Cultivation of the photosynthesis microorganism in a Taylor-Couette vortex flow with a small aspect ratio

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Abstract. This study focuses on the dynamics of the Taylor-Couette Vortex Flow (TVF) in a photo-bioreactor in which CO2 is changed to O2 with high efficiency by the photosynthesis ability of micro algae. Stirring by means of a screw propeller is generally used for a simple agitation. However, the problem is that there exists a very high shearing flow region just near the propeller, which causes the destruction of the alga cell by the shearing force. In contrast, the TVF mixing is expected to reduce such a local and random shearing force because of their column of steady and orderly vortices. In this study, the relationship between the microorganism growth rate and the flow structures in dilute suspensions of a TVF is investigated and the flow characteristics are measured by using an ultrasonic velocity profiler with a small aspect ratio of 3.

1. INTRODUCTION

Bio-reactors are utilized in many applications, for example, food, medical and environmental areas, because it provides desired products without complex processes and produces less by-products (secondary-compounds) by use of biocatalysts; animals and plants cells, and microorganisms. Bioreactors require agitating the animal and plant cells, otherwise those cells may be precipitated with coagulation, and nutrition doesn’t spread uniformly. Generally sedimentation with coagulation is an obstacle to the culture multiplication. Therefore, an appropriate stirring method is expected without destructing the cells; and mixing them uniformly with low shearing force. We adopted a stirring method based on Taylor-Couette Vortex Flow (TVF), which enables uniform and mild mixing without causing local shearing flows seen in screw-type stirring. Besides, recently, TVF has been applied intensively to bioindustry and medical field (Wreley et al., 1999).

TVF can be obtained typically as a vortex flow produced by two concentric cylinders, with the inner one rotating. When the aspect ratio between their gap and height is infinite, TVF has been investigated as the chaos with the spectrum evolution of the disturbance. The practical usage accelerates its compaction, and the study of TVF flow with finite boundaries (end-walls) and small aspect ratio. In the case of TVF with a small aspect ratio, the influence of the Ekman boundary is significant, and its flow pattern becomes more complex even with comparatively low Reynolds number, resulting in the decreased original mixing ability.

Nakamura (1988) studied the flow patterns with a variety of aspect ratios (1-8). Benjamin (1978) studied the mutation of primary flow by changing the length of comparatively short annulus. Mullin (1982) investigated the evolution of primary flow and the transition from N-cell mode to N+2-cell mode by the flow visualization. However the difficulty is in understanding the mode bifurcation by
measuring the spatiotemporal internal flow completely. For this reason, direct measurement is necessary to clarify the internal velocity field structures. The conventional methods, i.e. Laser Doppler Anemometry (LDA) and Particle Image Velocimetry (PIV) cannot be applied to opaque fluids, because their intransparency prevent the light incidence. Therefore, Ultrasonic Velocity Profiler (UVP) has been developed (Takeda, 1986; Kikura et al., 2004) for optically non-transparent liquid flows. Recently the velocity of a liquid-metal (opaque) flow, i.e. mercury was obtained by Takeda et al.(2002). Takada (1999) measured the spatiotemporal velocity field using an ultrasonic velocity profiler (UVP). The flow fields were analyzed by using two-dimensional Fourier transform and the orthogonal decomposition technique; the intensities of coherent structural modes were quantitatively obtained. With the respective Reynolds number, the intensities of various modes clearly showed that a transition behavior of the quasi-periodic state results from the wavy vortex mode, and the modulating waves were found to disappear suddenly. Kikura, et al. (1999) also used the UVP method and measured the dynamics of magnetic fluid (opaque) Taylor-Couette flows produced by two concentric cylinders with the inner one rotating. Hence, this UVP method for the flow fields has also been applicable to “the turbidity solution”. And also, the method has a merit that it can measure the fluid flow characteristics without intruding the flows.

The objective of this research is to investigate the influence of flow fields on the microorganism (biocatalyst) growth in the bioreactor. Recently life supporting systems in closed space have been intensively researched in the aerospace industries. In those areas, the researches on food supply and oxygen supply systems are of no small concern. We paid an attention to the oxygen supply system. Generally the oxygen supply system is operated mainly by the electric process or using O2 gas bottle. On the other hand we adopted the photosynthesis of plant cells that produces O2 from CO2. The cells are expected to produce O2 with high efficiency because of their additional abilities such as growing in the photosynthesis process. Many researchers have been studying photosynthetic bioreactors which use micro algae such as Chlorella and Spirulina. Mitsuhashi et al.(1995) examined the photosynthetic growth rate in a suspension medium of Spirulina at the temperature between 30°C and 40°C, and reported that the oxygen production rate was increased with the shear stress until a plateau was reached at about 0.3 Pa. This study could be the first precise report on the relationship between an oxygen production rate and shearing flow of Spirulina.

In this paper, we describe the relationship between the microorganism "Spirulina” growth rate and the flow structures in dilute suspensions of a TVF with a small aspect ratio of 3, moreover, we explain flow characteristics measured by an ultrasonic velocity profiler.

2. EXPERIMENT

2.1 Experimental Apparatus and Method
In this study, Spirulina Platensis (IAM M-135) was chosen as a sample of plant cells (Fig.1). Spirulina Platensis is one of high efficiency photosynthetic algae that are fast growing. The shape of this microorganism is spiral, and the length is about 200-500μm. Spirulina has been also spotlighted from the viewpoint of food problems. In the United Nations World Food Conference of 1974 it has been lauded as the best food for the future, and proposed by NASA (CELSS) as one of the primary food to be cultivated during long-term space missions.

We adjusted an initial Spirulina weight concentration, about 0.3g/liter in SOT medium, and then put its liquid into the TVF cylinders gap. SOT medium is alkaline aqueous solution containing minerals (Table.1). The schematic diagram of TVF between two concentric cylinders is shown in Fig.2. The apparatus consists of two concentric cylinders made of transparent acrylic material. The length of the cylinders is H = 75 mm, the outer radius of the inner cylinder is R1 = 15 mm, and the inner radius of the outer cylinder is R2 = 40 mm. They are positioned vertically and the space between the two cylinders is filled with medium containing Spirulina. In other words, the aspect ratio Γ is H/(R2-R1)=3, the radius ratio η is R1/R2=0.375. The outer cylinder is fixed, and the inner cylinder is rotated with the stepping motor located on the upper wall.
The experimental cultivation conditions are shown in Table 2. Illumination condition is kept constant by the incubator fluorescent light controller (3.5klx). The temperature is also controlled at 303K by the incubator. The experimental vortex mode is 2 cell, and the growth phase condition is liner phase.

![Spirulina platensis](image1.png)

![UVP measurement of Taylor-Couette Vortex Flow (TVF)](image2.png)

Table 1  SOT medium

| Ingredient        | Quantity       |
|-------------------|----------------|
| FeSO$_4$·7H$_2$O  | 0.1g/50ml      |
| Na$_2$·EDTA·2H$_2$O | 8g/500ml     |
| MgSO$_4$·7H$_2$O  | 20g/500ml      |
| CaCl$_2$·2H$_2$O  | 4g/500ml       |
| NaNO$_3$         |                |
| NaCl             |                |
| K$_2$SO$_4$      |                |
| NaHCO$_3$        |                |
| K$_2$HPO$_4$     |                |
| H$_3$BO$_3$      |                |
| MnSO$_4$·5H$_2$O |                |
| ZnSO$_4$·7H$_2$O |                |
| CuSO$_4$·5H$_2$O |                |
| Na$_2$MoO$_4$·2H$_2$O |          |
| Distilled water  |                |

Fig. 2  UVP measurement of Taylor-Couette Vortex Flow (TVF)
The ultrasonic transducer is installed on top of the vessel to measure the axial velocity distribution, as shown in Fig.2. In the system with the outer cylinder fixed, and the small Reynolds number, the fluid in the annular gap moves as a plane in the perpendicular direction to the cylinder axis. Reynolds number was described by Eq.(1), where, $\Omega$ is the rotation rate of an inner cylinder and $\nu$ is the kinematic viscosity of a medium containing Spirulina.

$$\text{Re} = \frac{d \cdot U}{\nu} = \frac{(R_i - R_o) \cdot \Omega R_i}{\nu}$$  

Table 2 Experimental cultivation conditions

| Microorganism breed     | Spirulina platensis (IAM Culture collection No. M-135) |
|-------------------------|--------------------------------------------------------|
| Medium                  | SOT                                                    |
| Initial concentration   | 0.3                                                    |
| of Spirulina in SOT     | [g/liter]                                              |
| Illumination            | 3.5                                                    |
| Temperature [K]         | 303                                                    |
| TVF mode                | Two cell                                               |
| Growth phase            | Linear growth phase                                    |

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The UVP monitor used in this study is the UVP-DUO model (Met Flow Inc.). The principle of the ultrasonic Doppler method is based on the echography for position detection, and the Doppler shift for velocity detection. The ultrasound transducer was operated with a basic frequency of 8MHz and a beam diameter of 3mm. The channel distance (the distance between two adjacent measurement volumes) was 0.75mm. Liquid measurement requires the ultrasonic reflectors, because UVP is based on Doppler shift. In the case of Spirulina, by means of using them as the reflectors, we suppose it is possible to measure culture solution flows and the organism behaviors without additional reflectors, because the Spirulina size is larger than ultrasonic wavelength. However, when Spirulina is used as reflectors, it is feared that acoustic impedance is not strong enough since the organisms contain lots of aqueous fluid internally.

2.2 Multiplication Characteristics Evaluation

For evaluating the multiplication characteristics, the growth rate $Q$ of the Spirulina was described by Eq.(2), where, $C_0$ and $C_1$ are the initial concentration and the 1-day later concentration of Spirulina respectively. The concentration is measured by absorption spectrum of 560nm (before this measurement, we made the calibration line between Spirulina concentration and absorption spectrum). The sampling unit of about 5 ml was taken from the test section after 1-day cultivation.

$$Q = \frac{C_1 - C_0}{C_0}$$  

3. RESULTS AND DISCUSSION

3.1 Multiplication Experiment Results

Fig.3 shows the photographs of the cylinders side views under several Re flow conditions. Fig.4 shows the relation between the growth rate $Q$ and Reynolds number Re. $Q$ increases with the increase of Reynolds number. Shown in the photographs, after one day with no stirring, Spirulina coagulate with each other as connecting upper and bottom wall. In contrast, after 1-day TVF agitation, it was observed that the floating algae increase with Re increase. Therefore, we can guess that the growth
rate Q increases with Re increase because the floating algae don't shade the light each other, in other words, they improve the optical conditions.

Fig. 3 The side view of TVF cylinders
3.2 Flow Characteristics of a Diluted TVF

3.2.1 Ultrasonic Velocity Profile Measurement using Spirulina Plaensis

Fig.5(a) shows the profiles (by UVP) of time averaged velocities in Spirulina medium. The horizontal axis is a distance from the upper wall, the vertical axis is velocity. Plots in the figure are time averaged values, and the vertical short lines are the standard deviations from them. For the comparison under the same conditions, the time averaged velocity results are shown in Fig.5(b), when 80μm nylon particles (Japan Laser Corp.) are used as ultrasonic reflectors in SOT medium without Spirulina.

In the case of TVF of 0.37 g/litter Spirulina medium, as well as the results of the medium fluid velocity with nylon particles reflector, the formation of two vortices was observed in the cylinder axial direction. As the results, in the flow field measurement in a bio-reactor, the applicability of Spirulina as an ultrasonic reflector is verified.

In the case of spirurina medium, the velocity profile differences were seen at the near plus and minus maximum peak. This phenomenon are most likely due to the several effects such as the Spirulina’s weight, shape and population changing phenomena while incubation, and the effects of O₂ small bubbles produced by Spirulina, and the acoustic effects derived by the relative angle of the spiral-shaped Spirulina against the ultrasonic beam axis. However, further investigation will be required for the accurate understanding of this phenomenon.

![Graph showing Spirulina growth rate after 1-day incubation](image)

**Fig. 4** Spirulina growth rate after 1-day incubation

![Graph showing velocity profile differences](image)

**Fig. 5** Velocity profile difference between Spirulina plaensis and nylon particles reflector (Re=1600)
3.2.2 Time Averaged Velocity Profile Measurement Results of Spirulina Medium
(under Multiplication Experiment Re Condition)
The flow patterns were measured using an Ultrasound Doppler Profiler. Fig. 6 shows the time averaged velocity profiles (concentration of Spirurina in SOT is about 0.3 g/liter). The transducer was set up on the top at the radial direction 3mm position inward from the outer cylinder and the ultrasonic wave is emitted from the upper to the bottom region. From Fig. 6 in each Re condition, it is well observed that the flow has two vortices, and the averaged velocity are larger as Reynolds number increase. Therefore, in the case of dilute suspensions of a TVF with a small aspect ratio of 3, we found that the agitation effect becomes larger as Reynolds number increase within the range of Re between 0 and 7000. As a result, we guess that these flow structures can influence the growth rate. However, further study is necessary to precisely understand the effects of flow structures on the growth rate.

4. CONCLUSIONS
Our purpose is to investigate the effects of TVF on the bio-catalytic microorganism growth --- in bio-reactors. In the case of the dilute suspensions of a TVF with a small aspect ratio of 3, we found that the agitation effects become larger as Reynolds number increase. As a result, it was found that the growth rate Q increases as Reynolds number increase within the range of Re = 0 to 7000.

For further understanding of the flow structure effects on the microorganism growth, we will carry out the simultaneous 2 phase measurement (Spirulina:solid and medium:liquid) in the near future, by using 2 types of wavelength transducers.

NOMENCLATURE
\[ H \] length of cylinders \[ \text{[m]} \]
\[ R_i \] outer radius of inner cylinder \[ \text{[m]} \]
\[ R_2 \] inner radius of outer cylinder \[ d \] gap between inner and outer cylinder \[ Re \] Reynolds number

\[ C_0 \] initial Spirulina concentration in SOT \[ C_1 \] Spirulina concentration in SOT after 1-day incubation

\[ Q \] Spirulina growth rate

**Greek Letters**

\[ \Gamma \] aspect ratio \[ \eta \] radius ratio

\[ \nu \] kinematic viscosity of medium \[ \Omega \] rotation rate of inner cylinder

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