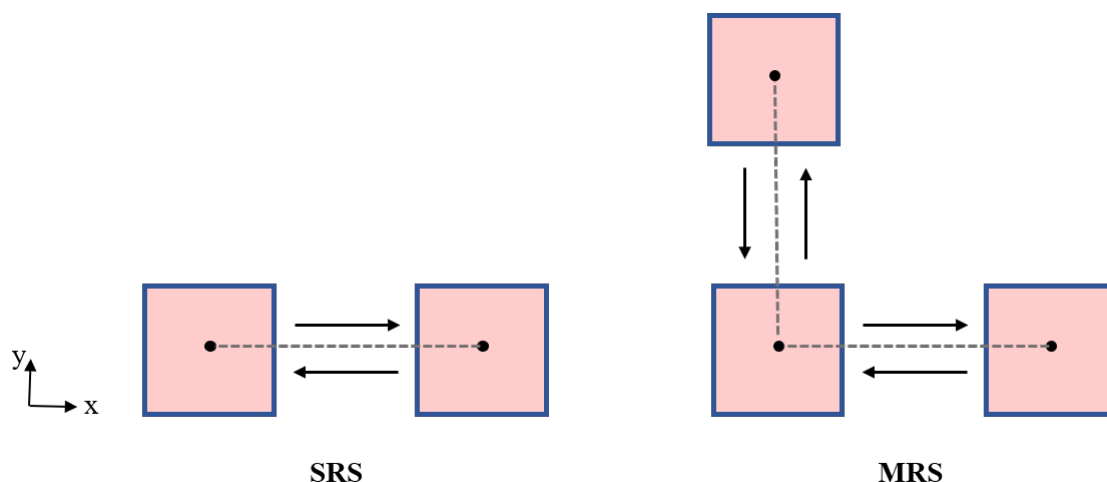


Figure 1.4 Sketch of reciprocal shaking bioreactor.

1.3.2 Rotating wall bioreactor

Another bioreactor that allows agglomeration-free formation is the rotating cell culture system (RCCS), which was originally developed by the US national aeronautics and space administration (NASA) to simulate cell cultivation under microgravity environment since the beginning of the last century [26]. The configuration is shown in Figure 1.5. Rotating wall bioreactor has been successfully utilized for culturing and differentiating various stem cells, considering the fact that it provides homogeneous culture conditions, such as a relatively low fluid shear stress and oxy-

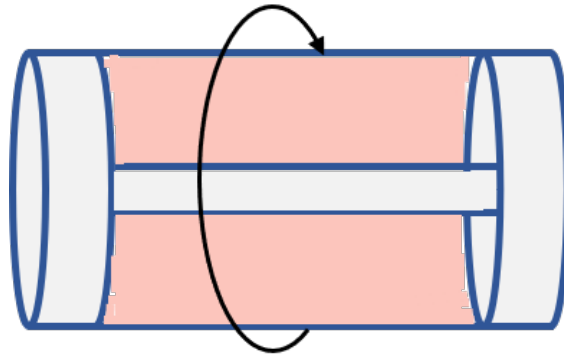


Figure 1.5 Sketch of rotating wall bioreactor.

genation by diffusion [27]. In such a bioreactor, the cells' layer could be alternately submerged in media or exposed to air, required for differentiation and growth of the cells, and the gas and medium could be continuously exchanged [28].

The RCCS with either a high aspect ratio vessel or a slow turning lateral vessel (STLV) is frequently used in practical application and experimental research. The STLV manner produces cell colonies in smaller sizes and increases cell yield, while aggregation of the cell is observed in the high aspect ratio vessel system, leading to significant cell death [29]. In general, a rotating wall bioreactor is difficult to scale up and may not be suitable to produce large quantities of cells needed for clinical applications [30].

1.3.3 Rocking bioreactor

Singh [31] firstly introduced a disposable wave-induced bioreactor, called rocking bioreactor, consisting of a vessel on a rocking tray mixing the cell culture with a wave-like movement, as shown in Figure 1.6. It is partially filled with cells and placed on a rocking plate. Aeration is performed via the head-space of the vessel. The rocking motion induces undulation ensuring mixing, an enlarged and constantly renewed surface to increase oxygen transfer. The rocking bioreactor developed to provide excellent mixing and mass transfer with the reduced shear force for the culture of cells highly sensitive to hydrodynamic condition, which depends on gravity with multidimensional oscillation has been widely applied in industrial settings owing to its attractive

design and advantages [32].

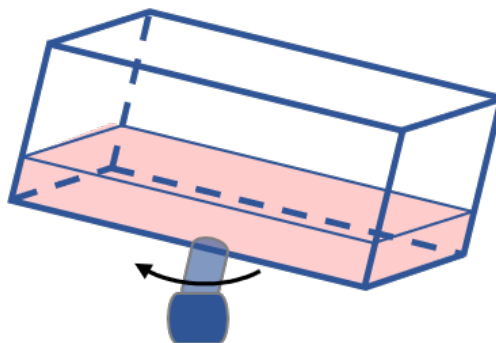


Figure 1.6 Sketch of rocking bioreactor.

Generally, the rocking bioreactor can be shaken flexibly with a gentle motion from one side to another, or with a stronger motion that ultimately leads to a breaking wave on the surface of the culture liquid [33]. In addition, the design of this bioreactor is simpler than the stirred-tank bioreactor due to the absence of an impeller. It has been observed in practice that the usage of this bioreactor results in marked differences for the corresponding applications [34]. However, the drawback of the bioreactor is the limited volume only with 2 – 50 L. With the development of technology, researchers have successfully overcome this disadvantage. Presently, this system is available up to working volumes of 500 L [35].

1.3.4 Orbitally shaken bioreactor

Orbitally shaken bioreactor (OSR) has become the most frequently used bioreactor for the cultivation of mammalian and stem cells owing to its manageable and easy-to-operate characteristics. The conventional shaking bioreactor is a cylindrical tank and rotates along a fixed orbit at an adjustable angular rate, as shown in Figure 1.7. In addition, this type of bioreactor could improve mass transfer and ensure efficient oxygenation of the medium while keeping a relatively low fluid shear force [36, 37]. For this reason, orbitally shaken bioreactor is increasingly utilized as an efficient system for a wide range of different tasks.

Nonetheless, the common orbital shaking bioreactor hardly meets the anticipated demands since it exhibits a lower suspension ability compared with the stirred tank bioreactor [38]. It is

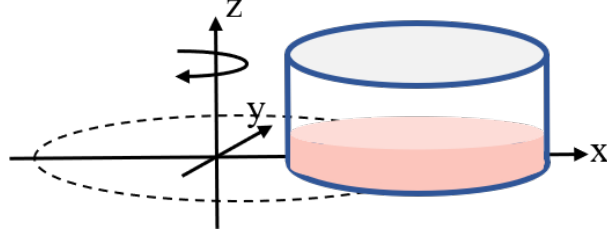


Figure 1.7 Sketch of orbitally shaken bioreactor.

well-known that the selection of a suitable culture system is especially important for biomedical development. Thus, it is advisable that a novel culturing technique for optimizing the process of orbitally shaken bioreactor is urgently developed.

1.4 Related researches and status

In order to meet the practical demand, many studies either experimental researches or numerical simulations on the process control and optimization of cultivation environment have been conducted. All the optimizations aim to provide suitable conditions and understand the impact of shear stress for cell proliferation by investigating the hydrodynamic characterization.

1.4.1 Experimental study on cell cultivation

To date, efforts have been made to optimize the flow condition of a spinner flask or a stirred-tank bioreactor (STR) in experimental laboratories. Several groups have attempted to improve expansion iPS cells using different impeller agitation rates [39–41]. More importantly, Gupta et al. [42] focused on the effect of agitation speed in a spinner flask through numerous experimental trials, and proposed an optimal agitation speed ($= 25$ rpm) for the expansion of iPS cells while maintaining their essential characteristics. It is well-known that there are other factors needed to be investigated in such kind of bioreactor. Meng et al. [43] optimized the critical operating steps and factors that affect iPS cells growth and production in stirred-suspension culture, including inoculation conditions, seeding density, and cell aggregate size. They demonstrated that the optimized protocol could generate 10^9 viable iPS cells keeping a pluripotent state in a 1000-mL

stirred-tank bioreactor which would be sufficient for clinical applications.

Based on the study of a stirred-tank bioreactor, an improvement of the structure has emerged. Serra et al. [44] combined microcarrier technology and stirred-tank bioreactor in perfusion mode. This bioreactor operating in perfusion resulted in a significant improvement in cell yields. In their study, they demonstrated the importance of controlling oxygen concentration to improve cell growth. This concentration allowed for higher energetic cell metabolism drastically increased growth rate and enhanced culture performance under the perfused condition. In addition, Abecasis et al. [45] also emphasized the importance of controlling the dissolved oxygen concentration perfusion to increase cell growth in a stirred-tank bioreactor. A scalable protocol for continuous expansion of iPS cell aggregates in the bioreactor was implemented using mechanical dissociation for aggregation disruption. Furthermore, a rolled scaffold that could provide a large-scale and reliable platform for shear-sensitive cell culture in biopharmaceutical industries was exploited by YekrangSafakar et al. [46], which was made from PET film with repeating spacers, and the film was rolled to form a cylindrical shape with numerous channels. Unlike stirred-tank bioreactors, oxygen is delivered through convection in a laminar flow with higher efficiency, which drastically reduced the hydrodynamic shear damage to cells.

Recently, orbital shaking technology has gradually caught researchers' attention. Several groups have successfully employed this technique for scalable cell culture. Stettler et al. [47] developed disposable shaking bioreactors for highly efficient cell production in different working-volume container ranging from 50 mL to 50 L. It was showed that the orbital shaking container was preferable for cell cultivation due to its benefits, in terms of extended cell culture viability and increased product yield for cell cultivation conditions. Thereafter, the performance of two kinds of height conical bottom geometries associated shear stress levels in a cylindrical orbital shaking bioreactor was investigated by Rodriguez et al. [48]. It indicated that the conical bottom geometry with the greatest height was much easier to generate strongly intensive vortical structures spanning over the entire bioreactor at lower shaking speeds in comparison to a flat bottom geometry, thus resulting in lower shear stresses. Furthermore, the newly designed bioreactor was more efficient for cell sus-

pension owing to higher lifting force, avoiding the cell sedimentation at the center of the bottom wall. Further, Horiguchi et al. [49] developed a novel culture bag with orbital shaking manner, which was suitable for high reproducibility of cells in massive production with a homogeneous size by providing sufficient oxygen to the aggregated cells. The novelty of this bag was attributed to its hollow center configuration. The cells, therefore, were prevented from accumulating at the bottom center region.

Another alternative is the investigation and optimization of the rocking bioreactor. The experimental results indicated that the rocking bioreactor may be a replacement to a stirred-tank bioreactor especially for an attachment-dependent cell culture [50]. Weber et al. [51] thought that the optimization of the operational conditions was essential for the growth of cells to obtain high cell densities in a rocking bioreactor. Thus, they established a favorable environment for cell cultivation due to its simplicity of operation and cost based on their experimental studies. It is well-known that oxygen transfer was the main limiting factor that affects cell viability. Therefore, a novel perfusion-rocking bioreactor was developed and characterized for highly efficient production [52]. They demonstrated that the significance of control strategy on perfusion rate and defined an optimal medium to achieve high cell density and production. Overall, the newly designed system greatly increased bioprocess flexibility and reduced costs due to the usage of disposable systems rather than stainless steel equipment.

1.4.2 Numerical investigation on cell cultivation

To study the fluid dynamics of a bioreactor during the process of cell cultivation, only depending on the experimental study is quite time-consuming and costly because of the lab intensively repetitive work and the complex cell cultivation process, and it is constrained by the availability of sophisticated measuring equipment and skillful technicians. Besides, it cannot meet the current research demand on the understanding of the hydrodynamic characterizations. Consequently, computational fluid dynamics (CFD) become more attractive applicable for providing an inexpensive, feasible, and effective solution towards obtaining reliably accurate predictions, and also

a much-needed insight into practical applications and scientific research. The prominent advantages, particularly for design optimization and visualization without expensive experiments, have gained researchers' attention in biochemical fields [53–55]. Also, flow characterization and hydrodynamic force are often neglected in the past for optimizing cell culture protocols as well as the bioreactor designs. As these properties are beyond the experts' expertise and ability, most studies correlate the cell production or oxygen supply, lacking understanding of the details of the flow behavior and mechanical interaction in the bioreactor. Hence, further investigations are carried out using numerical simulations to enable visualization of flow structure and understanding of the dynamics.

As already mentioned above, perfusion bioreactor plays an essential role in biological applications. Israelowitz et al. [56] introduced irises to overcome limitations in a conventional perfusion bioreactor by regulating the pressure and flow patterns. The new structure bioreactor would allow for improving the efficiency of cell proliferation and increasing cell density in a controlled flow environment. Guyot et al. [57] gave an insight into the dynamic microenvironment to cells exposed in a perfusion bioreactor. They found that the proposed strategy could control the delivered shear stress magnitudes to cells by adapting perfusion rate, alleviating costly and time-consuming operation during cell growth. Overall, control the flow rate in the perfusion bioreactor is an important operation process.

The stirred-tank bioreactor has been widely utilized as a device to proliferate cell to massive numbers. As a result, investigating and optimizing the bioreactor has also been a major research interest for the past few years. Borys et al. [58] reported the study of controlling the cell aggregate size for large-scale production in a stirred-tank bioreactor using different scale-up parameters such as volume average energy dissipation, impeller tip speed, power input, and maximum shear rate. It was suggested that keeping the volume average energy dissipation rate or maximum shear stress constant, is the best method to maintain cell aggregate size through scale-up. Zhu et al. [59] also evaluated the effect of baffle by analyzing the flow field in a baffled shaking bioreactor. It was proven that the baffle geometry could enhance the level of mass transfer more effectively compared

to the unbaffled one, but mass transfer capability was likely independent of the baffle installation height. Verma et al. [60] quantitatively assessed impact of impellers on the hydrodynamic shear stress with different operational impeller speed in a stirred-tank bioreactor. Therefore, the range of shear stress was successfully obtained. The optimum rotational speed was also achieved for the cultivation of shear sensitive cells by characterizing the performance of the bioreactor. More recently, Lu et al. [61] studied the effect of three kinds of baffle structures bioreactor: vertical baffle, inclined baffle and horizontal baffle on the flow field. It was found that the inclined baffle has the best performance especially for volumetric mass transfer coefficient, meanwhile, the shear stress increased significantly for the vertical baffle structure. It was not recommended to install the vertical baffle in such bioreactor.

Because of the inherent disadvantages occurring in the stirred-tank bioreactor, the researchers' interests in a disposable bioreactor – rocking bioreactor has tremendously increased. Currently, the available sizes of the rocking bioreactor have a wide range. It has been stated that the selection of these reactors leads to remarkably different results in the corresponding applications [34]. Therefore, Öncül et al. characterized the flow conditions such as liquid surface height, liquid velocity, and shear stress, in two sizes of rocking bioreactors using numerical simulations. The flow state was observed to be laminar with repeatedly gentle motion. It was concluded that the shear stress in the bioreactor was below the threshold value leading to cell death. Svay et al. [62] also investigated the details of the flow field in a rocking bioreactor with various rocking angles and rocking speeds. It was found that the volumetric oxygen transfer coefficient was proportional to power consumption by analyzing hydrodynamic statistics. This correlation predicted could be used to create an engineering framework for the scale-up of wave bag bioreactors at different scales. Analogous numerical studies were also carried out by Zhan et al. aiming at characterizing the flow conditions. They pointed out that in all the range of operational settings, a resonance phenomenon where shaking speed ($= 15$ rpm), had a predominant effect on the fluid motion, namely, it generated the highest fluid velocity, oxygen mass transfer rate and shear stress [35]. Thus, it was recommended that seek an approximate value of shaking frequency where the shear stress caused cell death with

an acceptable range.

In order to realize large-scale production for practical applications, more and more researchers have converted their attention to the improvement and optimization of orbitally shaken bioreactor. Hang et al. [63] developed an novel shaking bioreactor with inverted frustoconical structure (IFSB). The simulation results showed that the new bioreactor efficiently increased gas-liquid interface area, and decreased shear stress damage to cells compared with the flat-bottom bioreactor under the same operational conditions. Such condition was favorable for the cell growth in a high cell density. Thereafter, Ding et al. [64] evaluated the fluid field performance of different size IFSBs. It was found that the shear stress and specific mass transfer area gradually decreased as the size of the bioreactor increased. Instead, there was no significant difference in terms of the liquid surface shape, energy dissipation rate, and mass transfer coefficient. Zhu et al. [65] simulated the fluid characteristics of tubes with a conical bottom in an orbitally shaken manner with different filling volumes and shaking speeds. The simulation results showed that increasing the shaking speed was an effective way to enhance the oxygen supply which was sufficient for supporting the high-density culture of cells. In addition, it was confirmed that the average velocity in z -direction was a crucial parameter to maintain cells in a suspension state at any filling volume. Similarly, the fluid characteristics of a cylindrical bioreactor with wide conical bottom under different shaking speed and filling volumes were also investigated by [66]. They demonstrated that shaking speed has no significant effect on specific gas-liquid interface area but has a positive effect on mass transfer coefficient resulting from the intensively turbulent dissipation rate. Moreover, the average shear stress increased significantly with the increment of shaking speed, while it decreased with liquid filling volume. Furthermore, this group considered other geometrical changes at the bottom wall in the shaking bioreactor, including conical, ellipsoid, flat, and sphere bottom. In their study, it was interestingly found that a cone-shaped bottom structure is superior to others, because of its beneficial characteristics such as lower shear damage, high-efficiency oxygen transportation, and easy manufacture [67].

As demonstrated above, shear stress is one of the important parameter of cell growth. Stronger shear stress could result in an undesired effect on cell quality. Table 1.1 summarizes shear stress for a variety of bioreactors and operating conditions found in the literature. It is evident that the shear stress is always larger in a stirred-tank bioreactor than those in a rocking bioreactor and orbitally shaken bioreactor. Therefore, considering this merit, these two types of bioreactors are regarded as the main objective in the present numerical simulation on cell cultivation.

Table 1.1 Shear stress values available in the literature for different bioreactor.

Type	Volume [L]	Velocity [rpm]	Orbital diameter	Shear stress [Pa]	Reference
Wave	10	15, 22, 30	4–7 degree	0.005–0.06	Zhan et al.,2019
	2 or 20	15	7 degree	0.01 (max)	Öncül et al., 2009
STR	0.05	20–45	-	0.0984 (max)	Ismadi et al.,2014
	2.2	75-150	-	1.97-3.96	Verma et al., 2019
	10	80	0.05 m	0.2 (max)	Zhu et al., 2019
	2.5	60	0.08 m	0.0178 (max)	Lu et al., 2021
OSR	1.5-2.5	45,55	0.03 m	0.0089 (max)	Lu et al., 2020
	0.3	150-200	-	0.075-0.19	Tissot et al., 2011

1.4.3 Machine learning technology

The growth of data is rapid across most scientific disciplines in recent years, and gaining valuable information has become a new mode of scientific investigation. In response to the increasing volumes of data, advanced technologies in reduced computational consumption, and sophisticated algorithms, machine learning technology appears to extract information from these data [70]. Machine learning techniques encompass a broad range of algorithms and modeling tools dealing with evaluation and prediction based on the existing data. Its superiority for high accuracy instead of the implementation of costly and complex numerical calculation has increasingly caught researchers' attention in many fields [71–73].

To make machine learning accessible and flexible in industrial applications, various machine learning algorithms are considered as a candidate to deal with practical issues. Therein, a state-of-the-art algorithm – Bayesian optimization has shown great promise by assuming unknown

functions and continuous tuning [74], which has been also highly applicable in biotechnology and bioprocess to address the computational demand issues. Martínez et al. [75] employed the Bayesian optimization to optimize bioreactor productivity by proposing a probabilistic tendency models. Thereafter, this theory was successfully verified by Luna and Martínez [76]. In addition, the Bayesian optimization was also applied to seek the medium refreshment regime that would maximize cell growth in a perfusion bioreactor [77]. Hernández Rodríguez et al. [78] also performed Bayesian optimization to describe the profiles of viable and total cells with a high-accuracy prediction.

Obviously, Bayesian optimization has become an advanced technology, which is also a primary methodology employed in the present study. The detailed procedure for implementing Bayesian optimization can be found in Chapter 5.

1.5 Thesis outline

This thesis aims to give an insight into the hydrodynamic characteristics during the cell culture process. The frequently applied bioreactors including rocking bioreactor and orbitally shaken bioreactor have been investigated and optimized using numerical simulations. The chapter outlines of the present thesis are stated as follows.

In Chapter 2, the employed numerical methods including governing equations and numerical scheme for calculating the fluid flow and cell movement are introduced. Some reasonable assumptions based on the physical property of cell and liquid are made in order to simplify the computational model.

In Chapter 3, a series of numerical simulations are performed to investigate the situation of cell dispersion in the rocking bioreactor under various shaking velocities and shaking angles. The cell distribution can be characterized accurately, and the shear stress acting on cells is evaluated quantitatively. By analyzing the obtained data, an optimal condition for cell cultivation is proposed. The numerical model and computer-aided design presented herein provide a better understanding of rocking bioreactor .

In Chapter 4, to explore a suitable shaking manner in an orbitally shaken bioreactor, a novel shaking method with periodic alternate rotation (PAR) is proposed, based on the conventional method – one direction rotation (ODR). A systematic comparison between two shaking methods is conducted using numerical simulations. The newly developed shaking manner has apparent advantages especially in suspending cells. A further investigation of the PAR method is therefore carried out to capture the optimum culture condition concerning cells quality and energy consumption. The simulation results give new insight into bioreactor design and would be beneficial to the shaking techniques for cell cultivation in suspension culture.

In Chapter 5, numerical simulations are conducted to evaluate the performance of a newly developed orbitally shaken bioreactor, which is equipped with a vaulted “bump” at the bottom wall. The presence of a bump is aimed at reducing the cell aggregation in the center part, meanwhile improving the oxygen transport and cell suspension ratio in the culture medium without increasing shear damage. In addition, to enable a suitable cell culture environment for practical applications, the Bayesian optimization algorithm is employed to capture an optimal operating condition of the new bioreactor. The methodology used in this study is a feasible and reliable tool for performance prediction and process optimization in a bioreactor.

In Chapter 6, the results obtained in this thesis are summarized and concluded, and perspectives for future works are described as an extension of this work.

CHAPTER 2

NUMERICAL METHODOLOGIES

In this chapter, the governing equations for calculating the fluid flow and cell movement and relevant assumptions are introduced. All the governing equations associated with the present numerical simulation were solved using an open source software, OpenFOAM.

2.1 Assumptions related to liquid and cell

The cell aggregates in a culture medium were modeled as rigid spherical particles. Some reasonable assumptions based on the physical property of cell and liquid are made in order to simplify the computational model. The following assumptions are summarized in the simulation model:

- The culture liquid is Newtonian and incompressible.
- The number of particles (cells) is unchanged, and is much smaller than that in an practical process.
- The collision between particles is neglected since the probability of collision between the particles is quite small due to the low concentration of cells in culture medium.
- The temperature and physical properties of the system remain constant and homogeneous during the process.
- Biological, biochemical, or chemical reactions do not occur during the simulation.

2.2 Governing equations for rocking bioreactor

2.2.1 Liquid phase

The governing equations of the liquid phase, i.e., continuity and momentum balance, are expressed in the following forms:

$$\nabla \cdot \mathbf{v}_f = 0 \quad (2.1)$$

$$\frac{\partial(\rho_f \mathbf{v}_f)}{\partial t} + \nabla \cdot (\rho_f \mathbf{v}_f \mathbf{v}_f) = -\nabla p + \nabla \cdot (\mu_f \nabla \mathbf{v}_f) + \rho_f \mathbf{g} + \sigma k \mathbf{n} \delta \quad (2.2)$$

where $\mathbf{g}$ denotes the gravitational acceleration, which is defined in eq. (2.3). The last term in the momentum equation represents a surface tension referred to as the continuum surface force (CSF) model [79]. The surface tension showed in the equation (σ) is 0.0715 kg/s².

$$\mathbf{g} = \mathbf{g} \begin{pmatrix} \sin \theta \sin \omega t \\ 0 \\ 1 - (1 - \cos \theta)(1 - \cos \omega t) \end{pmatrix} \quad (2.3)$$

2.2.2 Solid phase

The discrete element method (DEM) is used to calculate the movement of the particle, which is governed by Newton's second law of motion, as expressed in the following form:

$$m_p \frac{\partial \mathbf{v}_p}{\partial t} = \mathbf{F}_D + \mathbf{F}_G - m_p \frac{\partial \mathbf{U}}{\partial t} \quad (2.4)$$

where $m_p = \pi \rho_p d_p^3 / 6$ is the mass of the particle.

$$\mathbf{F}_D = C_D A_p \frac{\rho_f |\mathbf{v}_f - \mathbf{v}_p|^2}{2} \frac{\mathbf{v}_f - \mathbf{v}_p}{|\mathbf{v}_f - \mathbf{v}_p|} \quad (2.5)$$

is the drag force from the liquid phase, $A_p = \pi d_p^2 / 4$ is the projected area of a particle, and

$$\mathbf{F}_G = m_p \mathbf{g} \left(1 - \frac{\rho_f}{\rho_p} \right) \quad (2.6)$$

is the net gravitational force acting on a particle considering the buoyancy force that results from the difference in density between the liquid and the particles. The empirical drag coefficient is expressed as

$$C_D = \begin{cases} \frac{24}{Re_p} \left(1 + \frac{1}{6} Re_p^{2/3} \right) & Re_p \leq 1000 \\ 0.424 & Re_p \geq 1000 \end{cases} \quad (2.7)$$

where $Re_p = |\mathbf{v}_f - \mathbf{v}_p| d_p / \nu$ is the Reynolds number of the local particle based on the relative velocity.

2.3 Governing equations for orbitally shaken bioreactor

2.3.1 Liquid phase

The governing equations for the liquid phase (air and water) are continuity and Naiver Stokes equations. By choosing the reference velocity as $U_{\text{ref}} = R\omega$ and the reference length as the diameter of the cylindrical tank $L_{\text{ref}} = d$, the nondimensional equations for $\mathbf{v}^* = \mathbf{v}_f/U_{\text{ref}}$ are obtained as follows.

$$\nabla^* \cdot \mathbf{v}^* = 0 \quad (2.8)$$

$$\begin{aligned} \frac{\partial \mathbf{v}^*}{\partial t^*} + \nabla^* \cdot \mathbf{v}^* \mathbf{v}^* = & -\nabla^* p^* + \frac{1}{Re_\omega} \nabla^{*2} \mathbf{v}^* \\ & + \frac{2}{Fr^2 Dr} \mathbf{e}_{z^*} - \frac{2\mathbf{a}^*}{Dr} + \frac{1}{We} \delta \kappa^* \mathbf{n}^* \end{aligned} \quad (2.9)$$

where $\mathbf{a}^*$ is the nondimensional acceleration caused by the centrifugal force:

$$\mathbf{a}^* = \begin{cases} -\cos(\omega t) \\ \sin(\omega t) \\ 0 \end{cases} \quad (\text{ODR}) \quad (2.10)$$

and for the PAR method, the tank alternates the rotation direction every period $T = 2\pi/\omega$.

$$\mathbf{a}^* = \begin{cases} [(-\pi^2 + \theta^2) \cos \theta + \theta \sin \theta] / 4 \\ [(-\pi^2 + \theta^2) \sin \theta - \theta \cos \theta] / 4 \\ 0 \end{cases} \quad (\text{PAR}) \quad (2.11)$$

where $\theta = \pi \cos(\frac{\omega t}{2})$, $|\mathbf{a}^*| = 1$ for the ODR method, and $|\mathbf{a}^*| = (1/4)\sqrt{(-\pi^2 + \theta^2)^2 + \theta^2}$ for the PAR method.

The nondimensional parameters in the equation (2.9) are Reynolds number (Re_ω), Froude number (Fr), Weber number (We) and eccentricity (Dr), which are expressed as:

$$\begin{aligned} Re_\omega &= \frac{R\omega d}{\nu} \\ Fr &= \frac{R\omega}{\sqrt{R|g|}} \\ We &= \frac{\rho_f R^2 \omega^2 d}{\sigma} \end{aligned}$$

$$Dr = \frac{2R}{d}$$

The Re_ω is orbitally shaken Reynolds number for liquid, and the Froude number (Fr) represents the ratio of the inertial force and gravitational force. The eccentricity (Dr) is the ratio of the diameter of trajectory and the cylindrical tank, and the Weber number (We) is used to measure the relative importance of the fluid inertia compared to its surface tension. In order to compare the ODR and PAR, the same value of the modified Froude number, Fr^* ($=A_m \times Fr$) is set for both methods. Fr^* means the external force applied to the system, which is defined as the ratio of the 4th and 3rd terms on the right side of equation (2.9). Therein, $A_m (= \sqrt{(1/T) \int_0^T |\mathbf{a}^*| dt})$ is the time-averaged amplitude of the centrifugal force. For the ODR method, $A_m = 1$, and for the PAR method, $A_m = 1.216$. Fr^* is an important control parameter in the present study for the numerical exploration and optimization.

2.3.2 Solid phase

The discrete element method (DEM) was applied to capture the behavior of the cells in the culture liquid [80, 81]. The motion of each particle is governed by Newton's second law:

$$m_p \frac{\partial \mathbf{v}_p}{\partial t} = \mathbf{F}_D + \mathbf{F}_G + \mathbf{F}_L + \mathbf{F}_{vm} - m_p \frac{\partial U}{\partial t} \quad (2.12)$$

The definition of drag force ($\mathbf{F}_D$) and gravitational force ($\mathbf{F}_G$) was given in the previous chapter. The expression of lift force ($\mathbf{F}_L$) caused by the flowing liquid around the particle and virtual mass force ($\mathbf{F}_{vm}$) is shown in the following form:

$$\mathbf{F}_L = C_L \mu_f |\mathbf{v}_f - \mathbf{v}_p| \left(\frac{d_p}{2} \right)^{\frac{1}{2}} \left(\frac{\nabla \times \mathbf{v}_f}{\mu_f} \right)^2 \quad (2.13)$$

The empirical lift coefficient is also defined:

$$C_L = \begin{cases} 6.46 \left(1 - 0.3314 \sqrt{\frac{\Omega_c}{2Re_p}} \right) \exp \left(-\frac{Re_p}{10} \right) \\ \quad + 2.1408 \sqrt{\frac{\Omega_c}{2Re_p}} & (Re_p \leq 40) \\ 0.3385 \sqrt{\frac{\Omega_c}{2}} & (Re_p \geq 40) \end{cases} \quad (2.14)$$

As particles move through the liquid, the moving particles could move some volume of surrounding liquid, and the liquid further exerts a force on the particles. This force is called “virtual mass force”, expressed as:

$$\mathbf{F}_{vm} = 0.5m_p \frac{\rho_f}{\rho_p} \frac{D\mathbf{v}_f}{Dt} \quad (2.15)$$

2.4 VOF method

From the multitude of numerical methods, the volume of fluid (VOF) method has been proven to be an appropriate choice for the simulation of the complicated free-surface flows due to their flexibility, accuracy and robustness e.g. in handling strong morphological changes as well as for their inherent mass conservation property [82], which was first proposed by Hirt and Nichols [83]. The volume fraction (α) of liquid in each cell ranging from 0 to 1 to describe the phase distribution. $\alpha = 0$ (or 1) implies the cell is only occupied by gas (or liquid) [84]. The VOF method herein is applied to represent the free surface of the gas-liquid interface in the rocking bioreactor. For the readers convenience, the theoretical concept and the main equations are given below,

$$\frac{\partial \alpha}{\partial t} + \nabla \cdot (\alpha \mathbf{v}_f) + \nabla \cdot ((1 - \alpha) \alpha \mathbf{v}_r) = 0 \quad (2.16)$$

where $\mathbf{v}_r$ is the relative velocity at the interface, expressed as:

$$\mathbf{v}_r = \mathbf{v}_l - \mathbf{v}_g \quad (2.17)$$

This function can be used to calculate the fluid properties in each phase as a weight function. In order to use a set of governing equations using the weight function, each fluid property should

be calculated based on the volume occupied by this fluid in the surface cell, defined as [85]:

$$\rho = \alpha\rho_l - (1 - \alpha)\rho_g \quad (2.18)$$

$$\mu = \alpha\mu_l - (1 - \alpha)\mu_g \quad (2.19)$$

2.5 Physical properties

The liquid phases have the same property of water and air at room temperature. The densities (ρ_f) of water and air are 996 and 1.2 kg/m³, respectively. The kinematic viscosity of ($\nu = \mu_f / \rho_f$) is 1.1×10^{-6} m²/s for liquid and is 1.5×10^{-5} m²/s for air. The surface tension (σ) of water is set as 0.0715 kg/s². For the solid phase, the particle density (ρ_p) and diameter (d_p) is 1075 kg/m³ and 1.75×10^{-4} m, respectively. The total number of particles is dependent on the type and area of the bioreactor, which would be stated separately in the each section.

2.6 Numerical procedure

The governing equations are discretized using the finite volume method (FVM). The second-order linear interpolation scheme is used for the spatial discretization except for the non-linear term in the advection equation. The spatial derivative for the non-linear term is the Van Leer scheme [86]. The implicit Euler method is adopted to represent time advancement. A VOF model is used to represent the free surface of the gas-liquid interface in a shaking tank [84]. The velocity and pressure fields are coupled using the pressure implicit with splitting operator (PISO) algorithm [87]. An open-source software, OpenFOAM is used for all calculations. The solver icoUncoupledKinematicParcelFoam which combines interFoam and Lagrange is applied for calculation of multi-phase flow and tracking particles. It should be pointed out that the validation has been performed in a previous study [88], and the computational accuracy of the numerical codes has been justified by comparing with the study of Salek et al. [89].

CHAPTER 3

NUMERICAL OPTIMIZATION OF CELL DISPERSION IN ROCKING BIOREACTOR FOR STATIC CULTURE

In this chapter, numerical simulations to investigate the situation of cell dispersion in the rocking bioreactor under various operating conditions are conducted. The cell distribution can be characterized accurately, and the shear stress is evaluated quantitatively. Further, we elaborate on the relations between the uniformity of cell dispersion and shear stress, and an optimal condition concerning a nondimensional ratio for cell cultivation is proposed.

3.1 Background

iPS cells are expected to contribute to the investigation of regenerative organs, disease modeling, and the development of drug screening. To put them into practical application, the technology of iPS cell cultivation is an essential process.

Conventional culture method using various bioreactors including the stirred-tank bioreactor, rotating wall bioreactor, and T-flask are employed in practical applications. Nevertheless, these bioreactors hardly fulfill the demands of manufacturing, as they are associated with low efficiency, high local shear stress, and a lack of stringent quality control [38, 90]. Furthermore, a novel square bioreactor with single-direction reciprocal shaking (SRS) and multi-direction reciprocal shaking (MRS) was investigated to optimize the dispersion of particles [25]. It was found that the MRS method exhibited an advantage for distributing particles when compared with the SRS method. However, the MRS method also consumed at least 10 times more power than the SRS method, which significantly increases its economic cost in industrial applications. Hence, an ideal and productive bioreactor should be developed to generate high-quality aggregated cells.

In contrast to classical bioreactors, rocking bioreactors that depend on gravity with multidimensional oscillation have been extensively used in industrial settings. This type of motion not only maintains cells undifferentiated but also reduces the damage of shear stress to some extent. Despite

this, there are few studies that investigate the operational process and mechanism. A systematic analysis and discussion of rocking bioreactor are carried out by using numerical simulation.

3.2 Methodology

The modeled rocking bioreactor has a length of 74 mm on each side and a height of 37 mm. The culture liquid phase is water and its height is 3 mm. The sketch and wave trajectory of the vessel are illustrated in Figure 3.1.

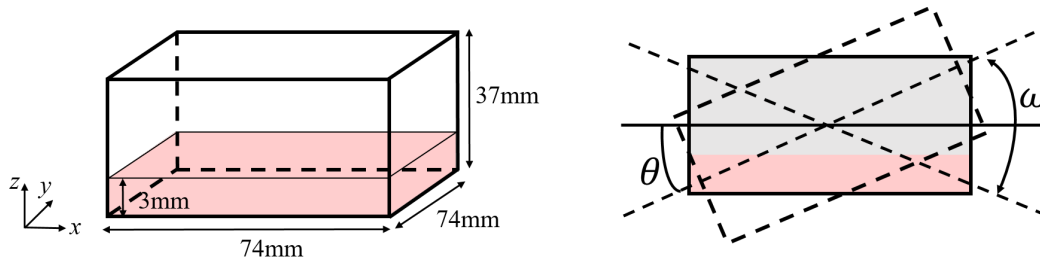


Figure 3.1 (Left) Computational domain in a rocking bioreactor. (Right) The orbit of rocking tank, where θ and ω are the angle of inclination and angular velocity, respectively.

3.2.1 Flow state analysis

To specify the flow state in the tank, a nondimensional parameter is introduced to characterize the onset of the fluid. Kurzweg et al. proposed an estimation of turbulence onset in unsteady oscillating flow as a function of the Womersley number (Wo) and a parameter β , defined as follows [91]:

$$Wo = \frac{L}{2} \sqrt{\frac{\omega}{\nu}} \quad \beta = \Delta x \sqrt{\frac{\omega}{\nu}} \quad (3.1)$$

where L denotes the characteristic length scale of the flow (which is, in the present case, represented by the length of the tank), Δx indicates the maximum liquid element displacement in the tank during one cycle.

The operating parameters in the present simulation are selected for speeds varying from 30 to 60 rpm and angles between 5° and 30° . In this range, the obtained maximum value for Wo is

88.12. A value of $Wo > 1$ corresponds to a flow strongly modified by unsteady effects, which should be avoided in the present configuration. The maximum value of β is 176.81. The transition to turbulence first appears in oscillating flows when β exceeds 700 for $Wo > 8.5$ [91]. However, considering the β values that are much less than 700, it can be concluded that the liquid flow is in the laminar state. Hence, with the selected settings, no turbulence model is needed for the present numerical calculations.

3.2.2 Operating conditions

To obtain the effect of operating conditions on the dispersion of particles in the wave tank, the angles of inclination and shaking velocity are, respectively, varied from 5° to 30° and 30 to 60 rpm with a reasonable increment, as shown in Table 3.1. The total number of particles is 990.

Table 3.1 Values of the control parameters used for optimization.

Case	Shaking velocity ω [rpm]	Angle of inclination θ [$^\circ$]
case 1	30	5
case 2		10
case 3		20
case 4		30
case 5	40	5
case 6		10
case 7		20
case 8		30
case 9	50	5
case 10		10
case 11		20
case 12		30
case 13	60	5
case 14		10
case 15		20
case 16		30

The total number of grid points in the simulation is 328,000. Initially, the particles are uniformly distributed in the center region of the tank, and the liquid is stationary. The no-slip condition is imposed on the flow velocity on the boundary. All of the simulations are quantified at 6 s after the shaking process is stopped, which confirms that the mean values are well converged.

3.2.3 Evaluation parameters

The domain of the tank bottom wall is divided by 20×20 bins; the number density (N_d) in a bin is used to define the dispersion of particles. In addition, the aggregation ratio of the particles (C_v), which is derived from N_d , could capture the final behavior of the particles. A low value of C_v indicates high uniformity of particle distribution. N_d and C_v are expressed as follows:

$$N_d = \frac{\text{Number of particles in a bin}}{\text{Total number of particles}}$$

$$C_v = \frac{\text{Standard deviation of } N_d}{\text{Average } N_d}$$

The shear stress acting on the particles is an essential factor to qualify the condition of cell suspension culture. By applying Stokes approximation for flow around a sphere, the magnitude of shear stress over the surface of a spherical particle is defined as:

$$|\tau| = \frac{2\mu_f |\mathbf{v}_f - \mathbf{v}_p|}{d_p} \quad (3.2)$$

And the associated nondimensional form can be expressed in the following form:

$$|\tau|^* = \frac{|\tau| S_p}{\mu_f \nu_f} \quad (3.3)$$

where S_p is the superficial area of particle, $S_p = \pi d_p^2$.

3.3 Optimization of wave numbers

In this section, the wave number N_R (where N_R is the number of wave during one period) is set for different values (4, 8, 12, 16, respectively) to obtain an optimal magnitude and reduce the calculation cost.

A typical snapshot of the particle position at the end of the wave process for $N_R = 8$ is presented in Figure 3.2 (a). The corresponding measurement of particle dispersion (N_d) is calculated as depicted in Figure 3.2 (b), where the yellow region represents highly accumulated particles.

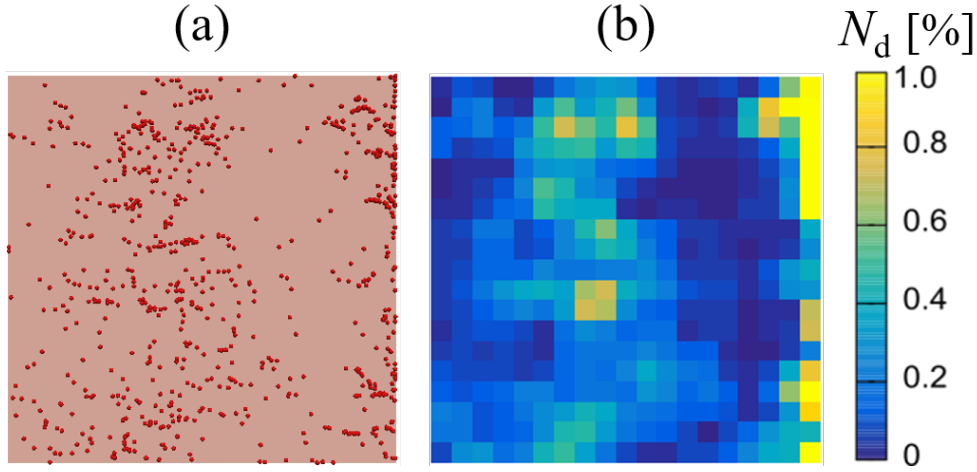


Figure 3.2 Top view of the square tank at a fixed wave number ($N_R = 8$) in the case of $\omega = 60$ rpm, $\theta = 30^\circ$. (a) The particle distribution is represented by red spots. (b) The corresponding number density of particles, N_d .

An intuitive perspective of particle distribution measured by the number density (N_d) for the different wave numbers is depicted in Figure 3.3. It is inevitably found that a high aggregation is formed in the right region of the vessel, which could be attributed to the high magnitude of the gravitational component at a large shaking angle ($= 30^\circ$). Further, it reveals that the particle dispersion is almost independent of the wave number for $N_R > 8$. We consider the energy consumption and the quality of cells because the longer wave process could damage cells. The wave number $N_R = 8$ is adopted in the following calculations.

3.4 Shear stress acting on the particles

As the cells are sensitive to shear stress, literature concerning cell cultivation has shown that a range of shear stress from 0.8 to 3 N/m² has been explored in a perfusion bioreactor [92], and the value exceeds 1 N/m² in a stirring bioreactor [93]. Furthermore, the shear stress threshold of 0.7 N/m² could harm the animal cells in cell bags [94]. Therefore, the shear stress should be further investigated for the selection of bioreactors without damaging the cells.

In the present simulation, the shear stress is calculated from the shaking speed, as depicted in Figure 3.4. It should be noted that the shear stress increases monotonically with increments in the

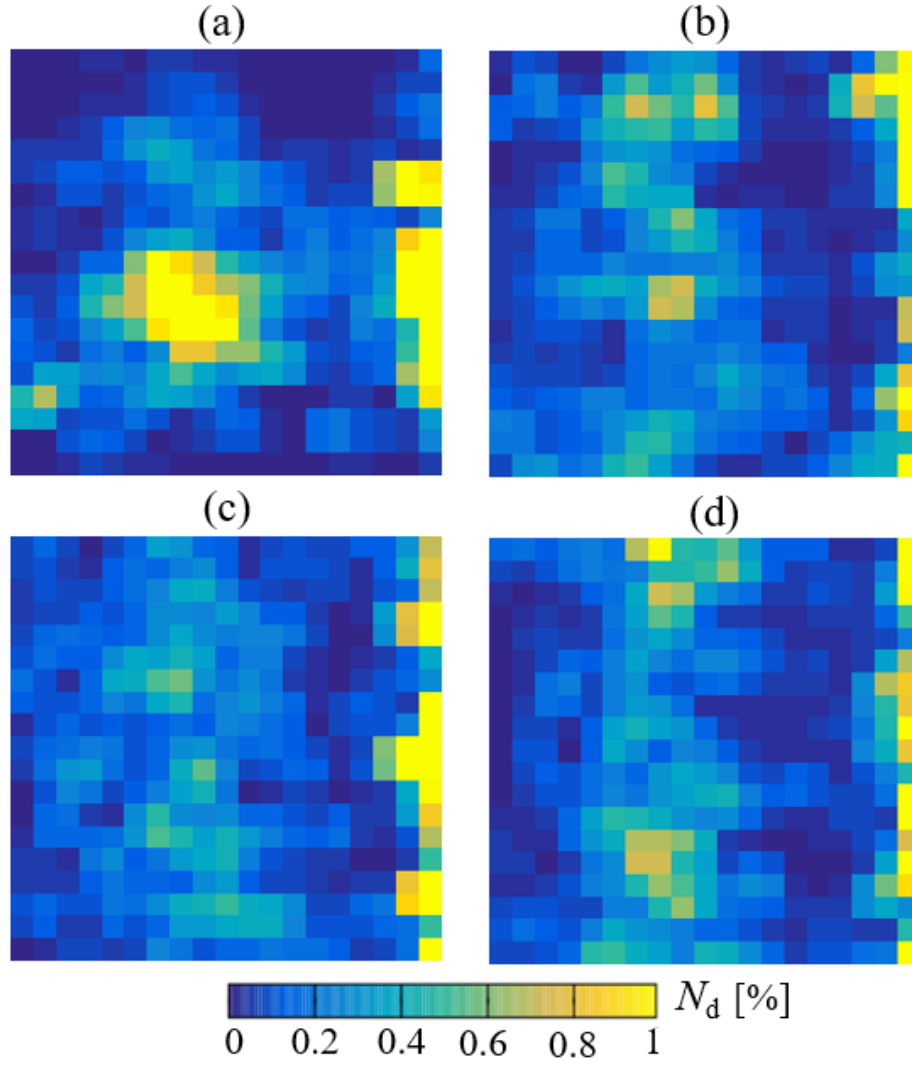


Figure 3.3 Top view of number density of particles (N_d) at various wave numbers of $\omega = 60$ rpm, $\theta = 30^\circ$. (a) $N_R = 4$, (b) $N_R = 8$, (c) $N_R = 12$, (d) $N_R = 16$.

angle and angular velocity. The relatively high velocity and angle could develop a chaotic flow, leading to a stronger velocity gradient. The present results exhibit very low values of shear stress (all values are less than 0.12 N/m^2) compared to those of the stirring bioreactor.

3.5 Comparison of particle dispersion

The effect of angle on the particle dispersion can be fully understood from Figure 3.5. For a smaller value of θ , the dense particle distribution occurs at the initial position, whereas the particles can be dispersed efficiently at larger values of θ ($\theta \geq 20^\circ$). The observed results could be

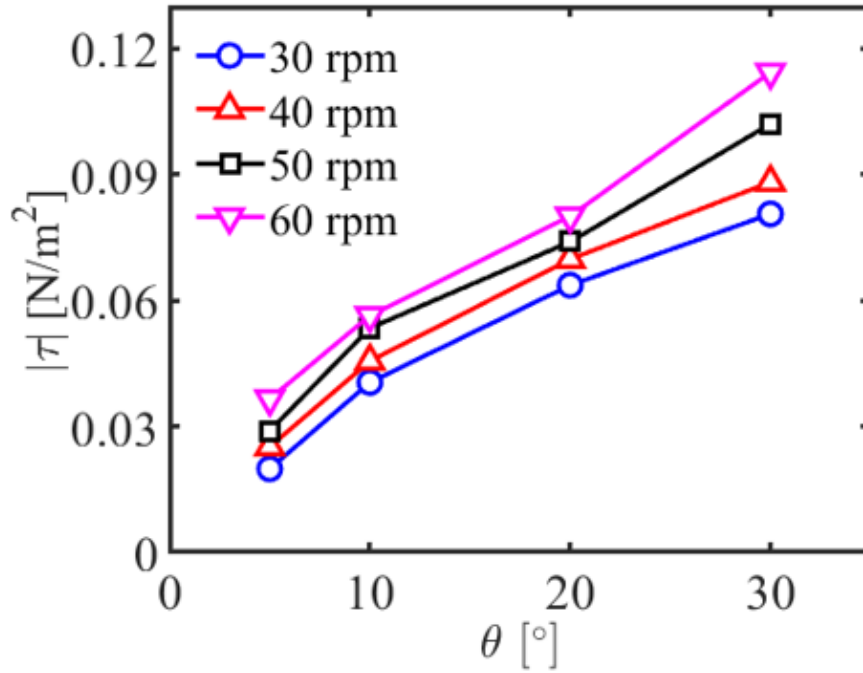


Figure 3.4 Variation of average shear stress as a function of angle at shaking velocities of 30, 40, 50, and 60 rpm.

partly explained by the strong fluctuation of the liquid at a large angle because of the alternating transform. Further, it should be noted that the particles tend to accumulate in the vicinity of the sidewall, which is mainly caused by the strong gravitational force in the vertical (z) direction.

In addition, the effect of shaking speed on the particle dispersion is studied, as depicted in Figure 3.6. The particles partly assemble near the center at a weak shaking speed as depicted in Figure 3.6 (a) and (b). However, as the shaking velocity increases, the uniformity of particle distribution is improved significantly owing to the stronger motion caused by the increase in shaking velocity. Similar results are also observed at other angles, but we will not discuss them here.

Further investigation on the uniformity of particle dispersion is conducted by estimating the aggregation ratio (C_v), as depicted in Figure 3.7. It is worth noting that the shaking speed has a significant effect on particle aggregation ratio for a smaller value of θ . Instead, the impact of larger θ ($\theta > 10^\circ$) on the distribution of particles could be negligible.

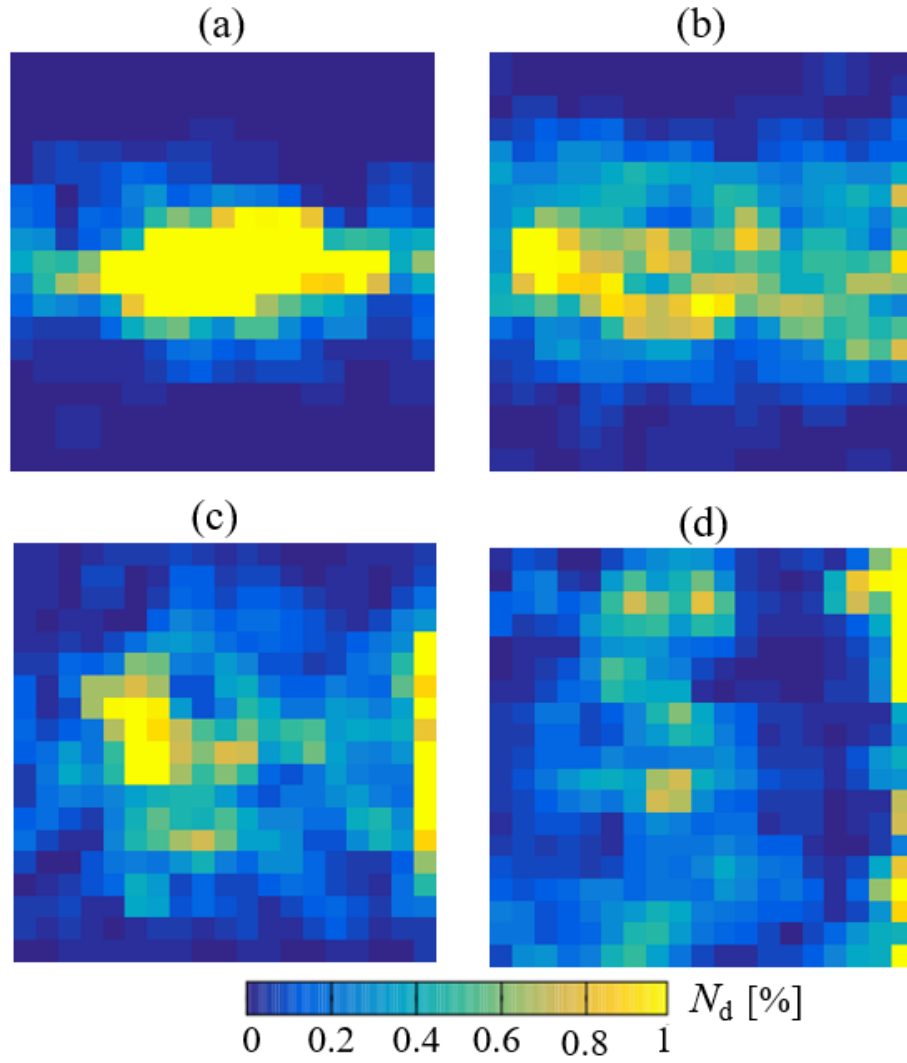


Figure 3.5 Top view of number density of particles, N_d , at $\omega = 60$ rpm. (a) $\theta = 5^\circ$, (b) $\theta = 10^\circ$, (c) $\theta = 20^\circ$, (d) $\theta = 30^\circ$.

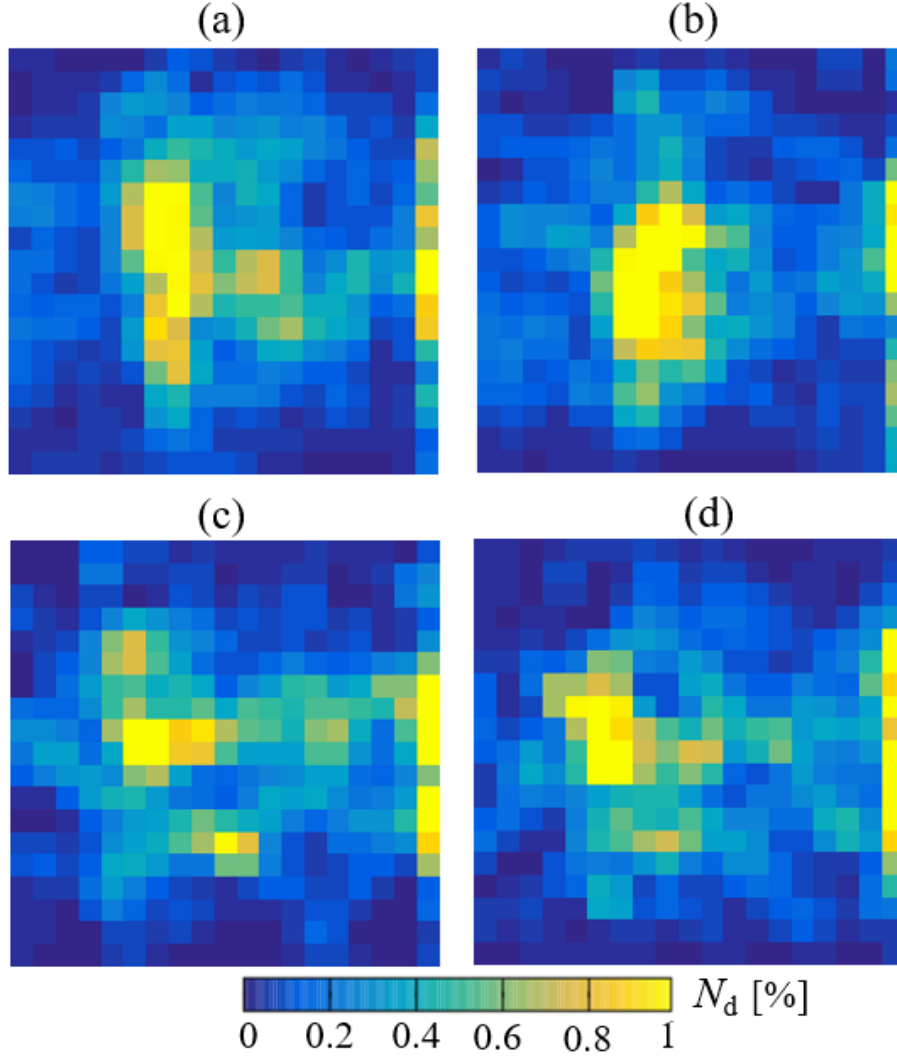


Figure 3.6 Top view of number density of particles, N_d , $\theta = 20^\circ$. (a) $\omega = 30$ rpm, (b) $\omega = 40$ rpm, (c) $\omega = 50$ rpm, (d) $\omega = 60$ rpm.

3.6 Analysis of optimized condition

To characterize the performance of the bioreactor, an optimal condition considering the shear stress and particle dispersion is explored, as depicted in Figure 3.8. It is interesting that C_v remains constant for $|\tau| > 0.05$ N/m², although it decreases drastically initially. Consequently, the uniformity of particle dispersion is not affected by higher shear stress directly.

As we have discussed the characteristics of shear stress (Figure 3.4) and aggregation ratio (Figure 3.8), the minimum magnitudes of both C_v and $|\tau|$ are desired. The shear stress and particle

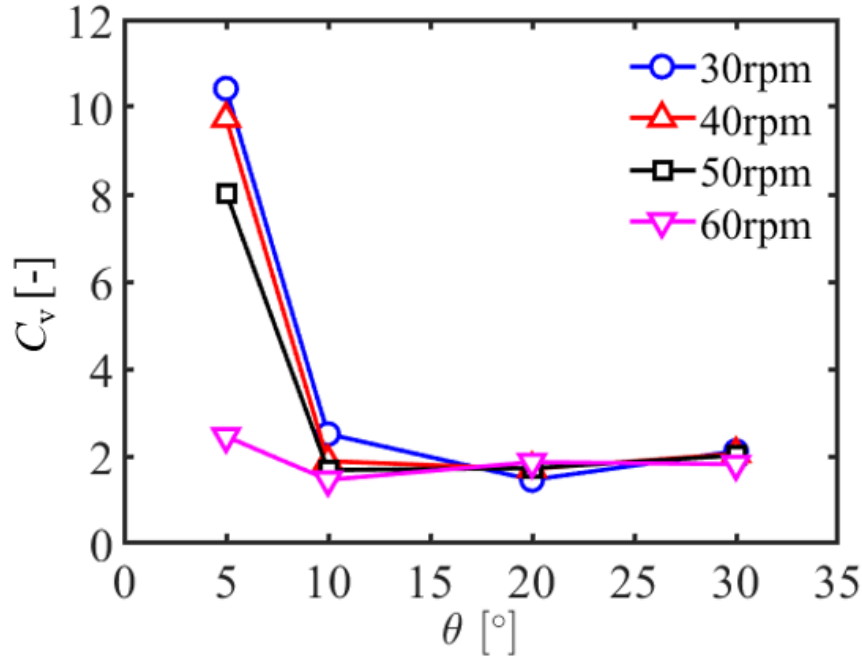


Figure 3.7 Profiles of aggregation ratio with angle at shaking velocities of 30, 40, 50, and 60 rpm.

dispersion are mainly controlled by drag force and gravitational force, respectively. Further, the inertial force (which can be expressed by Re) has an effect on the particle dispersion. Thus, a nondimensional ratio $Re(\mathbf{F}_G/\mathbf{F}_D)$ is used to evaluate the performance of the bioreactor. Here, the Reynolds number is defined as follows:

$$Re = \frac{L\omega\theta h}{2\nu_f} \quad (3.4)$$

The profiles of $C_v \cdot |\tau|^*$ with $Re(\mathbf{F}_G/\mathbf{F}_D)$ are presented in Figure 3.9. It should be noted that the data are scattered, but a minimum threshold of $C_v \cdot |\tau|^*$ appears in the fitted line at a concentrated value of $Re(\mathbf{F}_G/\mathbf{F}_D) \approx 7$. This suggests that an optimized condition for uniform particle dispersion is obtained from a nondimensional parameter while avoiding damage to the cells because of high shear stress.

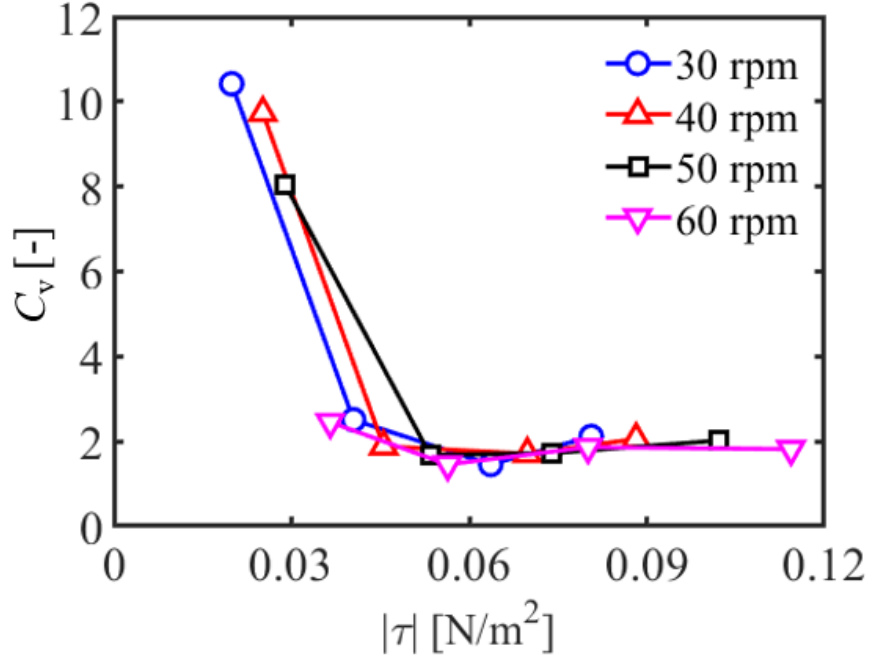


Figure 3.8 Profiles of mean shear stress with aggregation ratio at shaking velocities of 30, 40, 50, and 60 rpm.

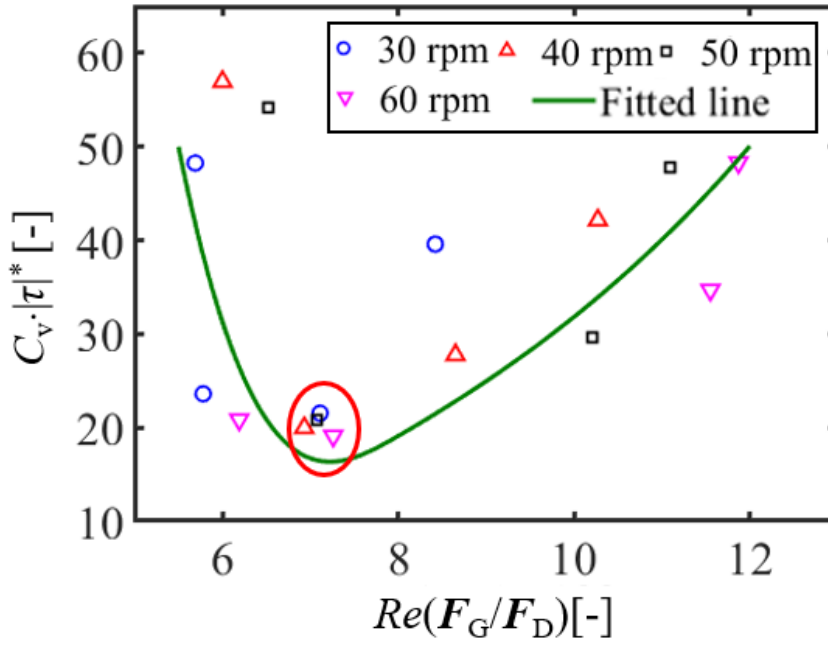


Figure 3.9 Profiles of $C_v \cdot |\tau|^*$ with the nondimensional ratio of $Re(F_G/F_D)$ at shaking velocities of 30, 40, 50, and 60 rpm.

3.7 Summary

The wave number (N_R) is set ranging from 4 up to 16 with a suitable increment under the same shaking velocity and shaking angle. It is found that the uniformity of cell distribution is not changed when the wave number is larger than 8 times. Thus, the optimal wave number (= 8) for one period is successfully obtained considering the cell quality and energy consumption. For the cell dispersion under the effect of different working conditions, although larger shaking velocity and shaking angle could efficiently distribute the cells, it is inevitable that such operating condition results in a stronger shear stress due to the larger velocity gradient. When the shaking angle reaches a certain value, the shaking velocity has no significant effect on the uniformity of cell distribution. Since the shear stress and the situation of particle dispersion are governed by gravitational force, drag force and inertial force, the correlation among these factors is established.

CHAPTER 4

NUMERICAL INVESTIGATION OF ROTATING METHOD IN ORBITALLY SHAKEN BIOREACTOR

In this chapter, to explore a suitable shaking manner in an orbitally shaken bioreactor, a newly shaking method with periodic alternate rotation (PAR) is proposed, based on the conventional method – one direction rotation (ODR). The characteristics of these two measures are quantified through the suspension ratio and shear stress by performing numerical simulations. Besides, a systematically physical analysis on the PAR method is conducted to illuminate the shaking mechanism.

4.1 Background

As previous stated, iPS cells have been applied in a broad range of fields including regenerative medicine. Nevertheless, several technical hurdles must be overcome before iPS cells are put into practical application. One of the obstacles is the scalable, reproducible production of cells in sufficient amounts for their successful clinical applications, which would be difficult to achieve economically using static culture method, since the static culture method has serious limitation of repeated handling operation [95]. The development of culture system that enables scalable expansion is a crucial process for cell production. This allows us to transfer attention to investigate the suspension culture method. The classical bioreactor used in suspension culture is an extensively used bioreactor – orbitally shaken cylindrical bioreactor. Nonetheless, the common orbital shaking bioreactor hardly meets the anticipated demands since it exhibits a lower suspension level and local cell agglomeration or attachment in the center region at the bottom [21, 38, 96, 97]. To this end, a novel culturing technique that is capable of producing required quantities and high-quality cells is urgently needed to be developed.

4.2 Methodology

In the present simulation, the movement of the cylindrical bioreactor follows a circular path in the horizontal plane during the shaking without self-rotation. The diameter (d_t) of the tank is 84 mm and the tank height (h_t) is 42.3 mm. The culture medium is water and the height (h_l) is 10 mm, as illustrated in Figure 4.1 (a).

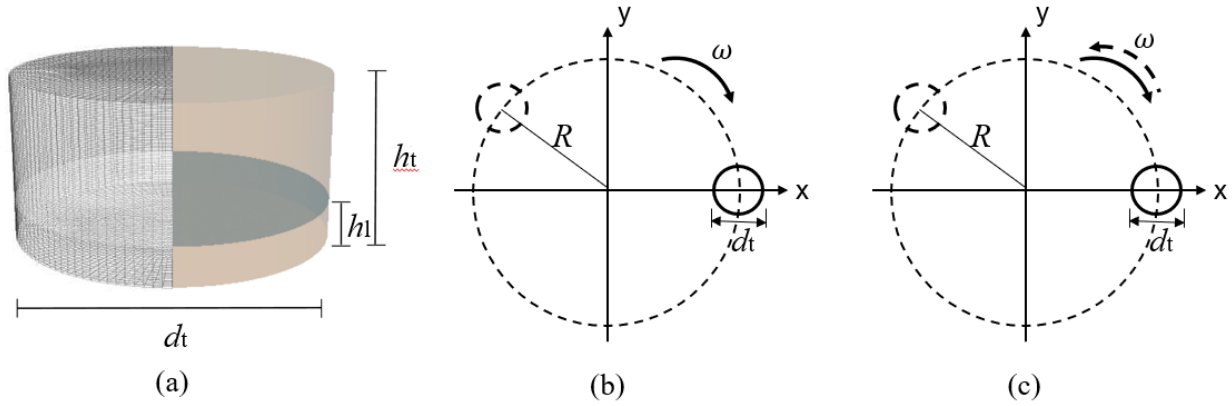


Figure 4.1 (a) Sketch and mesh of the computational domain in the orbital shaking tank. Top view of trajectory of (b) the ODR and (c) PAR method.

4.2.1 Shaking method

The tank revolves horizontally, and the track follows a circle. This shaking method here is called ODR, as shown in Figure 4.1 (b). The motion in the PAR method can be described as: the tank firstly rotates clockwise for one period, then turns to the reverse direction, finally repeating this movement periodically, as shown in Figure 4.1 (c). The center position of the tank in the ODR and PAR system is expressed in the following form:

$$\mathbf{r} = \begin{pmatrix} x \\ y \\ z \end{pmatrix} = \begin{pmatrix} R \cos \omega t \\ -R \sin \omega t \\ 0 \end{pmatrix} \quad (\text{ODR}) \quad (4.1)$$

$$\mathbf{r} = \begin{pmatrix} x \\ y \\ z \end{pmatrix} = \begin{pmatrix} -R \cos \left(\pi \cos \frac{\omega t}{2} \right) \\ -R \sin \left(\pi \cos \frac{\omega t}{2} \right) \\ 0 \end{pmatrix} \quad (\text{PAR}) \quad (4.2)$$

Since the flow state is not chaotic with the selected settings, no turbulence model is needed for this numerical simulation. The total number of grid points for the tank in the simulation is 840,000. Initially, the particles are uniformly distributed in the water, and the liquid is stationary. All the simulations are computed for 30 s, and it is confirmed that the mean values are well converged.

4.2.2 Evaluation parameters

The percentage of afloat particles can be expressed by a nondimensional parameter. Here, we introduce the suspension ratio P_S defined as:

$$P_S = N_S/N_A \quad (4.3)$$

where N_S is the number of floating particles, and N_A ($= 208$) is the total number of particles. We assume that the particle is afloat in the liquid if the center position of the particle in z -direction is larger than a certain height ($= 4.5 \times 10^{-4}$ m).

The shear stress acting on the particles is an essential factor to qualify the condition of cell suspension culture. The definition and specific explanation has been provided in the Chapter 3.

4.3 DEM validation

In this work, the force imposed on the particles including lift force (F_L) and virtual mass force (F_{vm}) are calculated, which are not considered in our previous study [38]. Therefore, the verification of the DEM model is conducted by comparing simulated free-surface shape and particle distribution with experimental results.

4.3.1 Experimental settings

The orbitally shaken bioreactor with ODR shaking manner filled with 2 L water and 0.28 m inner diameter is rotating. The shaking behavior with 0.025 m radius is shaken for 30 seconds at various shaking velocity such as 30, 40, 50, and 60 rpm. The particle number is 3,000,000. A video camera is installed at the top and side wall of the tank to record the free-surface shape. The experiment is carried out at a room temperature.

4.3.2 Simulation verification

Numerical simulation is performed under the identical experimental conditions except for the particle number. The particle number herein is 30,000, and the particle density and diameter is 1075 kg/m^3 and $1.75 \times 10^{-4} \text{ m}$, respectively. For the computational domain, the tank height is 0.15 m, and the mesh number is 2.6×10^6 .

Free surface shapes and particle position are recorded for several time steps, as shown in Figure 4.2. The shapes of the numerical results during the motion process are consistent with those observed in the video images. In addition, the liquid is uniform and stable at a smaller shaking velocity, such as $\omega = 30 \text{ rpm}$, and there are no particles afloat in the liquid medium. However, the conspicuous deformation of the free surface appears, especially for a large shaking velocity. Consequently, many particles suspend in the liquid under the action of intensive motion.

Figure 4.3 shows the particle distribution at a fixed shaking velocity from the top view. As can be seen, the particles have a tendency to gather in the center region with time proceeding, then form a circle. Both the experimental and simulation results have similar cell dispersion. Therefore, it can be concluded that the validation for the DEM model is successful.

4.4 Comparison of ODR and PAR method

The objective of this section is to compare the performance of the ODR and PAR method from the spatial–temporal data, to obtain a satisfying culture measurement by analyzing the suspension level and shear stress.

4.4.1 Particle behavior

A typical snapshot of the particle dispersion for both cases is illustrated in Figure 4.4. In the ODR system, the particles tend to migrate to the center and bottom of the tank because an unwanted secondary flow appears within the boundary layer, which is called Einstein’s tea leaf paradox as provided in (a). Similar aggregation was also found in the experimental study [21]. Such behavior should be avoided since it is deleterious for the cell growth.

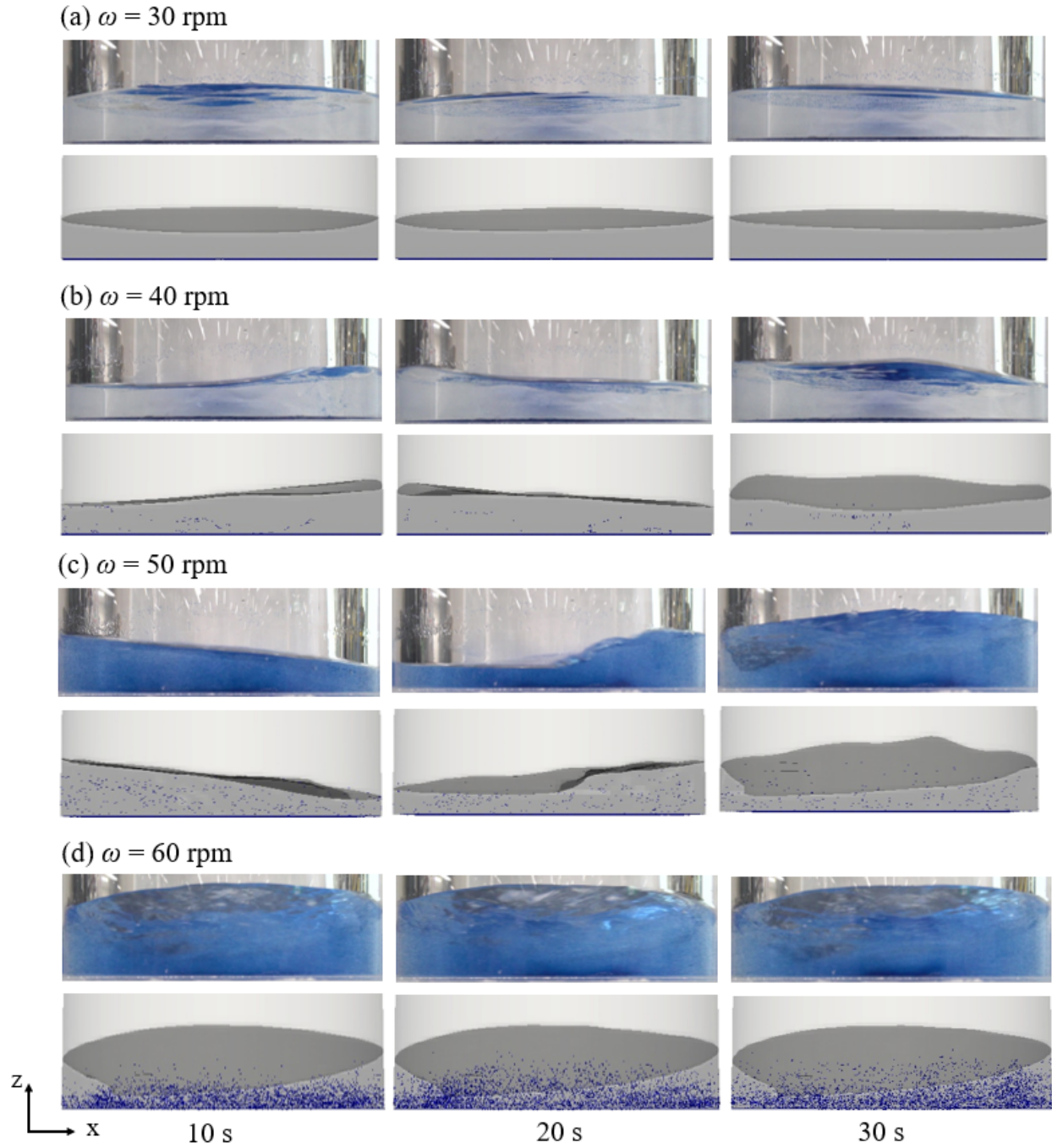


Figure 4.2 Comparison of free surface shapes and particle suspension state at various shaking velocities from side view. Liquid and particles are photographed in experiment (upper panels) or simulation (lower panels). The blue points represent particles.

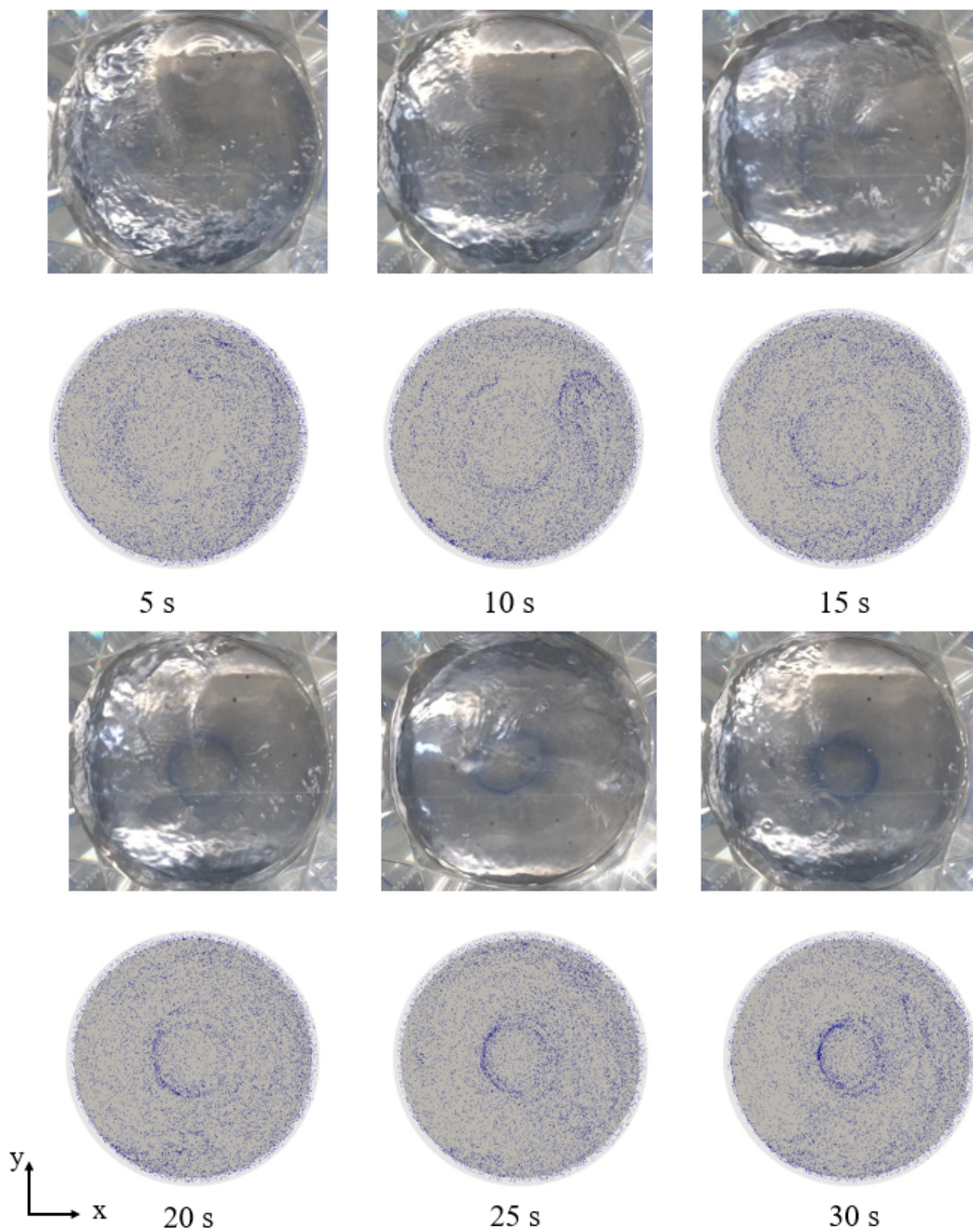


Figure 4.3 Comparison of particle distribution for several time steps at $\omega = 60$ rpm from top view. Liquid and particles are photographed in experiment (upper panels) or simulation (lower panels).

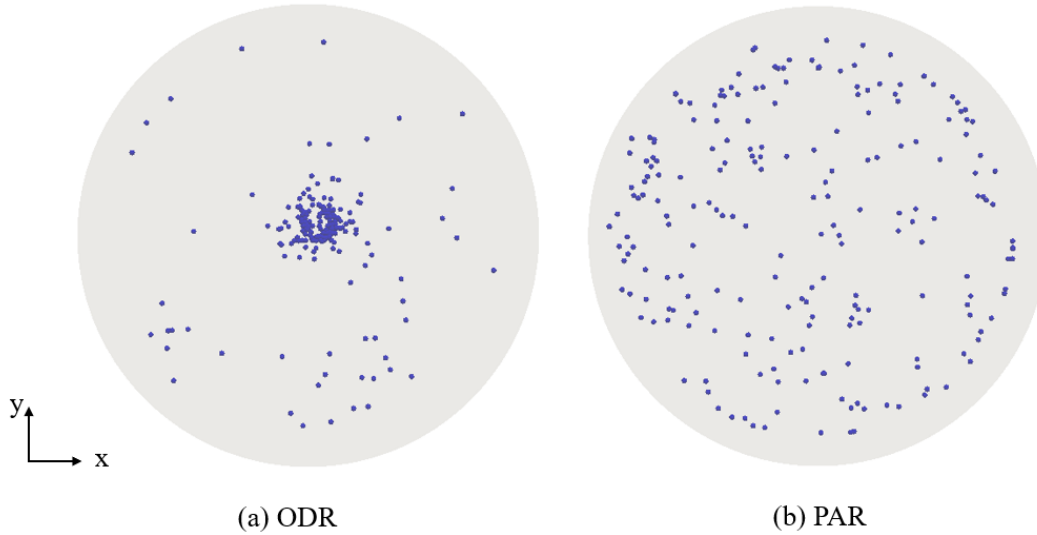


Figure 4.4 Top view of the particle distribution at the bottom is shown by the blue points at a certain time during the process of the (a) ODR and (b) PAR method.

However, the PAR method overcomes this shortcoming, whereby the aggregation is significantly reduced. This should be attributed to the difference in flow structure as seen in Figure 4.5. It is shown that there is a single vortex along the sidewall in the ODR method, since the liquid motion circulates mainly in the clockwise direction. Also, the flow gradually disappears in the central region, resulting in the local accumulation of the particles there. Nevertheless, in the case of the PAR, the velocity components are complex and extend to each direction due to the alternated rotation, which makes the particles fully dispersed in the whole tank.

4.4.2 Suspension ratio

A quantitative estimation regarding suspension ratio is provided, as shown in Figure 4.6. It is evident that the number of afloat particles gradually descends with time under the ODR condition, since all the particles ultimately sink at the bottom wall. In contrast, the particles can be lifted with the improved approach that enhances the movement of culture medium. This partially results from liquid velocity in z -direction is large. In addition, the lift force generated from the culture medium tremendously contributes to the suspension of particles. Based on these observations, the PAR method is deemed suitable for cell cultivation in suspension culture.

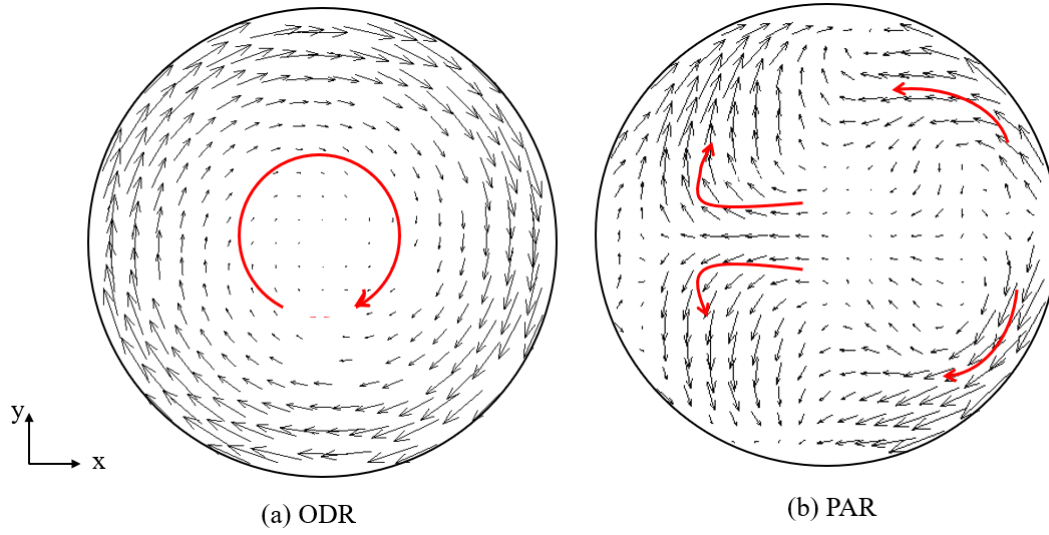


Figure 4.5 Top view of the mean velocity vector in the (a) ODR and (b) PAR method at a horizontal plane ($z = 5$ mm).

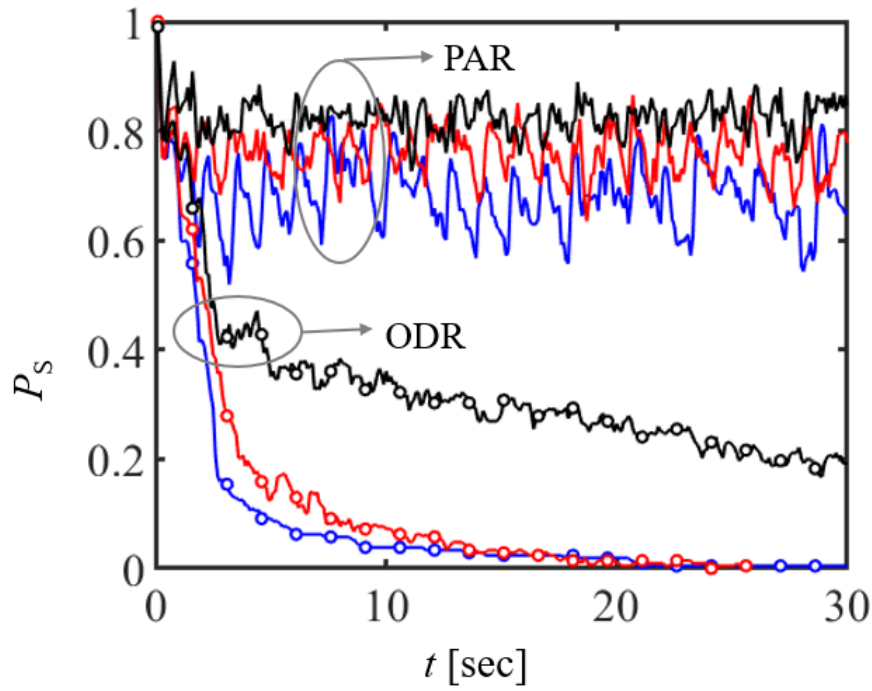


Figure 4.6 Suspension ratio (P_s) as a function of time for the ODR and PAR method at $Dr = 1$:
—, $Fr^* = 0.37$; —, $Fr^* = 0.40$; —, $Fr^* = 0.45$.

4.4.3 Shear stress acting on the particles

It can be predicted that the profiles of shear stress with time is similar to the tendency of suspension ratio, a stronger shear stress is accordingly generated in the PAR system due to the complicated fluid flow which is induced by the periodically rotated motion.

Additionally, the shear stress in the ODR and PAR system can be expressed by examining the magnitude of the slip velocity. The slip velocity are defined as:

$$|\mathbf{v}_{\text{slip}}| = |\mathbf{v}_f - \mathbf{v}_p| \quad (4.4)$$

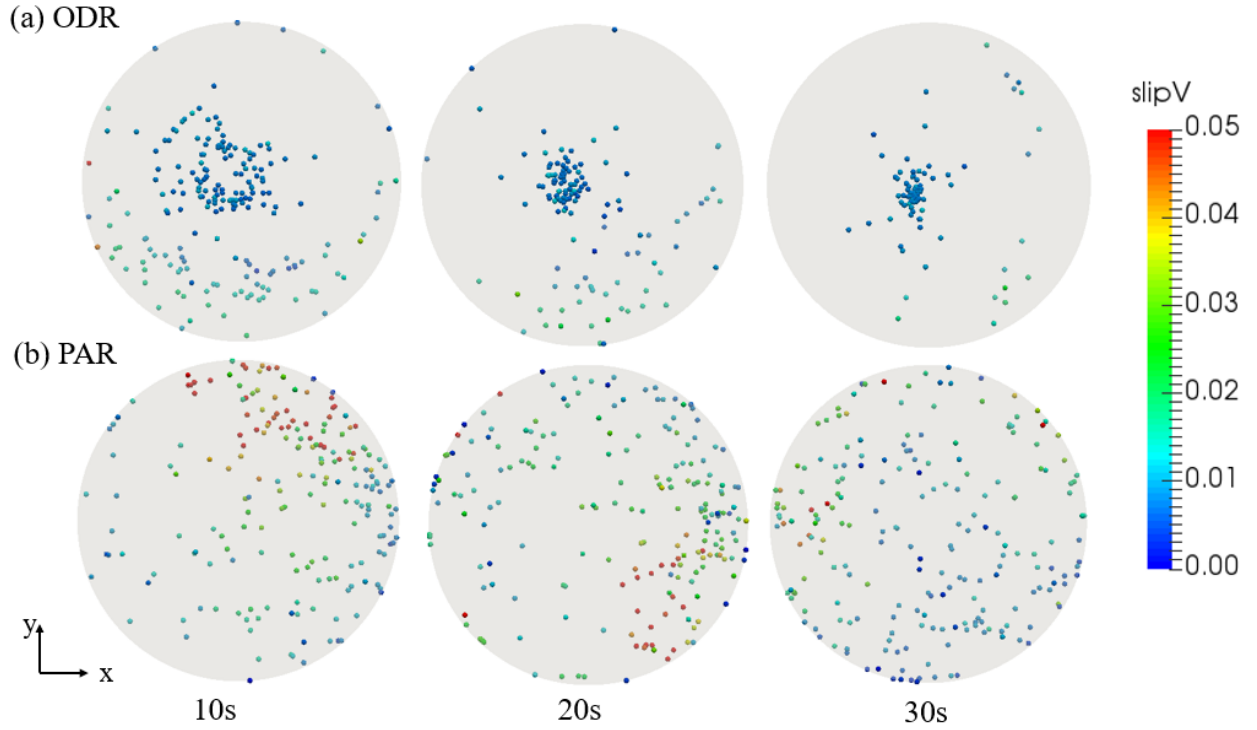


Figure 4.7 Top view of the vessel. The particles distribution at the bottom is shown by the red points at a certain time during the process of the (a) ODR and (b) PAR method.

The top view of the profiles of the slip velocity on the particles at initial (10 s), middle (20 s) and final shaking time (30 s) are presented in Figure 4.7, which are, as shown, significantly different. Notably, in the case of ODR, the minor slip velocity is generated whereby the shear stress is weak. On the other hand, a strong slip velocity occurs in the PAR method with sharp

velocity gradient due to the large velocity difference which is induced by the periodically rotated motion. As stated previously, the high shear stress may lead to death or differentiation of cells.

To further characterize the performance of the two methods, a specific analysis between suspension ratio and shear stress are conducted. The obtained P_S is compared quantitatively at the same level of the $|\tau|^*$, as presented in Figure 4.8. It can be seen that the PAR method exhibits apparent advantage in the aspect of suspending particles in all the range of the shear stress. In order to clarify the mechanism behind this phenomenon. Here a non-dimensional Reynolds number is introduced as follows:

$$Re_{\max} = \frac{U_{\max} L}{\nu_f} \quad (4.5)$$

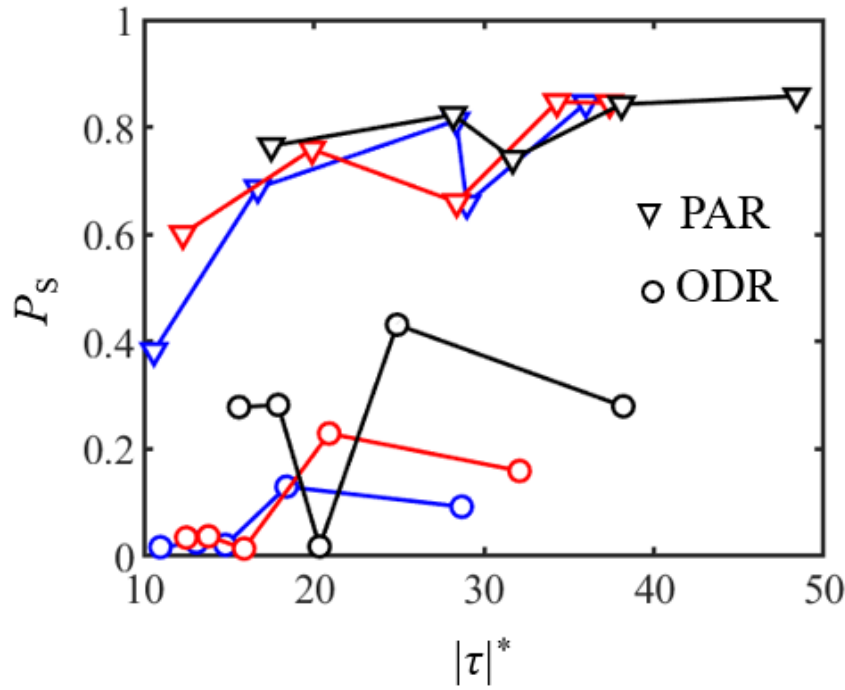


Figure 4.8 Suspension ratio (P_S) as a function of shear stress ($|\tau|^*$) in the ODR ($\text{---}\circ\text{---}$) and PAR system ($\text{---}\nabla\text{---}$). The meanings of the lines' color are the same as Figure 4.6.

From this calculation, it's found that the Reynolds number in the PAR method is larger than that in the ODR method. The higher $Re_{\max}$ implies that a complex and chaotic liquid motion is induced which can be attributed to the periodic shaking behavior. In light of this, the PAR method is more

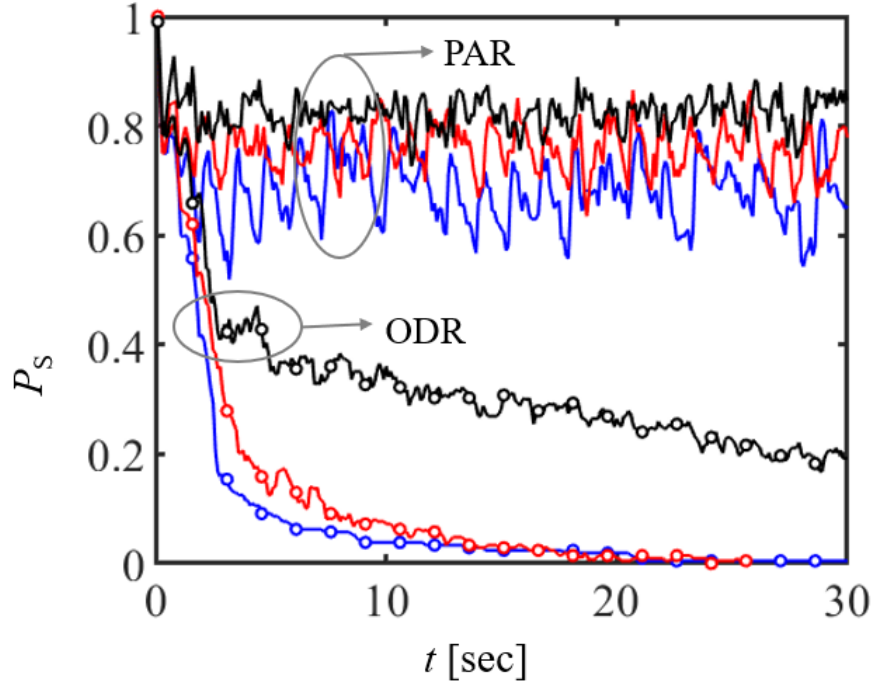


Figure 4.9 Suspension ratio (P_s) as functions of time for the PAR method: —, $Fr^* = 0.34$, $Dr = 0.24$; —, $Fr^* = 0.34$, $Dr = 1.90$; —, $Fr^* = 0.4$, $Dr = 1.90$.

favorable for the suspension of particles. Hence, we mainly focus on the parametric analysis of the PAR manner in the following section.

4.5 Characteristics of the PAR method

In this section, the performance of the PAR method is specifically estimated by calculating the suspension ratio and shear stress.

4.5.1 Suspension ratio

In the present study, the considered operating conditions assume the Fr^* from 0.3 to 0.45, and the Dr ranges from 0.12 to 1.9. Further reduction of the Fr^* is found to be inapplicable for consistent suspended operation, the suspension ratio of particles is close to 0.

Figure 4.9 shows the temporal variation of the P_s from the initial stationary state. A significant difference in the P_s is observed with respect to various Fr^* and Dr as time progresses. At a fixed Dr ($= 1.90$), Fr^* is proportional to the shaking frequency. Therefore, a higher Fr^* ($= 0.4$) is

responsible for a larger P_S , at which the buoyant force is dominant. On the other hand, at a given Fr^* ($= 0.34$), the P_S can be greatly increased owing to the violent liquid flow by adjusting Dr as shown by the red and blue lines in Figure 4.9.

In addition, the average suspension ratio for various Fr^* is systematically analyzed. The P_S keeps increasing as Fr^* increases, as shown in Figure 4.10. This phenomenon should be attributed to the enhancement of inertial force at larger Fr^* . Noteworthy that all the profiles exist a peak value of P_S , which is roughly independent of Dr (≈ 0.25) and Fr^* . A specific explanation will be given in the following part.

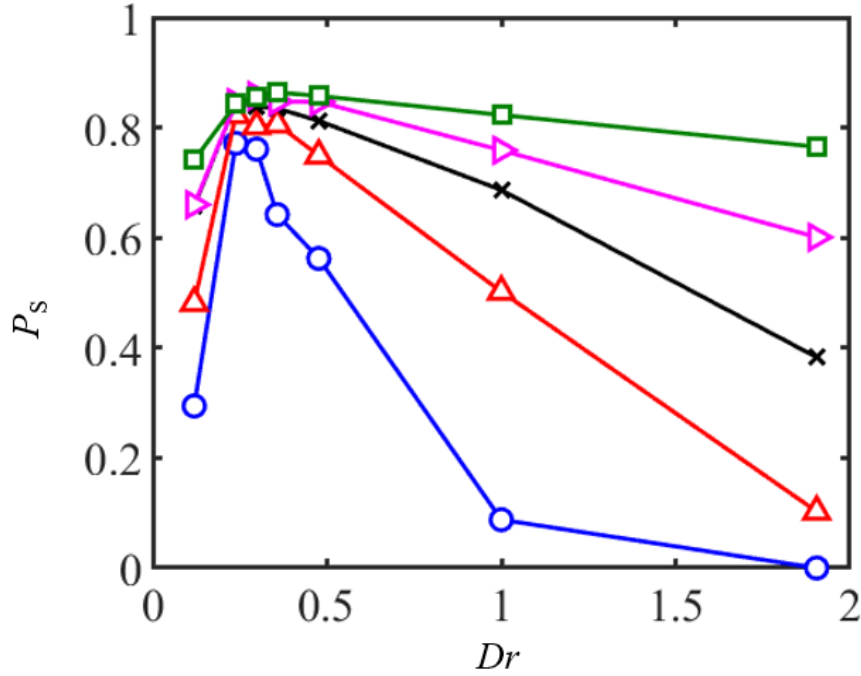


Figure 4.10 Time-averaged suspension ratio (P_S) as a function of Dr for the PAR method. —○—, $Fr^* = 0.31$, —△—, $Fr^* = 0.34$, —×—, $Fr^* = 0.37$, —▷—, $Fr^* = 0.40$, —□—, $Fr^* = 0.45$.

To gain further insight to the variation of suspension ratio, the flow pattern at a certain Fr^* ($= 0.34$) for different Dr is depicted in Figure 4.11. Since the Dr is inversely proportional to ω , the effect of inertial forces is dominant at smaller Dr ($= 0.12$), and wall impacts strongly rise, which develop a locally largest velocity in the vicinity of the sidewall, as shown in Figure 4.11 (a). The motion of strong shaking frequency is characterized as an "out-of-phase" phenomenon,

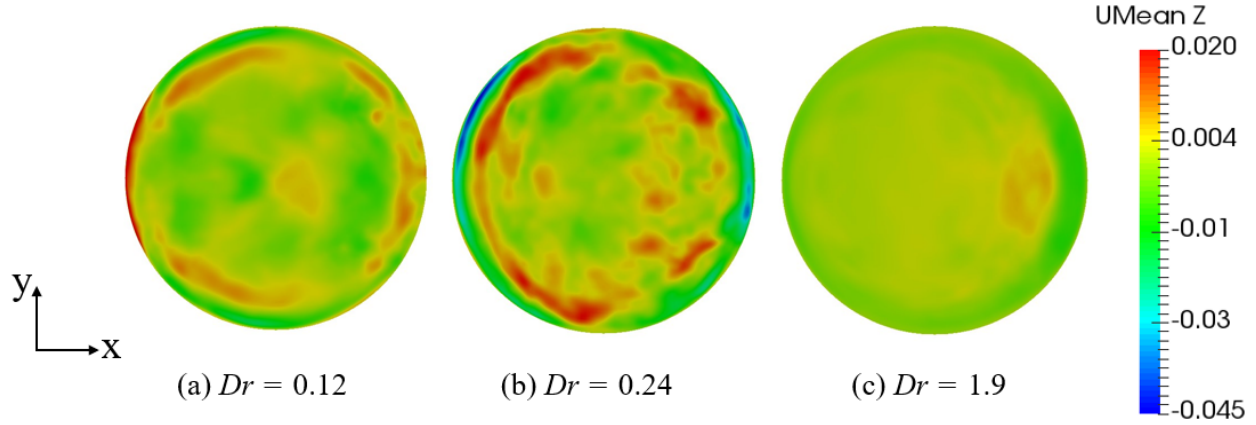


Figure 4.11 Contour of mean velocity in vertical (z) direction for a horizontal plane for 30 s, $Fr^* = 0.34$, $z = 5$ mm.

as explained in the literature [98] for more details. Moreover, the velocity is overall largest at $Dr = 0.24$, as presented in Figure 4.11 (b). The upward vertical velocity which promotes particles floating is larger so that the suspension ratio increases appreciably. The vertical velocity decreases with a further increment of Dr due to the slow rotation speed, as shown in Figure 4.11 (c). The particles consequently move to the bottom wall of the tank.

4.5.2 Shear stress acting on the particles

Despite the high level of shear stress affects the viability and proliferation of cells, the critical shear stress value damaging the cells is still bewildering. The influence of shear stress is inevitably considered for the selection of suspension culture devices. In this part, we would like to estimate its characteristic imposing various shaking frequency.

The shear stress is a conditioned average only for the floating particles. As one could expect, the profiles of shear stress are similar to the variation of suspension ratio, as provided in Figure 4.12. The shear stress increases with Fr^* enhances, which is the product of the large liquid velocity gradient caused by a necessary increase in shaking frequency. Therein, a slight deviation for the maximum value at the case of the largest Fr^* (green line) is observed. This appearance is caused by the complexity of the liquid flow, while the overall trend is unchanged. With further increasing Dr value ($Dr > 0.25$), the $|\tau|^*$ is inhibited as the shaking speed is greatly reduced.

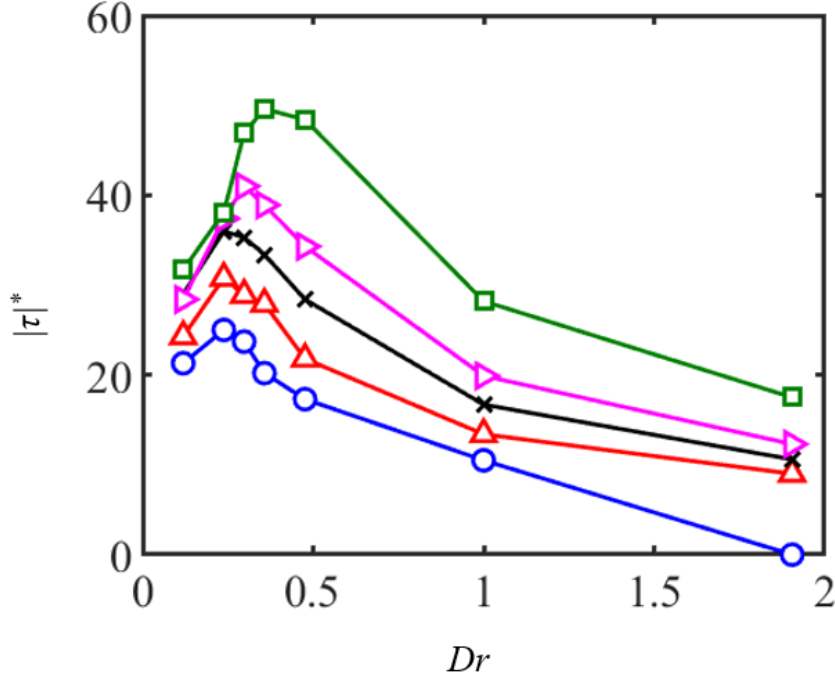


Figure 4.12 Time-averaged shear stress ($|\tau|^*$) acting on the particles as a function of Dr for the PAR method. The meanings of the lines are the same as Figure 4.10.

4.5.3 Analysis of the maximum value for suspension ratio and shear stress

In this section, the maximum value of suspension ratio shear stress with different Fr^* numbers is depicted in Figure 4.13. The profiles show that for the Fr^* number ranges studied in this research, the maximum suspension ratio increase gradually and then almost keep constant with further increment of Fr^* number. Nevertheless, the maximum shear stress increases monotonically, as presented in Figure 4.13 with blue line. Continuous improvement of Fr^* number should be avoided due to the production of strong shear stress. It is obvious that the enhancement in Fr^* number could not appreciably increase suspension of particles. This could be explained by the fact that, even for high Fr^* number, a small region near the free surface keeps steady or poorly concentrated in particles.

An analytical theory – potential flow theory is employed to explain the maximum value exhibited in the variation of suspension ratio and shear stress, which is a powerful tool to predict the movement of flow in a shaking tank [99]. For the method used in the present research, a nat-

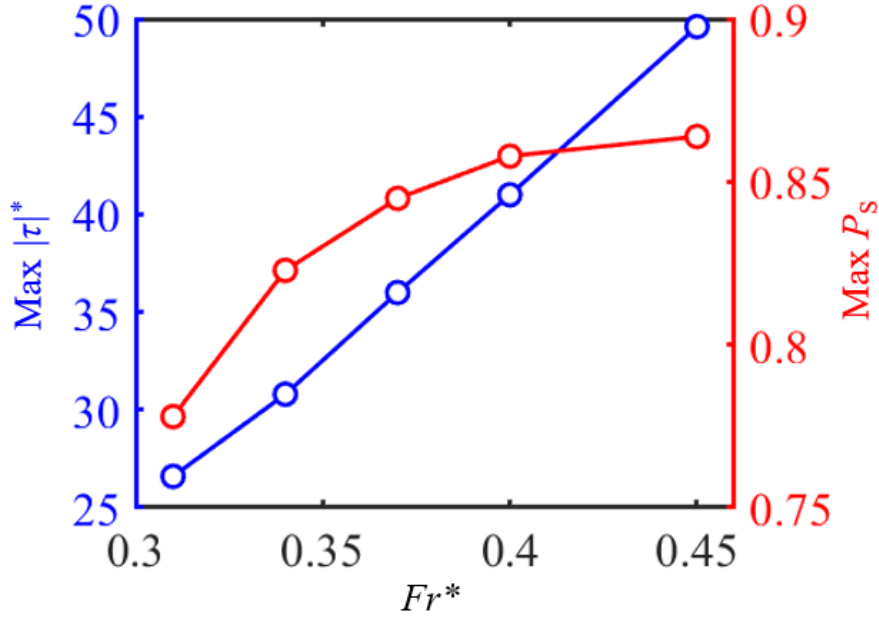


Figure 4.13 Maximum value of suspension ratio (P_s) and shear stress ($|\tau|^*$) as a function of Fr^* for the PAR method. The red line means the profile of suspension ratio. The blue line is profile of shear stress.

ural frequency ω_{1n} of the mode (1,n) given by the relationship $\omega_{1n}^2 = g\lambda_{1n} \tanh(\lambda_{1n}h)$, where $\lambda_{1n}=2\epsilon_{1n}/d$, and the ϵ_{1n} for each mode is a constant. Since the flow is dominated by the first mode $n=1$ [100], the shaking frequency and the first mode of natural frequency ω_{11} are compared. It is interestingly noted that the threshold appears when the shaking frequency is approximately equal to the natural frequency (ω_{11}), which implies resonance phenomenon is generated. The resonance strengthens oscillation of the culture liquid in the shaking tank.

4.6 Summary

The conventional shaking manner with one direction rotation (ODR) is hard to fulfill the practical demand due to its local accumulation and particle sedimentation. A novel shaking method with periodic alternate rotation (PAR) therefore is proposed, which is beneficial to suspend cells in the culture medium. Thus, further study the PAR is carried. And there are two important nondimensional parameters (Fr^* and Dr) that mainly affect the flow state. Thus, the dependence of Fr^* and Dr on the suspension ratio and shear stress has been investigated for the PAR method. Both

the suspension ratio and shear stress increase with the enhancement of Fr^* . However, as for different values of Dr , suspension ratio and shear stress firstly increase reaching to a threshold, then gradually decrease. The mechanism behind the peak value for suspension ratio and shear stress is also studied. It is interestingly found that this value always appears where the shaking frequency of the bioreactor is close to the natural frequency, which means a resonance phenomenon occurs, and has an important influence on the fluid motion. This finding corresponds with the results in a rocking bioreactor [35].

CHAPTER 5

BAYESIAN OPTIMIZATION FOR CULTURE CONDITION IN ORBITALLY SHAKEN BIOREACTOR WITH A BUMP BOTTOM

In this chapter, a numerical model is developed to improve the performance of the orbitally shaken bioreactor, which features a vaulted bump at the bottom wall. Quantitative evaluation of the mass transfer, suspension level, and shear stress of the new reactor is conducted and compared with the classical system under the same operating conditions. Further, the Bayesian optimization algorithm is employed to provide accurate predictions with the aim of obtaining the optimal operating conditions of the new bioreactor.

5.1 Background

Although the suspension ability has been greatly enhanced with the new developed PAR method, the energy consumption, however, is 10 – 100 times higher compared with that of the traditional shaking tank [101]. This compels us to concentrate on the optimization of conventional shaking method (ODR), and proceed to consider the geometrical changes of bioreactors. A cylindrical bioreactor featuring a hollow wall was developed [102]. The oxygen transfer and shear stress were estimated under various ratios between hollow and vessel diameter, while the potential behavior of the cells was not considered. A novel attempt with respect to change the bottom structure of the shaking bioreactor was considered [66]. In their study, it was demonstrated that a cone-shaped bottom structure is superior to others such as ellipsoid, flat, and sphere bottoms, because of its beneficial characteristics, including lower shear damage and high-efficiency oxygen mixing [67]. The potential behavior of the cells, however, was not considered. Even though bioreactors have already contributed significantly to the enhancement of bioprocess development, it has been argued that there is still considerable room for further improvements to the existing configurations [103, 104]. Thus, to this end, in this chapter, a new cylindrical orbitally shaken bioreactor with a bump at the bottom wall is proposed for cell cultivation.

As can be easily guessed, the optimization of the three control parameters of this new configuration, i.e., bump height (h_b), shaking velocity (ω), and shaking radius (R), significantly increase the computational cost of the numerical simulation. To reduce the associated computational cost, the Bayesian optimization technique, which is a model-based machine learning technology for process optimization and control, has increasingly caught researchers' attention for its higher accuracy without the need to implement costly and complex numerical calculations [105–108]. The technique is also well suited to bioprocesses, and in fact it has already been adopted in biotechnology to reduce the computational cost [74–76]. Accordingly, the Bayesian optimization is employed in this study to predict the optimal culture condition.

5.2 Methodology

In this study, the approximations made on the properties of cells and liquid have been described in the Chapter 2. The sketch and shaking trajectory of the modeled shaking bioreactor are illustrated in Figure 5.1. The cylindrical geometry has a diameter (d_t) of 0.75 m, and its height (h_t) is 0.225 m. The diameter of the bump (d_b) is 0.60 m. The culture liquid phase is assumed to have the same physical properties of water, and the volume (V_l) is 20 L. Further, the shaking tank rotates in the horizontal plane with various radii (R), and the trajectory follows a circle. The equation is as follows:

$$\mathbf{r} = \begin{pmatrix} x \\ y \\ z \end{pmatrix} = \begin{pmatrix} R \cos \omega t \\ -R \sin \omega t \\ 0 \end{pmatrix} \quad (5.1)$$

5.2.1 Operating conditions

Governing equations of the liquid phase and solid phase are same as those shown in Chapter 2. The total number of particles is 1000. In addition, the discrete valued of control parameters including the bump height (h_b), shaking velocity (ω), and shaking radius (R) with a reasonable increment are listed in Table 5.1. Initially, all the particles are uniformly distributed at the bottom wall, and the liquid is stationary. The no-slip condition is imposed on the flow velocity on the walls

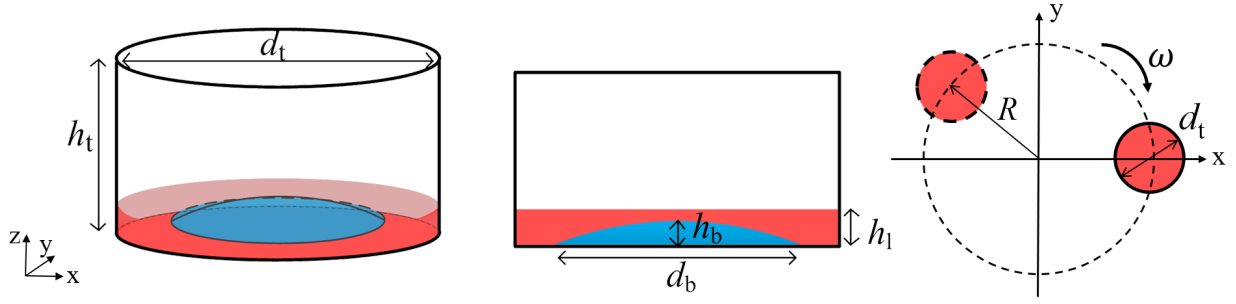


Figure 5.1 Sketch of the orbitally shaken bioreactor with a bump, where ω and R are the shaking velocity and radius, respectively.

of the bioreactor. All of the simulations are quantified in 60 s, which is confirmed that the mean value are well converged.

Table 5.1 Discrete values of the control parameters used for optimization.

h_b [m]	ω [rpm]	R [m]
0.01	20	0.01
0.02	40	0.02
0.03	60	0.03
0.04	80	0.04
0.05	100	0.05

5.2.2 Concentration equation

Continuous species transfer (CST), an analogous approach, is consistent with VOF model while satisfying mass balance [109, 110], which is used to calculate the mass transfer across the gas-liquid interface. The concentration of the interface is governed by the classical advection-diffusion equation,

$$\frac{\partial C}{\partial t} + \nabla \cdot (C \mathbf{v}_f) = \nabla \cdot (D \nabla C) - \nabla \cdot \left(D \frac{H - 1}{H\alpha + (1 - \alpha)} C \nabla \alpha \right) \quad (5.2)$$

where the C is the concentration of oxygen. and the second term in the right hand is caused by the concentration jump at the gas-liquid interface. The concentration (C) is expressed by combining both the concentration in the liquid phase and in the gas phase. And according to Henry laws,

the concentration in the liquid phase is proportional to that in the gas phase. Henry constant (H) herein is equal to 0.03. Thus, the expression of the concentration is written as follows:

$$C = \alpha C_l + (1 - \alpha) C_g \quad C_l = H C_g \quad (5.3)$$

And the diffusion coefficient (D) concerning the harmonic diffusion coefficient (D_h) and turbulent diffusion (D_e) is defined as:

$$D = D_h + D_e \quad (5.4)$$

where

$$D_h = \frac{D_l D_g}{\alpha D_g + (1 - \alpha) D_l} \quad D_e = \frac{\nu_e}{Sc_e} \quad (5.5)$$

where D_l ($= 2.01 \times 10^{-9} \text{m}^2/\text{s}$) and D_g ($= 1.92 \times 10^{-5} \text{m}^2/\text{s}$) is the species diffusion coefficient in the liquid and gas phase, respectively. The turbulent Schmidt number (Sc_e) is set 1000 in the present study.

5.2.3 Turbulence model

The flow with high rotating velocity in the tank is quite complex, which is not satisfied by either the standard or the RNG $k - \varepsilon$ models. Meanwhile, the $k - \varepsilon$ model has the capability to handle various fluid flow conditions, as it has a simple format, strong performance, and high accuracy [111]. To realize a more precise prediction of turbulent flow features, the realizable $k - \varepsilon$ turbulence model is applied because of the modified coefficient [112]. The transport equations related to the turbulent kinetic energy (k) and the dissipation of turbulent energy (ε) are:

$$\frac{\partial k}{\partial t} + (\mathbf{v}_f \cdot \nabla) k = \nabla \cdot \left[\left(\nu_f + \frac{\nu_e}{\delta_k} \right) \nabla k \right] + G_k - \varepsilon \quad (5.6)$$

$$\frac{\partial \varepsilon}{\partial t} + (\mathbf{v}_f \cdot \nabla) \varepsilon = \nabla \cdot \left[\left(\nu_f + \frac{\nu_e}{\delta_\varepsilon} \right) \nabla \varepsilon \right] + C_1 \frac{\varepsilon}{k} G_k - C_2 \frac{\varepsilon^2}{k + \sqrt{\nu_f \varepsilon}} \quad (5.7)$$

The term for the production of turbulent kinetic energy G_k is common in many of the turbulence models studied and is defined as:

$$G_k = -\nu_e (\nabla \cdot \mathbf{v}_f)^2 \quad (5.8)$$

where the turbulent viscosity is expressed as below:

$$\nu_e = -C_\mu \frac{k^2}{\varepsilon} \quad (5.9)$$

where C_μ represents the coefficient of turbulent viscosity. The model constant C_1 , C_2 , δ_k and δ_ε are determined according to the boundary layer, and defined as [112]:

$$C_1 = 1.44, C_2 = 1.9, \delta_k = 1.0, \delta_\varepsilon = 1.2$$

5.2.4 Evaluation parameters

The nutrition delivery to cells mainly depends on the gas exchange on the liquid surface, which greatly affects on the quality of the cells [113]. The oxygen volumetric mass transfer coefficient ($k_L a$) is regarded as an important parameter to evaluate the level of nutrient supply, which is the combination of oxygen mass transfer coefficient (k_L) and gas-liquid specific mass transfer area (a). k_L and a are expressed in the following forms by applying the eddy cell model [114]:

$$k_L = K \sqrt{D_1} \left(\frac{\bar{\varepsilon}}{\nu} \right)^{\frac{1}{4}} \quad (5.10)$$

$$a = \frac{S_i}{V_l} \quad (5.11)$$

where K is an empirical value, which is set to be 0.4 in the present study, and $\bar{\varepsilon}$ is the average dissipation rate of turbulence kinetic energy in the liquid phase. S_i is the gas-liquid interface area. Also, the suspension ratio (P_S) and shear stress ($|\tau|$) are regarded as an important factor in this chapter. The definitions have been stated in the Chapter 3 and Chapter 4.

5.2.5 Bayesian optimization algorithm

Bayesian optimization refers to a probabilistic-based method for seeking the optimum value of an objective function (f) based on the initial dataset (χ). The initial dataset is defined as:

$$\chi = \{(\mathbf{x}_1, y_1), (\mathbf{x}_2, y_2), (\mathbf{x}_3, y_3), \dots, (\mathbf{x}_n, y_n)\} \quad (5.12)$$

In regard to the unknown value (x_{n+1}, y_{n+1}), the Gaussian process is a reliable and powerful algorithm to predict the distribution due to its flexibility and tractability [74]. The formulation is expressed as [107]:

$$P(y_{n+1} | x_{n+1}, \chi) = \mathcal{N}(\mu(\mathbf{x}), \sigma(\mathbf{x})) \quad (5.13)$$

where the predictive mean function ($\mu(\mathbf{x})$) and standard deviation function ($\sigma(\mathbf{x})$) under the Gaussian process prior are defined as:

$$\mu(\mathbf{x}) = \mathbf{k}_*^T \mathbf{K}^{-1} \mathbf{y}, k_{**} \quad (5.14)$$

$$\sigma(\mathbf{x}) = \mathbf{k}_*^T \mathbf{K}^{-1} \mathbf{k}_* \quad (5.15)$$

where

$$K_{ij} = k(\mathbf{x}_i, \mathbf{x}_j) \quad (5.16)$$

$$\mathbf{k}_* = [k(\mathbf{x}_{n+1}, \mathbf{x}_1), k(\mathbf{x}_{n+1}, \mathbf{x}_2), \dots, k(\mathbf{x}_{n+1}, \mathbf{x}_n)] \quad (5.17)$$

$$k_{**} = k(\mathbf{x}_{n+1}, \mathbf{x}_{n+1}) \quad (5.18)$$

$k()$ is a kernel function, and here the Matérn 5/2 kernel is employed:

$$k(\mathbf{x}_i, \mathbf{x}_j) = \left(1 + \frac{\sqrt{5}r}{l} + \frac{5r^2}{3l^2}\right) \exp\left(-\frac{\sqrt{5}r}{l}\right) \quad (5.19)$$

where $r = |\mathbf{x}_i - \mathbf{x}_j|$, l is a hyper parameter. The parameter x_{next} obtained from acquisition function $a(\mathbf{x})$ is used to regarded as the next point for the CFD-DEM calculation, expressed as:

$$x_{\text{next}} = \operatorname{argmax}_x a(x) \quad (5.20)$$

The used acquisition function herein is the expected improvement (EI) as it performs well in maximization problems, and the expression is:

$$a_{\text{EI}} = (f(x_{\text{best}}) - \mu(x))\Phi(\gamma) + \sigma(x)\phi(\gamma) \quad (5.21)$$

$$\gamma = \frac{f(x_{\text{best}}) - \mu(x)}{\sigma(x)} \quad (5.22)$$

$$x_{\text{best}} = \text{argmax}_{x_n} f(x_n) \quad (5.23)$$

where Φ and ϕ are the cumulative density function and the probability density function of the standard normal distribution, respectively. The maximum value obtained by EI algorithm is regarded as the next calculation parameter. The Bayesian optimization is carried out using Gpyopt python library [115]. This process is repeated until the maximum value of objective function is converged. As an aid to a comprehensive understanding of the methodology, the framework for implementing Bayesian optimization is shown in Figure 5.2. The mentioned initial database (χ) for the optimization procedure is given in Table 5.2.

Table 5.2 Initial dataset of the control parameters and the corresponding (P_S^* , $|\tau|^*$, f) used for optimization.

h_b [m]	ω [rpm]	R [m]	P_S^* [-]	$ \tau ^*$ [-]	f [-]
0.01	20	0.01	0	0.014	-0.014
0.01	20	0.05	0	0.062	-0.062
0.01	100	0.01	0.002	0.284	-0.282
0.01	100	0.05	0.056	0.739	-0.683
0.05	20	0.01	0	0.016	-0.016
0.05	20	0.05	0	0.062	-0.062
0.05	100	0.01	0.013	0.275	-0.262
0.05	100	0.05	1	0.679	0.321

In the present simulation, the considered suspension level and shear force associated with the control parameters are important evaluation factors. Thus, the objective function is defined as:

$$f = P_S^* - |\tau|^* \quad (5.24)$$

where the normalized suspension ratio (P_S^*) and shear stress ($|\tau|^*$) are expressed as:

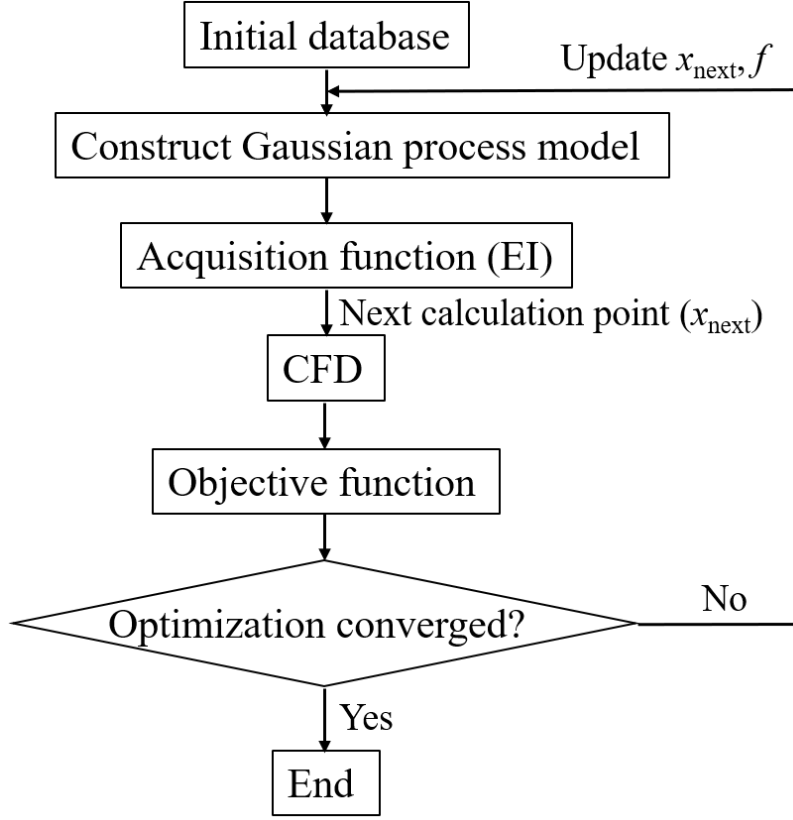


Figure 5.2 Framework of Bayesian optimization.

$$P_S^* = P_S / P_{S\max} \quad |\tau|^* = |\tau| / |\tau|_{\max} \quad (5.25)$$

where $P_{S\max}$ and $|\tau|_{\max}$ are the maximum values of suspension ratio and shear stress, respectively, in all time steps.

5.3 Effect of the bump

As the bump at the bottom of the bioreactor plays an essential role in the proliferation of stem cells, the first part of this study aims to evaluate the effect of the bump, under identical shaking modes ($\omega = 80$ rpm, $R = 0.03$ m), on the oxygen volumetric mass transfer coefficient ($k_L a$), suspension ratio (P_S), and shear stress ($|\tau|$).

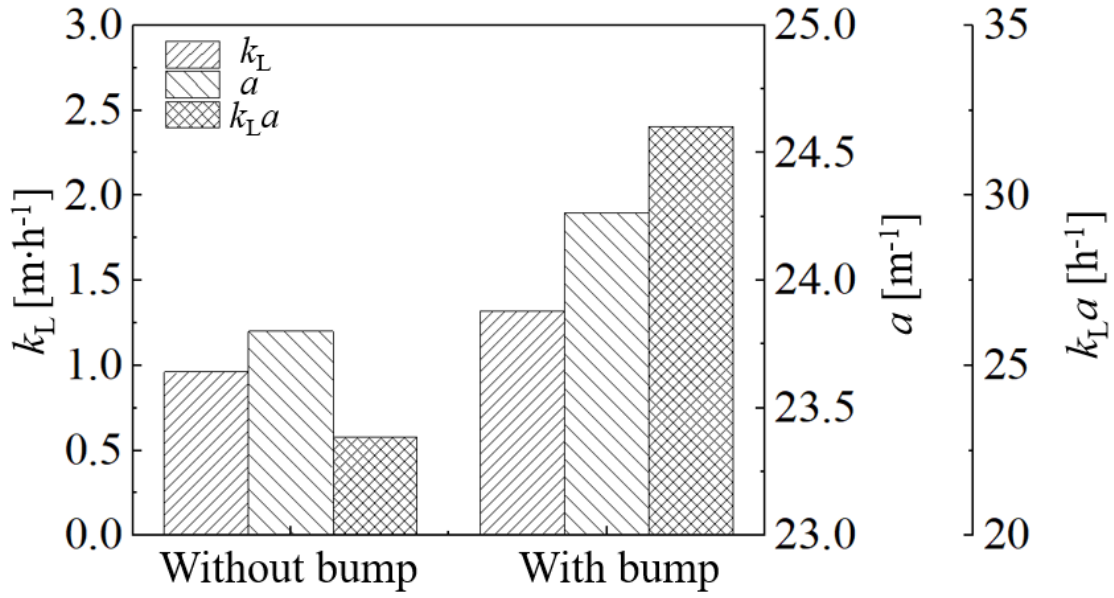


Figure 5.3 Effect of the bump on mass transfer coefficient (k_L), specific gas-liquid interface area (a), and oxygen volumetric mass transfer coefficient ($k_L a$). The parameters are set as: $h_b = 0$ or 0.04 m, $\omega = 80$ rpm, and $R = 0.03$ m.

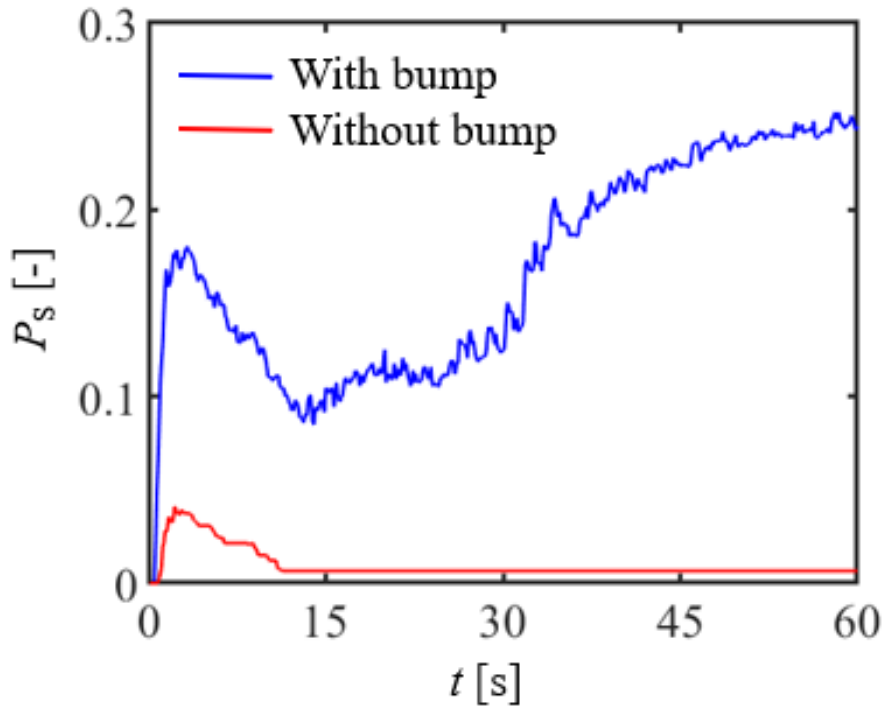


Figure 5.4 Effect of the bump on suspension ratio (P_s) at the same control parameters as in Figure 5.3.

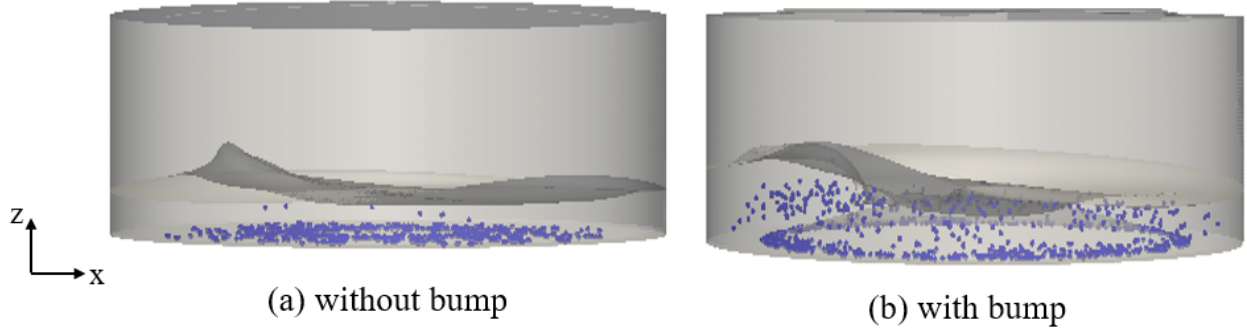


Figure 5.5 Snapshot of the particle distribution in the culture liquid at $t = 60$ s of (a) without bump and (b) with bump. The blue points represent particles.

The oxygen mass transfer capability, which includes k_L , a , and $k_L a$, is a crucial factor in the design, optimization, and scale-up of the cell culture process [116]. The results calculated from Equation (5.10) for two bottom configurations (with and without the bump) are given in Figure 5.3. The results show a clear distinction between these two cases. The k_L value, which depends only on the turbulent dissipation rate (ε), increases by 37% in the “new bioreactor” (the one with the bump). This is because the presence of the bump makes it easier for the flow to become turbulent. At the same time, the value of a is also greatly enhanced because of the bump. Consequently, the associated $k_L a$ value increases too. Across the board, it can be stated that the new bioreactor design introduced here optimizes the culture conditions; in particular, the mass transfer is enhanced.

To obtain a comprehensive understanding of the new bioreactor, the suspension ratio and shear stress are further quantitatively evaluated. Variations of the suspension ratio with time in both cases, the bioreactors with and without the bump, are illustrated in Figure 5.4. As can be seen, the configuration with the bump is more efficient for suspending particles. This may be attributed to the development of an upward vertical flow velocity component due to the presence of the bump at the bottom. A more intuitive observation of cell suspension state is provided in Figure 5.5. It is evident that the large deformation of free surface is formed under the action of the bump. Accordingly, the majority of particles are afloat in the liquid. Nonetheless, the fluid flow state is relatively steady. All the particles accumulate and sink in the center region at the bottom wall. Such local cell agglomeration and attachment were also observed experimentally [21], and it is

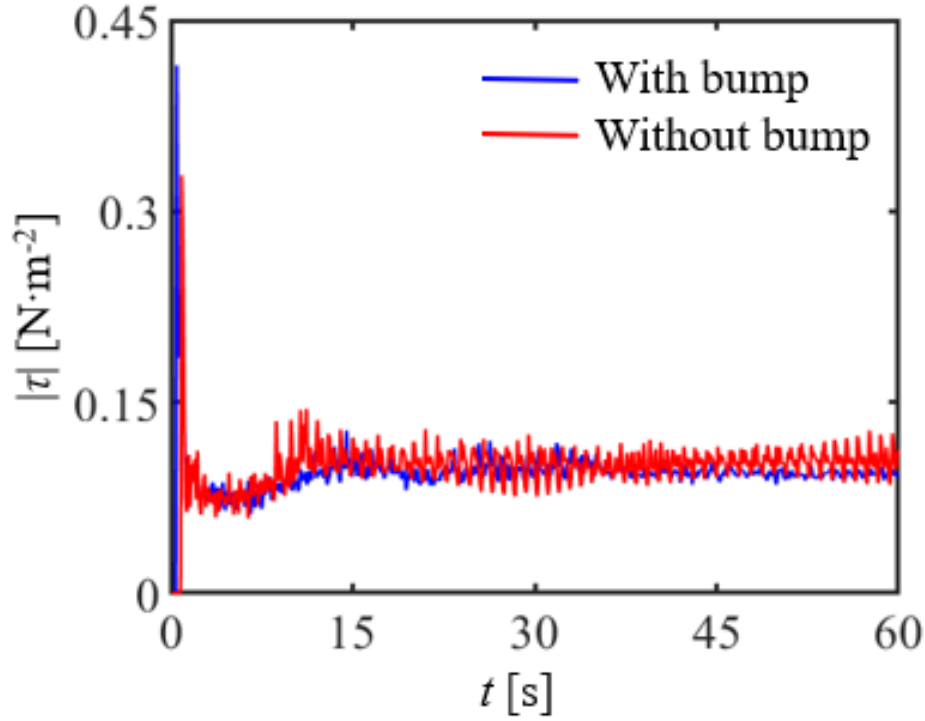


Figure 5.6 Effect of the bump on shear stress ($|\tau|$) at the same control parameters as in Figure 5.3.

well known that this behavior can damage cells.

Nevertheless, the shear stress caused by the flow velocity gradient at a certain shaking velocity and radius is almost identical for these two cases, as shown in Figure 5.6. This means that the bump does not increase the shear stress. In other words, the cell colonies experience the same range of shear stresses in the new bioreactor, and consequently, the shear stress profiles are similar in both configurations. This could be attributed to velocity vector along axial direction, as presented in Figure 5.7. There is no obvious difference of the vector in the horizontal plane, which means the bump structure does not contribute to the increase of shear stress.

From the above results, one can state that the new bioreactor design developed here would offer a better process environment for sustaining adequate oxygen supply as well as suspension ability during cell culture processing. This confirms that the bioreactor designed with a bump can be considered as a better candidate for cell cultivation. Therefore, the pursuit of further investigation of this new bioreactor design is the key objective in the following sections.

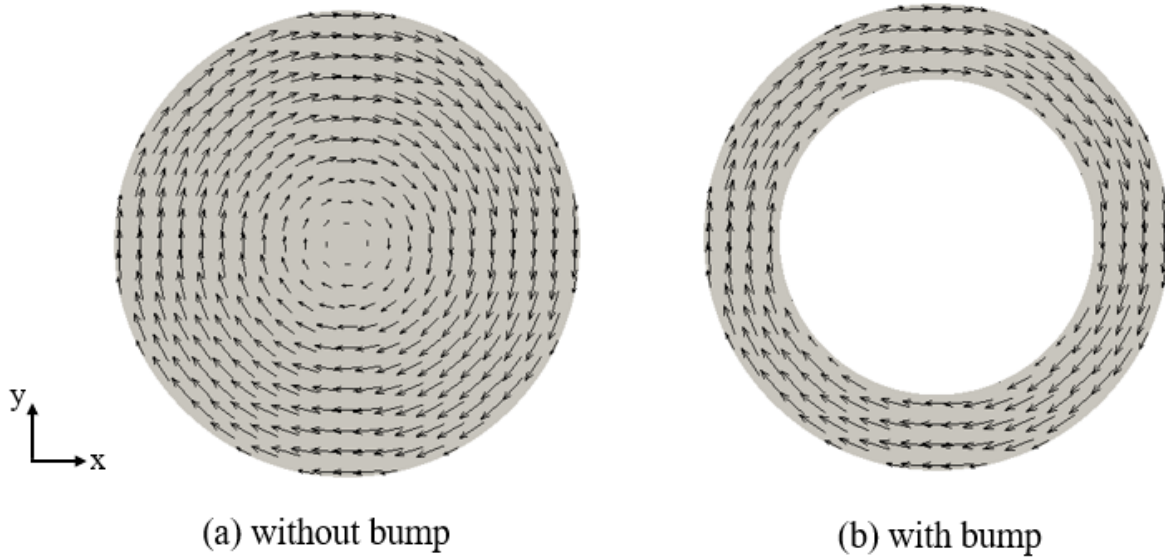


Figure 5.7 Top view of the time-averaged velocity vector at $z = 0.01$ m for the case of (a) with bump and (b) without bump.

5.4 Optimization of control parameters

In this section, Bayesian optimization is implemented to find an optimum parameter for the bioreactor with the bump. Almost all possible operating variables are considered in the optimization process. The values of control parameters (h_b , ω and R) and corresponding results at each iteration are presented in Table 5.3. It is noteworthy that although a regular numerical simulation would generally require 125 ($5 \times 5 \times 5$) calculations for the combination of three control parameters, only 24 calculations are conducted with the Bayesian optimization algorithm, which reduces the computational cost by approximately 80%. A more clear illustration for the variation of the objective function is provided in Figure 5.8. As can be seen, the value of the objective function, f , is large in the initial stage of iteration. It should be noted that the value gradually becomes smaller, especially after 12 steps. As a consequence, it is recommended the optimal value of the objective function is selected from the initial iteration step when the calculation is converged.

The calculation run is considered complete when the maximum value of the objective function (f) approaches a constant value. The variation in the maximum f associated with each iteration step is given in Figure 5.9. The maximum f curve quickly evolves into a horizontal line. Hence,

we assume that the present iterative steps are sufficient to reach a high convergence level.

Table 5.3 Calculated parameters, h_b , ω and R and results during the Bayesian optimization process at each iteration.

$Iter$	h_b [m]	ω [rpm]	R [m]	P_S [-]	$ \tau $ [-]	f [-]
1	0.05	80	0.05	0.0039	0.30	-1
2	0.04	100	0.05	0.72	0.20	-0.28
3	0.05	100	0.04	0.65	0.15	0.34
4	0.04	100	0.03	0.70	0.15	0.41
5	0.05	100	0.03	0.33	0.098	0.11
6	0.04	100	0.04	0.30	0.10	0.058
7	0.03	100	0.04	0.45	0.15	0.083
8	0.03	100	0.03	0.66	0.22	0.13
9	0.04	80	0.04	0.51	0.11	0.29
10	0.04	80	0.04	0.25	0.091	0.017
11	0.03	80	0.03	0.36	0.11	0.094
12	0.04	60	0.04	0.037	0.16	-0.49
13	0.03	80	0.03	0.019	0.25	-0.82
14	0.05	80	0.05	0.098	0.091	-0.18
15	0.03	80	0.03	0.049	0.11	-0.32
16	0.04	100	0.04	0.042	0.096	-0.27

Once the simulation calculation has converged, the optimal condition is immediately captured. To obtain the specific parameters, the distribution of P_S and $|\tau|$ for the objective function f with each step is shown in Figure 5.10. As emphasized earlier, the large value of the index of f for the best performance is expected because an improved suspension ability and small shear stress are realized. Thus, an optimal condition (point A in Figure 5.10) is successfully predicted throughout the calculation process, and the corresponding control parameters are:

$$\begin{pmatrix} h_b \\ \omega \\ R \end{pmatrix} = \begin{pmatrix} 0.04 \text{ m} \\ 100 \text{ rpm} \\ 0.04 \text{ m} \end{pmatrix}$$

To gain further insight into the optimized bioreactor with the bump at the bottom, various descriptive statistics such as the dissolved oxygen concentration (C), suspension ratio (P_S) and shear stress (τ), are analyzed in the next section.

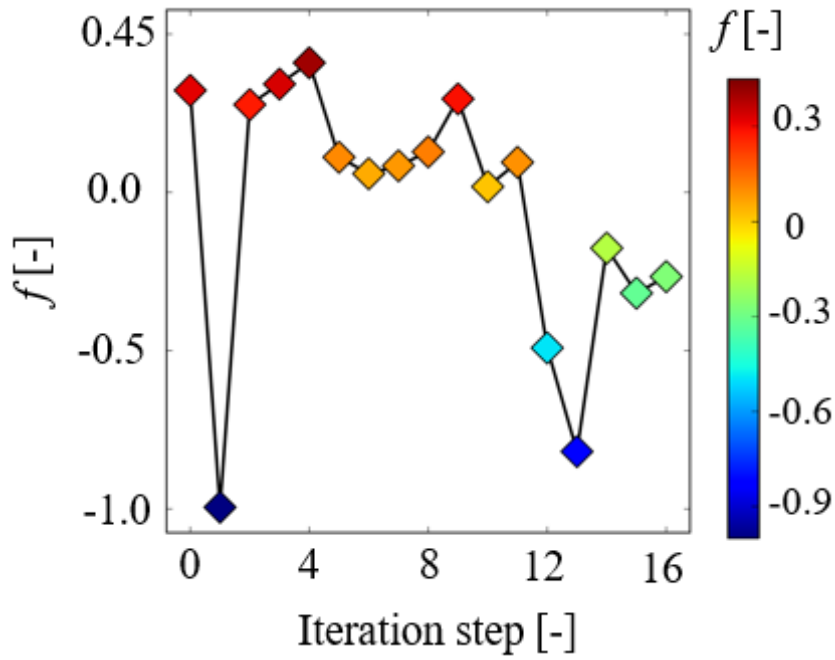


Figure 5.8 Variation of the value of the objective function, f with the iteration step during the Bayesian optimization process, where the color of point represents the magnitude of f .

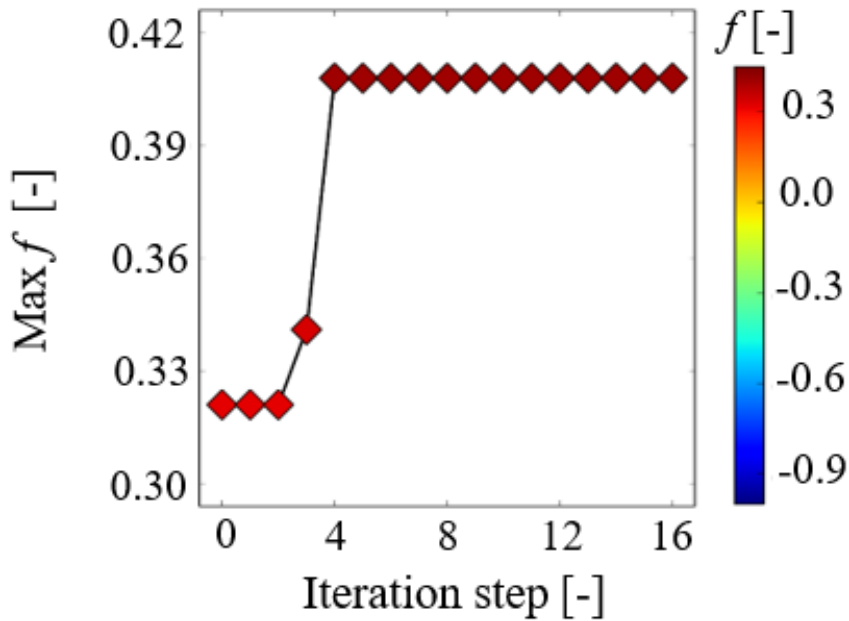


Figure 5.9 Variation of the maximum objective function (f) with the iteration step during the Bayesian optimization process.

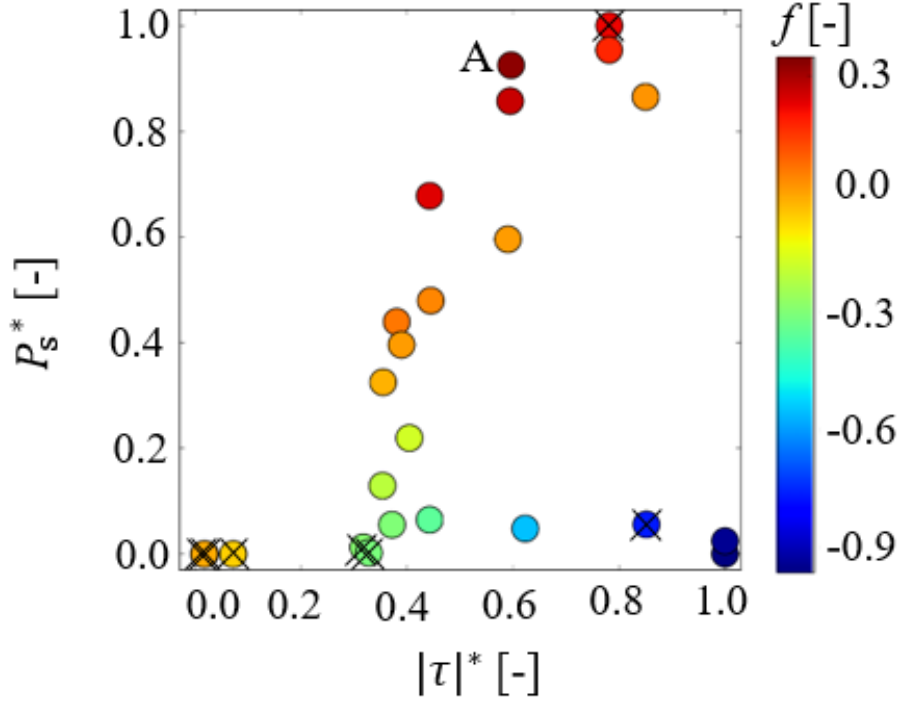


Figure 5.10 Distribution of P_s^* and $|\tau|^*$, where the color of the point represents the magnitude of the objective function, and the points with crosses indicate the initial parameters for Bayesian optimization.

5.5 Characteristics of the optimal state

Along the lines described in Sect. 5.3, a comparison between the optimal and initial bioreactors is conducted. It must be mentioned that the initial calculation parameters used here are:

$$\begin{pmatrix} h_b \\ \omega \\ R \end{pmatrix} = \begin{pmatrix} 0.04 \text{ m} \\ 80 \text{ rpm} \\ 0.03 \text{ m} \end{pmatrix}$$

Due to the low solubility of the culture medium, oxygen is the only nutrient that is required for cell growth throughout the cultivation process. If sufficient oxygen is not supplied, the viability and proliferation rate of the cell will decay. Thus, the ability to maintain an adequate oxygen concentration level is commonly regarded as a crucial factor for the selection of a bioreactor. Figure 5.11 depicts the spatial visualization of the oxygen concentration distribution and time-averaged velocity vector. As can be seen, the oxygen concentration of the initial bioreactor (with the bump) is larger across the whole liquid region than that of the conventional bioreactor (without

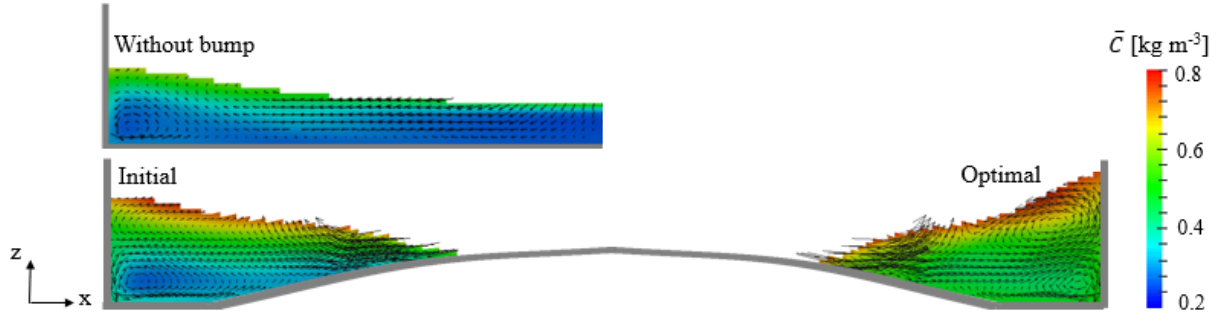


Figure 5.11 Time-averaged velocity vector and oxygen concentration distribution at a vertical section for the conventional (without the bump), initial, and optimal bioreactors. The cross section is located at $y = 0$.

the bump), which agrees with Figure 5.3. Again, it is confirmed that the newly designed bioreactor exhibits better performance in terms of oxygen transfer. Further, it is evident that two vortices form in the vicinity of the sidewall in the case of the optimal state, but such vortices are not easily observed under the initial operating condition. The swirling motion observed in the optimal state enhances oxygen mass transfer due to the large shaking velocity and radius. This leads to a high oxygen concentration locally, especially in the gas-liquid interface. Overall, the optimal bioreactor shows better oxygen transfer performance than that the initial bioreactor.

Likewise, the comparison of different shaking strategies is presented in Figure 5.12. Compared with the initial operating conditions, a noticeable increase by a factor of 3.5 in the suspension ratio under the optimal conditions is observed. This significant increase could be attributed partially to the fact that the upward flow velocity is stronger in the vicinity of the bump, as seen in Figure 5.11. On the other hand, a dimensionless parameter, the Froude number ($Fr = R\omega/\sqrt{d_t g}$), which is the ratio of the inertial and gravitational forces, is introduced to shed light on the mechanism involved. It should be noted that the main difference between the initial and optimal states is the ω and R values. Therefore, the large value of the Froude number indicates that the effect of the flow inertia in the optimized system is more predominant during the shaking process, which enables particles to float easily in the culture medium. Moreover, a similar result has been demonstrated in our previous work [117].

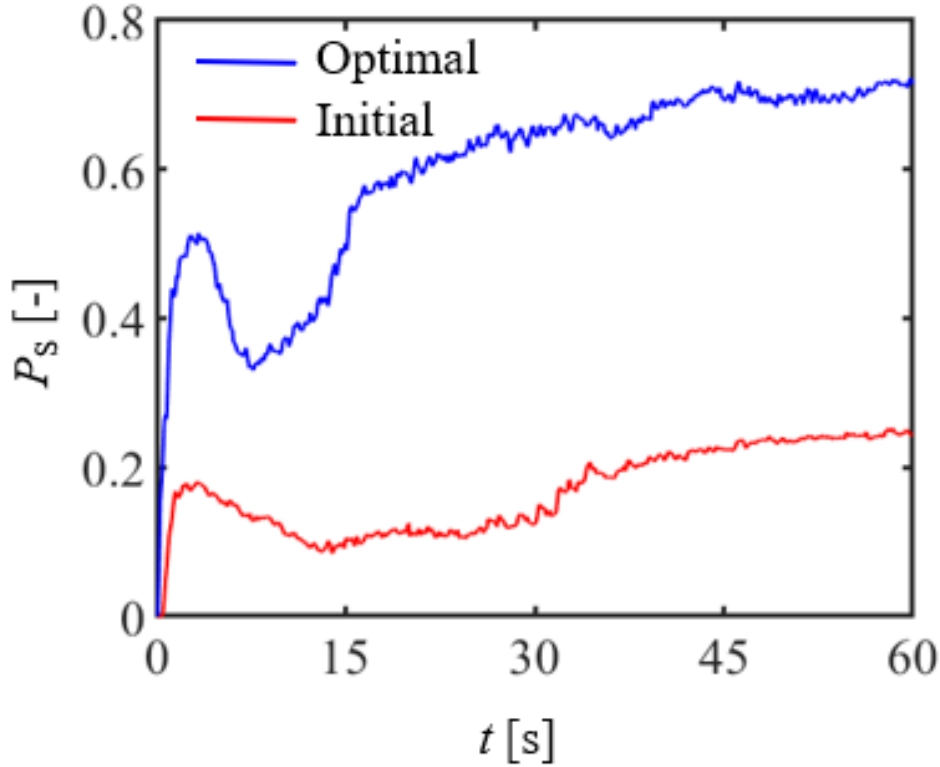


Figure 5.12 Variation of suspension ratio (P_s) as a function of time for the initial and optimal bioreactors.

With respect to the shear stress, as described above, the shear stress is a key factor in determining whether a bioreactor is suitable for the cultivation of shear-sensitive cells such as plant cells, mammalian cells, and stem cells [97]. Figure 5.13 shows the variation of shear stress with time in the cases of the initial and optimal operations. As can be seen, although the magnitude of the shear stress is approximately 1.5 times higher in the optimal condition, the increased amplitude is not significant compared with the profiles of the suspension ratio. In addition, it should be pointed out that an acceptable level of shear stress acting on the particles is achieved, and the average value in the optimal case is approximately equal to 0.15 N/m^2 , which is much lower than the threshold value of 0.4 N/m^2 that has been reported as leading to cell death [118].

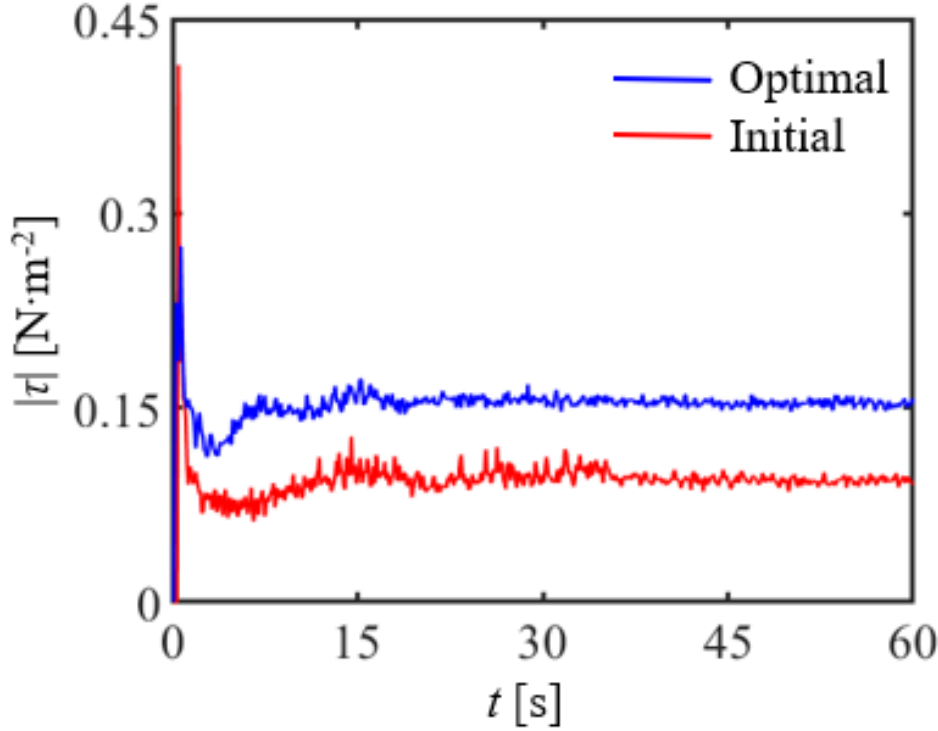


Figure 5.13 Variation of shear stress ($|\tau|$) as a function of time for the initial and optimal bioreactors.

5.6 Summary

We develop a novel orbitally shaken bioreactor installed with a vaulted bump on the bottom wall. It is evident that the presence of the bump has a positive influence on the oxygen volumetric mass transfer coefficient ($k_L a$) and the suspension ratio (P_S) of the particles, whereas it has less effect on the shear stress ($|\tau|$). Because a great number of calculations are required to seek for a suitable operation condition including the bump height (h_b), shaking velocity (ω), and shaking radius (R), the implement of the Bayesian optimization algorithm greatly reduces the calculation cost by nearly 80% with a high accuracy. The obtained optimal parameters are preferable to culture cells due to the sufficient oxygen supply and high suspension capability. Although the shear stress is a litter higher, it is much lower than the threshold value that causes cell death.

CHAPTER 6

CONCLUSIONS AND FUTURE PERSPECTIVES

6.1 Numerical optimization of cell dispersion in a rocking bioreactor for static culture

Developing a suitable condition in a bioreactor plays an essential role in culturing iPS cells meanwhile remaining cells' viability and speciality. A series of CFD simulations are performed to characterize a bioreactor with various configurations. The cells behavior and fluid flow characteristics in the bioreactors have been thoroughly investigated under different operational conditions.

A rocking bioreactor that depends on gravity with multidimensional oscillation has been widely used in industrial settings. The behavior of the cells as well as shear stress in the bioreactor are studied using numerical simulation. The following conclusions are drawn from the analysis.

- The shaking number strongly affects particle dispersion. Considering the energy consumption and quality of cells, an optimal shaking number which is equal to 8 times is predicted.
- Under the present settings, with the increments in angular velocity and angle, the shear stress increases monotonically owing to the stronger velocity gradient.
- A larger shaking velocity and angle could distribute the cells effectively. Therefore, choosing a suitable operational condition concerning the practical application is necessary.
- The effect of shaking velocity on the aggregation ratio can be negligible at a larger angle and shear stress. Further, an optimal condition considering the uniformity of particle dispersion and shear stress is characterized by a nondimensional ratio.

6.2 Numerical investigation of rotating method in an orbitally shaken bioreactor

CFD simulations are performed to characterize the two rotation methods in an orbitally shaken bioreactor. The suspension level and shear stress acting on cells are examined for different operational conditions. The main conclusions drawn are :

- A new periodic alternate rotation (PAR) method is introduced, and able to extremely increase the quantity of afloat cells compared with the common one direction rotation (ODR) method.
- In the PAR system, the suspension ratio and shear stress highly depend on the controlling parameters, Fr^* and Dr .
- The maximum values for P_S and $|\tau|^*$ are found, where the shaking frequency of the tank is close to the natural frequency. It should be mentioned that a resonance phenomenon has an important influence on the fluid motion and brings explicit understanding for the shaking bioreactor design.

6.3 Bayesian optimization for culture condition in an orbitally shaken bioreactor with a bump bottom

Numerical simulations are carried out to investigate the performance of an orbitally shaken new bioreactor with a bump on the bottom wall. The following conclusions are drawn from the study.

- The simulation results show that the presence of a bump in the bioreactor has a positive influence on the oxygen volumetric mass transfer coefficient ($k_L a$) and the suspension ratio (P_S) of the particles, whereas it has less effect on the shear stress ($|\tau|$).
- The new bioreactor is optimized in terms of bump height (h_b), shaking velocity (ω), and shaking radius (R), is carried out using the Bayesian optimization algorithm. The Bayesian optimization reduces the computational cost by approximately 80%.
- The optimal cultivation conditions are much more favorable from the standpoint of culturing cells owing to its sufficient oxygen supply and high suspension level compared to those of the initial state.

6.4 Future perspectives

The work presented in this thesis has investigated the hydrodynamic characteristics and features of the rocking bioreactor, and cylindrical orbitally shaken bioreactor with various configurations

and shaking manners using numerical simulations. However, there are still remaining problems that need to be further studied.

- The culture medium has the same physical property with water in the current work, which is quite different with the that in the experimental study. From a process engineering perspective, it is necessary to consider the effect of properties of culture medium on the cell cultivation.
- As discussed, the bottom structure has an significant effect on the characteristics of the flow field. Nevertheless, the bump diameter considered in this work is a fixed value. Further optimization of bump diameter is required to obtain an optimal bottom geometry.
- The Bayesian optimization algorithm appears to be a powerful, feasible and reliable methodology for the optimization of orbitally shaken bioreactors. It is hoped that this technology is applicable to further design and improvements of other bioreactors, such as rocking bioreactor, stirred-tank bioreactor, rotating wall bioreactor.

APPENDIX

RELATION BETWEEN NUMBER OF AFLOAT PARTICLES AND SHEAR STRESS

In the case of the PAR method, the effect of Fr^* and Dr on suspension ratio and shear stress has been illustrated in the Chapter 4. However, the relation between suspension ratio and shear stress for different external force (Fr^*) is not clear. Herein, the profile of number of afloat particles (N_s) as a function of shear stress ($|\tau|^*$) is shown in Figure A.1. As can be seen that, the enhancement of Fr^* can significantly increase the number of afloat particles in the same range of shear stress. It also should be noted that the difference is not obvious where the shear stress is 28.3, because of the little distinction of Fr^* . Overall, the large value of Fr^* is beneficial to suspend adequate amount of particles in the liquid, which is an indispensable factor of orbitally shaken bioreactor for suspension culture.

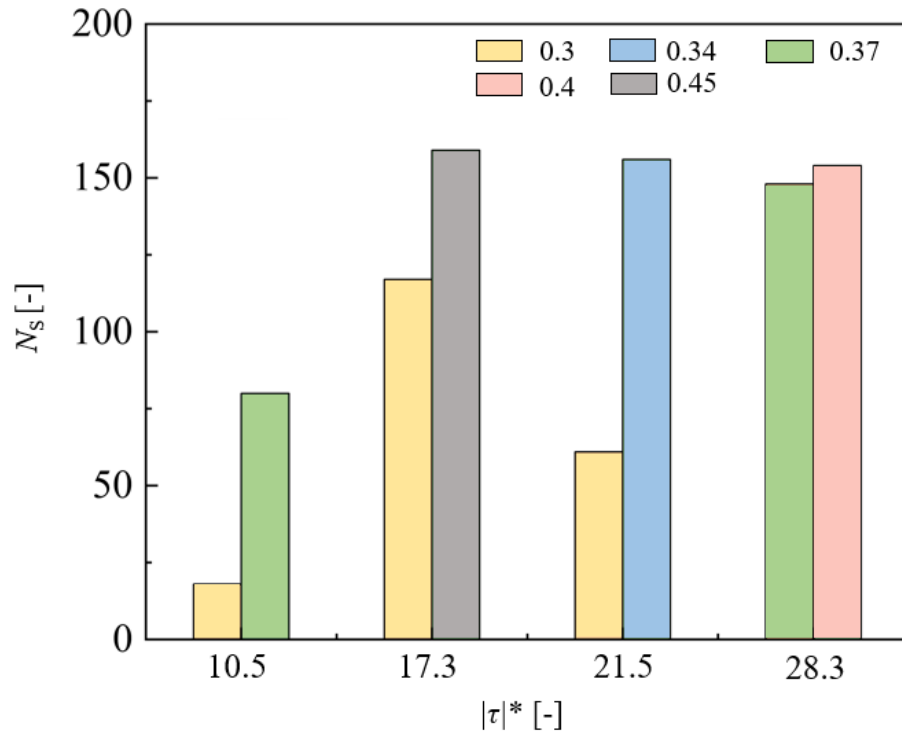


Figure A.1 Number of afloat particles (N_s) as a function of shear stress ($|\tau|^*$).

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LIST OF SYMBOLS

Nomenclature

a	gas–liquid specific mass transfer area [m^{-1}]
$\mathbf{a}$	acceleration [-]
a_{EI}	acquisition function [-]
A_{m}	compensation coefficient [-]
A_{p}	projected area [m^2]
C	concentration [kgm^{-3}]
C_1	constant [-]
C_2	constant [-]
C_{D}	drag coefficient [-]
C_{L}	empirical lift coefficient [-]
C_{μ}	coefficient of turbulent viscosity[-]
C_v	aggregation ratio [-]
d	diameter [m]
D	effective diffusion coefficient [m^2s^{-1}]
D_{h}	harmonic diffusion coefficient [m^2s^{-1}]
Dr	eccentricity [-]
$\mathbf{e}_z$	unit vector [-]
f	objective function [-]
$\mathbf{F}$	external force [N]

Fr	Froude number [-]
$\mathbf{g}$	gravitational acceleration [ms^{-2}]
G_k	production of turbulent kinetic energy [ms^{-2}]
h	height [m]
H	Henry constant [-]
k	turbulent energy [m^2s^{-2}]
$k()$	kernel function [-]
K	constant [-]
$\mathbf{K}$	matrix [-]
k_L	mass transfer coefficient [m^2s^{-2}]
$k_L a$	volumetric mass transfer coefficient [s^{-1}]
L	characteristic length scale [m]
l	hyper parameter [-]
m	mass [kg]
$\mathbf{n}$	unit vector normal to the liquid surface [-]
N_A	total number of particles [-]
N_d	number density [-]
N_R	wave number [-]
N_S	number of floating particles [-]
p	pressure [Nm^{-2}]
P	Gaussian process regression [-]
P_S	suspension ratio [-]
r	weight norm [-]

$\mathbf{r}$	position of the vessel center [m]
R	shaking radius [m]
Re_p	particle Reynolds number [-]
Re_ω	shaking Reynolds number [-]
R	rotating radius [m]
S	surface area [m ²]
Sc_e	turbulent Schmidt number [-]
S_i	gas–liquid interface area [m ²]
t	time [s]
T	characteristic period [-]
$\mathbf{U}$	shaking tank velocity [ms ⁻¹]
$\mathbf{v}$	velocity [ms ⁻¹]
V	liquid volume [m ³]
$\mathbf{v}_r$	relative velocity [ms ⁻¹]
$\mathbf{v}_{slip}$	slip velocity [ms ⁻¹]
We	Weber number [-]
Wo	Womersley number [-]
x_{next}	next evaluation point [-]

Greek letters

α	volume fraction [-]
β	nondimensional parameter of turbulence onset estimation [-]
χ	initial dataset [-]

ε	dissipation rate of turbulent kinetic energy [m^2s^{-3}]
δ	delta function [m^{-1}]
δ_ε	turbulent Prandtl number for dissipation rate [-]
δ_k	turbulent Prandtl number for kinetic energy [-]
γ	standard normal deviate [-]
μ	viscosity [$\text{Pa}\cdot\text{s}$]
$\mu(\mathbf{x})$	predictive mean function [-]
κ	curvature of liquid-gas interface [-]
λ	constant [-]
ν	kinematic viscosity [m^2s^{-1}]
ν_e	eddy viscosity [m^2s^{-1}]
$\mathcal{N}$	normal distribution [-]
ρ	density [kgm^{-3}]
τ	shear stress [Nm^{-2}]
θ	angle [-]
σ	surface tension [Nm^{-1}]
$\sigma(\mathbf{x})$	standard deviation function [-]
ω	angular velocity [rads^{-1}]
Ω_c	local particle vorticity magnitude [s^{-1}]
ϕ	probability density function [-]
Φ	cumulative density function [-]
Δx	maximum liquid element displacement [m]

Subscripts

b		bump
D		drag term
e		turbulent eddy
f		liquid phase
g		gas phase
G		gravitational term
L		lift force term
max		maximum value
p		solid phase
ref		reference scale
t		tank
vm		virtual mass term
x		x direction
y		y direction
z		z direction
$*$		nondimensional value

LIST OF ABBREVIATIONS

Computational fluid dynamic	CFD
Continuous species transfer	CST
Discrete element method	DEM
Embryonic stem	ES
Expected improvement	EI
Finite volume method	FVM
Hematopoietic stem	HS
Induced pluripotent stem	iPS
Inverted frustoconical structure	IFSB
Multi-direction reciprocal shaking	MRS
National aeronautics and space administration	NASA
Neural stem	NS
One direction rotation	ODR
Orbitally shaken bioreactor	OSR
Periodic alternate rotation	PAR
Pressure implicit with splitting operator	PISO
Rotating cell culture system	RCCS
Single-direction reciprocal shaking	SRS
Slow turning lateral vessel	STLV
Stirred-tank bioreactor	STR

Volume of method	VOF
World health organization	WHO

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LIST OF PUBLICATIONS

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[Conferences]

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