Fachtagung "Lasermethoden in der Strömungsmesstechnik"
8. – 10. September 2009, Erlangen

BIOKOMPATIBLE VISUALISIERUNG VON MIKROSTRÖMUNGEN UND DER CILIAREN BEWEGUNG VON OPERCULARIA ASYMMETRICA MITTELS MIKRO PARTICLE IMAGE VELOCIMETRY UND MIKRO PARTICLE TRACKING VELOCIMETRY

BIO-COMPATIBLE VISUALISATION OF MICRO FLOW AND PERIODIC RECIPROCATING CILIA BEAT INDUCED BY OPERCULARIA ASYMMETRICA WITH MICRO PARTICLE IMAGE VELOCIMETRY AND MICRO PARTICLE TRACKING VELOCIMETRY

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μ-PIV, μ-PTV, flow visualisation, cilia motion, GAS, SBR

Abstract

The aerobic granulation in a sequencing batch reactor (SBR) is a novel and promising technology in the field of wastewater treatment. Ciliates, namely, *Opercularia asymmetrica* which live on the surface of granules allow and initialise the formation of granular activated sludge (GAS), due to their cilia beat and the hereby induced micro-flow in the near field of the ciliates. Thus, a higher settling velocity of the GAS in comparison to conventional activated sludge can be achieved and the separation time can be reduced. The investigation of the cilia beat during the feeding time of the ciliates is a very interesting issue and can be utilized in different technical applications such as the development of micro-mixers. In the present work, the visualization of the micro-flow and the cilia beat are carried out by using micro particle image velocimetry (μ-PIV) and micro particle tracking velocimetry (μ-PTV), respectively. To visualize the flow and the cilia movement in micro-scale an Axiotech 100 microscope (Carl Zeiss) is used with a 50-fold and 100-fold objective, respectively. In order to make the biological processes visible in a bio-compatible way, tracer particles from milk-emulsion are used and a moderated light intensity of the microscope is set. The images are recorded with a high-speed CCD camera (Mikrotron GmgH) for μ-PIV and a high-speed CMOS camera (PCO AG) for μ-PTV. The evaluation of the fluid velocity is carried out by the commercial software PIVview2C (PIVTEC GmbH). A method is developed by using MATLAB (Math Works) to analyze the cilium displacement.

Introduction

The processes in a sequencing batch reactor (SBR) are optimal to produce granular activated sludge (GAS). Granules have a high density (approx. 1.05 *g/ml*) and thus a high settling ability. Their form is ellipsoidal and the diameter is up to 5 *mm*. Hence, they can be successfully used in biological purification of wastewater. Nevertheless, the granule formation remains still not completely understood because many factors influence the formation and the structure of GAS. As shown by Etterer and Wilderer (2001), the substrate composition, the superficial gas velocity (SGV), extra cellular polymer substances (EPS), feast-famine

regime and a sufficient settling time are important factors during this process. Fluid-mechanical investigations of the fluid were carried out by Zima–Kulisiewicz et al. (2007) and show that buoyancy forces, drag forces as well as collision forces (particle–wall, particle–particle) influence the granulation process. The normal and tangential strain, affect the formation and destruction of granules. Bio-granulation is a multi-scale phenomenon. Investigations of the micro-flow induced by the periodic reciprocating cilia beat of *Opercularia asymmetrica* should be also taken into account. According to Weber et al. (2007), the development of granules takes place with the aid of ciliates in three different phases. At the beginning, ciliates settle on other micro-organisms or particles and bulky growth of ciliates commences. The cilia beat of the ciliates during their feeding time induces a flow towards the biofilm. This flux improves the colonization process. In the second phase, the granule grows and the core zone is developed. Here, a lot of ciliate cells are completely overgrown by bacteria and die. Consequently, a dense core of bacteria and remains of ciliate stalks is formed as well as gradually a mature granule is developed. The above description emphasises the importance of ciliates during the granulation process.

The flow induced by ciliates is still not completely understood. Visualization of this fluid flow demands powerful imaging and flow analysis methods. First investigations with *Opercularia asymmetrica* were carried out by Delgado et al. (2007) as well as by Hartmann et al. (2007), Petermeier et al. (2007) and Kowalczyk et al. (2007). Above studies indicate that the employed measuring and flow visualization techniques guarantee the bio-compatibility criteria and they do not affect the investigated bio-system. The flow structure which consists of two counter rotating vortices is investigated for a single ciliate and a colony of ciliates.

In order to understand the actuation of the micro flow, in the present work the cilia movement at the mouth of *Opercularia asymmetrica* is observed qualitatively and quantitatively. The beating of the peristomial cilia of *Stentor polymorphic* has been observed and modelled before by Sleigh (1960). The elliptical motion of one cilium was implemented before by Vilfan and Jülicher (2006) in order to calculate the hydrodynamic flow field generated by the periodic beat of cilia which are attached on a surface.

Materials and methods

The above described granules are the biotopes of the investigated micro-organisms. These granules are selected from a SBR. The flow induced by *Opercularia asymmetrica* is analyzed by using micro particle image velocimetry (μ-PIV). The analysis of cilia motion is carried out by using micro particle tracking velocimetry (μ-PTV).

The micro-flow and the cilia beat are observed by using a transmitted light microscope Axiotech 100 (Carl Zeiss) with 50-fold and 100-fold optical magnification, respectively. Since the cilia length is approx. 10-15 μm an objective with 100-fold magnification is necessary. The numerical aperture of this objective is 1.3. The depth of the focal plane is consequently 325.44 *nm*. The GAS probes are taken out from the SBR and are added together with a small amount of tracer particles on a glass plate. The prepared sample is afterwards covered with a thin glass plate. The prepared probes can now be analyzed with the microscope.

As explained in the introduction, in order to obtain lifelike results bio-compatibility must be ensured. Thus, appropriate light source and tracer particles must be implemented. The intensity of the illumination must not exceed a certain level that can be endured by the micro-organisms; otherwise the viability of protozoa is drastically inhibited and reduced (Petermeier et al., 2007). Here, light built in the microscope is used as only light source (6 Watt lamp). Laser which is often used for PIV or PTV investigations is inapplicable in this study. The

seeding particles which are applied to trace the flow should also fulfil the bio-compatibility requirement. Therefore, tracer particles from milk-emulsion are used. Moreover, the diameter of the particles is in the range of 0.3 to 3 *µm*. These tracer particles show a very good compatibility and are used as nutriment by the investigated micro-organisms.

The images for µ-PIV are recorded by a high-speed CCD camera (Mikrotron GmbH) with an exposure time of 15 *ms*. They have a resolution of 860x1024 pixels. For the µ-PTV measurements a high-speed CMOS camera (PCO AG) with an exposure time of 2 *ms* is used. The resolution is in this case: 1280x1024 pixels. In order to determine the out of focal plane velocity component a piezo-element (piezosystem jena) that moves the objective in *z*-direction is used. Figure 1 depicts the used µ -PIV and µ-PTV systems.

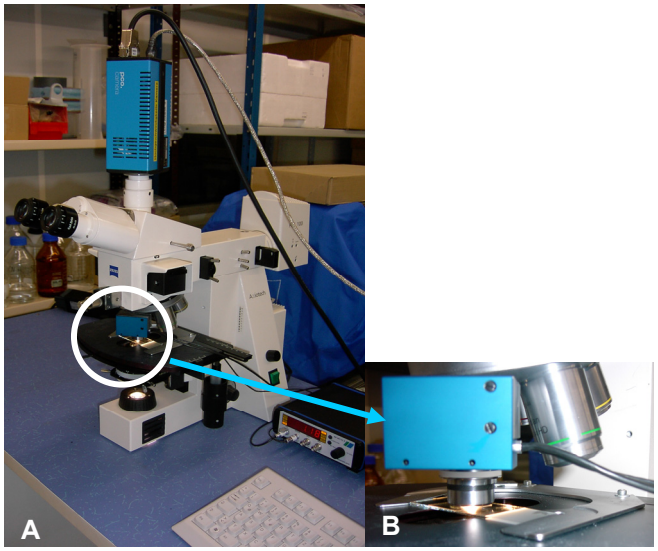

Figure 1 Experimental setup: microscope with CMOS (A) camera. Piezo-element (B) for the objective displacement in *z*-direction

The PIVview2C software (PIVTEC GmbH) is employed to evaluate the fluid velocity (Raffel et al. 1998). In order to extract the particle displacement from the acquired images a cross correlation was applied. Here, the interrogation window and the grid size are chosen as 32x32 pixels and 20x20 pixels, respectively. The sub-pixel displacement of the correlation peak is obtained by a three point Gauss fit. It selects the four closest neighbours of a correlation maximum and fits a three point Gaussian curve for each of the major axis (Willert and Gharib, 1991). After measuring the velocity of the flow further parameters that characterize the flow like the convective kinetic energy, the normal and shear strain rate are calculated.

An evaluation method was developed by using MATLAB (Math Works) to determine the velocity magnitude of the cilia displacement. First the two velocity components of the cilia in the focal plane are measured. Then, the three velocity components are measured separately. The three velocity components contain the out of plane component as well. v_s is the resulting velocity in the focal plane whereas v_{sz} contains additionally the out of plane component. As a result of the two dimensional measurements, further parameters like Reynolds number, added mass and acceleration force can be calculated. Knowing the velocity field of the flow, the total specific kinetic energy can be calculated and compared with the specific kinetic energy of the cilia movement. Thus the effectiveness of the micro-process can be determined. A model that approximates the movement of one cilium and of the whole cilia collective is proposed.

Results and Discussion

µ-PIV

The characteristic flow pattern induced by the periodic beat of *Opercularia Asymmetrica* consists of two counter rotating vortices. These can be observed by the µ–PIV investigations of the flow. Figure 2 shows the flow pattern generated by one ciliate and a colony of ciliates. For the colony the velocity magnitude increases. Hence, the convective kinetic energy, the normal and shear strain are also increasing with accretive number of ciliates which improves the effectiveness of the micro-mixing phenomena

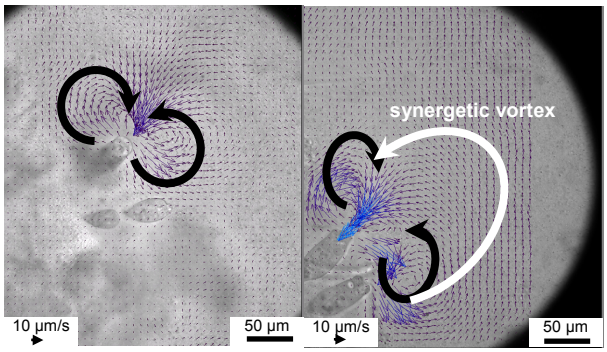

Figure 2 Velocity field for a single ciliate and a colony induced by *Opercularia Asymmetrica*

µ-PTV

In the case of µ-PTV investigations it should be first mentioned that the cilia of *Opercularia Asymmetrica* are arranged in two parallel rows on the mouth of the micro-organisms. The periodic reciprocating beat is synchronous for both rows, inversely and undulatory due to the phase shift in time (Δt) between each cilium. Since the diameter of the mouth amounts 9 µm, the cilia number n for each row is approximately 32. Figure 3 displays the coupled sinusoidal (in focal plane) and elliptical (out of focal plane) motion of a row and one cilium, respectively.

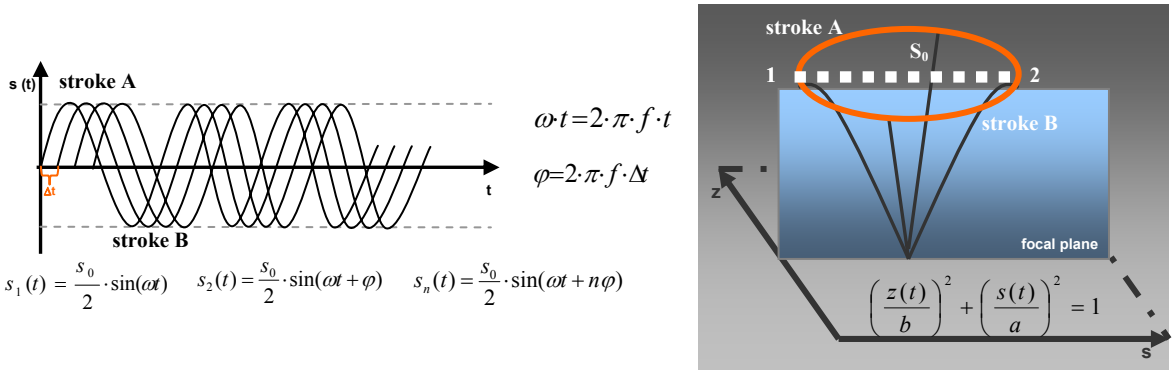

Figure 3 Sinusoidal motions of a cilia row (n = number of cilia); elliptical motion of one cilium

For the sinusoidal functions of n cilia, the strokes A and B can have the same size in time if the velocity of the cilia during stroke A is equal to the velocity during stroke B and they can be different if the two velocities are unequal. The amplitude of this function remains always unmodified since s_0 is constant. The cilium is moving during one cycle (stroke A + B) along an ellipse. For time consecutive cycles a steady state is observed. The frequency as well as the velocity of stroke A and B are equal. The elliptical equation contains the constants a and

b which represent the depth and the half diameter of the ellipse, respectively. These quantities can also be measured. If these constants are known including the time dependent position *s (t)* of the cilium in the focal plane, the position in z-direction *z (t)* can be calculated.

Since only in the focal plane sharp images can be acquired, the magnitude of the velocity (v_s) during one stroke is determined (Figure 3 points 1 → 2). The frequency is measured from the time which is necessary to execute one cycle. In Figure 4, the cilium velocity and the corresponding frequencies are plotted. The added mass forces (F_{AM}) and the acceleration forces (F_A) are calculated for a whole cilia collective (2xn = 64) by using the velocity v_s, the cilium geometry (cylindrical with 0.12 μm diameter and 10μm length) and the density of the surrounding medium.

$$F_{AM} = 0.5 C_A \rho_{medium} V_{cilium} \frac{d}{dt}(v_s) \qquad C_A = 2.1 - \frac{0.132}{A_C^2 + 0.12} \qquad A_C = \frac{|v_s|^2}{D_P \left| \frac{d|v_s|}{dt} \right|} \tag{1}$$

$$F_A = m_{cilium} \frac{d}{dt}(v_s) \tag{2}$$

These two forces are illustrated in Figure 5. It can be observed that with increasing frequency

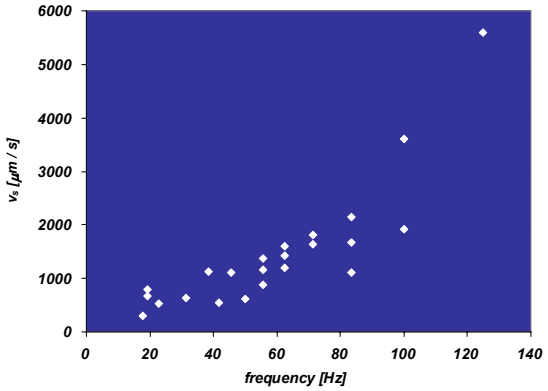

Figure 4 Cilium velocity (v_s) and frequency

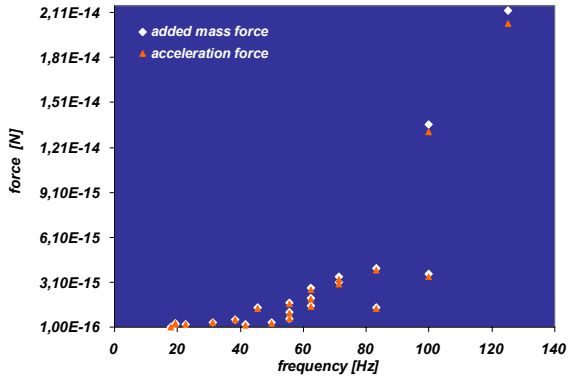

Figure 5 Added mass and acceleration force of the cilia collective (2xn = 64)

of the cilia beat their velocity is also increasing. Frequencies till 100 *Hz* are the most dominant ones. The maximum frequency is measured at 125 *Hz*. For one constant frequency different stroke velocities are measured. Although the velocities of the strokes A and B can be different during one cycle the net frequency can remain the same. This result is expected since the cilia beat is a random process. Furthermore, the added mass and the acceleration forces are equal for the investigated frequency range. Only for very high cilia frequencies the added mass force is greater than the acceleration force. Consequently, from the total energy released by the ciliates one half is used to overcome the added mass of the surrounding media and the rest is utilized to accelerate the fluid.

The investigated micro process is dominated by acceleration and inertia, hence, the Reynolds number is evaluated from the measured velocity (v_s), the length of one cilium (approx. 10 μm) and the kinematic viscosity of the surrounding medium (1.2×10^{-6} m^2/s). Figure 6 shows the exponential behaviour of the Reynolds number with respect to the frequency.

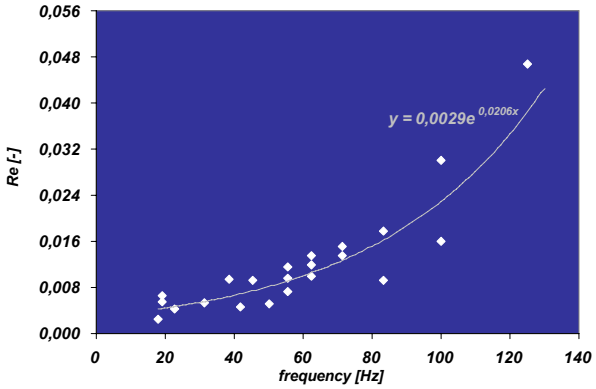

Figure 6 Reynolds number determined by the velocity v_s

The phase shift in time between two cilia could also be determined. Since the investigated micro processes are at random, it is very difficult to find a sample where in one focal plane, two successive cilia are visible. Hence in 325 *nm* depth of the focal plane two cilia exist and their space shift is visible. From the measured space shift and the velocity v_s which is equal for both successive cilia, the phase shift in time (Δt) can be determined. In Figure 7 the time shift and the cilia velocity are plotted. The higher the velocity of the cilia, the shorter is the time phase shift between the first and the next cilium. Since the velocities v_s of the two cilia are equal, this certainty can be assigned for the whole row of cilia. The only difference is that there is a shift in time between each cilium.

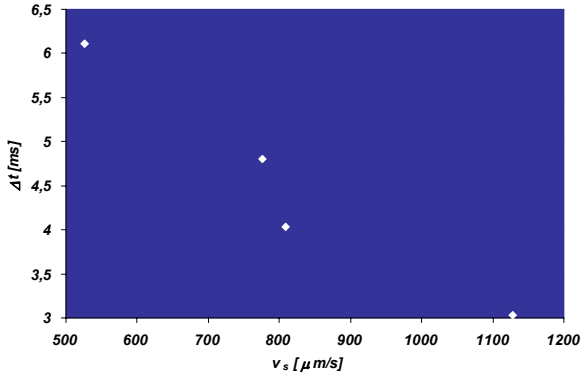

Figure 7 Phase shift in time (Δt) between two successive cilia in dependency from the velocity v_s in the focal plane

In order to evaluate the efficiency of this process the specific kinetic energy of the cilia motion is compared with the specific kinetic energy of the total flow. The former energy is calculated by the velocity v_s, whereas the latter one is calculated by the velocity and the density of the fluid. A set of μ-PTV and μ-PIV measurements are carried out to measure the cilia and the flow velocity, respectively. This line-up is depicted in Figure 8. The average specific kinetic energy of the flow is 3.25×10^{-10} $J/\mu m^3$ and of the cilia displacement 1.79×10^{-9} $J/\mu m^3$. Consequently, an effectiveness factor of 18.19% is achieved and the residual energy is dissipated.

The cilium velocity is determined in the focal plane and out of the focal plane. After many trials one example could be found where the displacement of the cilium backwards during one stroke could be tracked and three sharp images of the cilium were acquired. These three

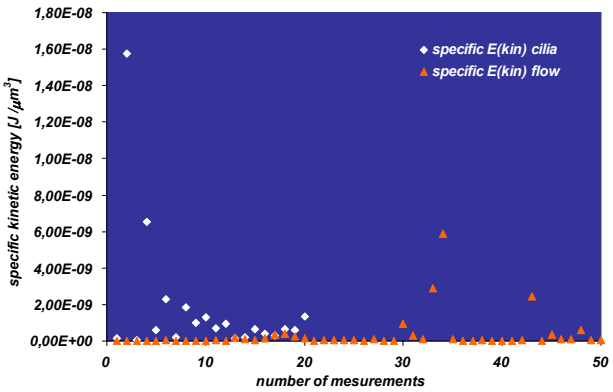

Figure 8 Specific kinetic energy of the 2D flow and the cilia movement

positions are shown in Figure 9. The velocity of the piezo-element is 250 µm/s which corresponds to the cilium velocity in z-direction. After measuring the in plane velocity (v_s), the magnitude of the total velocity (v_{sz}) is 893.55 *µm/s* with a corresponding frequency of 55.56 *Hz*. Lastly, the constants of the elliptical equation *a* and *b* are 2.40 *µm* and 1 *µm*, respectively.

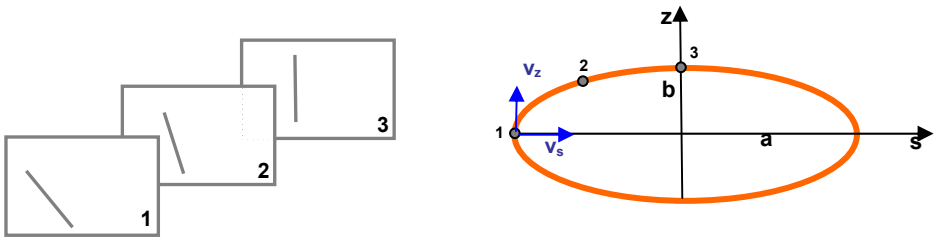

Figure 9 Sketch of the three tracked cilia positions during the displacement of the piezo-element in *z* direction

Conclusions

The two dimensional flow pattern induced by *Opercularia asymmetrica* is determined and characterized. In a colony the kinetic energy transferred on the flow is higher than for a single ciliate. In order to describe the cilia motion a model is proposed which can approximate the motion of one cilium and the whole cilia row. The motion of one cilium is elliptical whereas the motion of the row is sinusoidal with a certain time shift. If the time dependent cilium position in the focal plane, the depth and the diameter of the ellipse are known, the time dependent position of the cilium along the ellipse can be determined. Further parameters are evaluated like the two and three velocity components, the Reynolds number as well as the added mass and acceleration forces. One half of the micro-organismic energy is utilized to accelerate the added mass of the surrounding medium and the rest is used for the fluid acceleration. After calculating the mean specific kinetic energy of the total flow and the cilia motion, an effectiveness factor is calculated. This natural process just like many other technical processes is associated with much dissipation. From the produced acceleration energy 18.19% is transferred on the fluid.

Acknowledgement

This work has been funded by the DFG within DFG-SPP 1147 „Bildgebende Verfahren zur Strömungsanalyse“. This work has been done in cooperation with the Erlangen Graduate School of Advanced Optical Technologies (SAOT).

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