

DETAILED BIOCOMPATIBLE VISUALIZATION OF MICRO FLOW INDUCED BY *OPERCULARIA ASYMMETRICA* WITH MICRO PARTICLE IMAGE VELOCIMETRY

B.E. Zima-Kulisiewicz, E. Botello-Payro, A. Delgado

Lehrstuhl für Strömungsmechanik, Friedrich-Alexander-Universität Erlangen-Nürnberg, Cauerstraße 4, 91058 Erlangen

μ -PIV, visualization, *Opercularia asymmetrica*, GAS, SBR

Abstract

Aerobic granulation in Sequencing Batch Reactor (SBR) is a novel, promising technology in wastewater treatment. Ciliate *Opercularia asymmetrica* living on granules surface influences significantly the Granular Activated Sludge (GAS) development. Characteristic microorganismic flow generated by cilia beats of ciliates during their feeding improves colonization process of bacteria on *Peritrichia* stalks. Although several fluid dynamic investigations of multiphase flow in Sequencing Batch Reactor have been carried out in the macro scale, only few studies concern the micro scale. Thus, in the present work a detailed visualization of micro flow induced by *Opercularia asymmetrica* is carried by using micro Particle Image Velocimetry (μ -PIV). Micro-fluid flow is observed by using an Axiotech 100 microscope (Carl Zeiss). Effective bioflow visualization can be obtained with appropriate biocompatible seeding biotracers. Application of milk as seeding substance combined with a high optical magnification (50-fold) enables detailed fluid flow analysis in the ciliates vicinity. Moreover, biocompatibility is guaranteed by implementing built in microscope white light with moderate intensity. Images are recorded by high speed CCD camera (Mikrotron GmbH). The calculation of the fluid velocity is carried out with help of the software PIVview2C (PIVTEC GmbH).

Introduction

Sequencing Batch Reactor (SBR) process can be treated as optimal way for production of Granular Activated Sludge (GAS). Granules due to high density (ca. 1.05 g/ml), ellipsoidal form with length up to 5 millimetres, high settling ability can be successfully used in biological purification of wastewater. Nevertheless, granules formation remains still not completely understood. Many factors influence the formation and structure of aerobic granular sludge. As shown by Etterer and Wilderer (2001), substrate composition, Superficial Gas Velocity (SGV), Extracellular Polymer Substances (EPS), feast-famine regime as well as sufficient settling time play a decisive role in the granulation process. Fluid dynamical investigations carried out by Zima-Kulisiewicz et al. (2008) show that buoyancy forces, drag forces as well as collisional forces (particle-wall, particle-particle collision) influence the granulation process. The induced, normal and tangential strains affect granules formation and destruction. However, it should not be forgotten that biogranulation is a multiscale phenomenon. Thus, investigations in micro scale should be also taken into account. According to Weber et al. (2007), the granules development takes place with the aid of ciliates in three different phases. At the beginning ciliates settle on other organisms or particles and bulky growth of ciliates commences e.g. *Epistylis sp.* Stalks and zooids are colonized by bacteria. Cilia beats of the ciliates providing a continuous nutrient flux toward biofilm improve 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. Gradually a mature granule is devel-

oped. Finally, granules are composed of two zones: core zone and loose structured fringe zone, and serve as new substrate for swarming ciliates. Above description emphasises decisive ciliates role in granulation process.

However, flow induced by ciliates is still not completely understood. Visualization of this fluid flow demands powerful imaging and flow analysis methods. Sleigh and Barlow (1976) analysed for the first time flow induced by peritrichous ciliates. Vopel et al. (2002) studied flow field of marine peritrichous ciliates. 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 measuring and flow visualisation techniques employed guarantee biocompatibility, i.e. they do not affect investigated biosystems. Unfortunately, this restricts possibilities for optimizing the image generation in comparison to other flow field visualisation problems in which no biological systems are present. In consequence, images of lower quality leading to erroneous artefacts are obtained. Thus, either novel detection techniques that are able to overcome these disadvantages or advanced evaluation methods enabling the sophisticated analysis and description of flow fields are requisite. As shown by Petermeier et al. (2007), handling of artefacts could be performed by the hybrid using *a priori* knowledge of the flow physics formulated in numerical expressions and the enormous potential of Artificial Neural Networks (ANN) in predicting artefacts and correcting them. In the present work detailed micro-flow investigations with biotic seeding particles are proposed.

Experimental Setup

Microorganisms are selected from granules which grow in laboratory SBR (see Zima-Kulisiewicz et al., 2008). Flow induced by *Opercularia asymmetrica* during feeding movement is analyzed by using micro Particle Image Velocimetry (μ -PIV).

Micro - fluid flow is observed by using transmitted light microscope Axiotech 100 (Carl Zeiss) with 50- fold optical magnification and the phase contrast method (differential interference contrast - DIC). Here, GAS probe taken out from SBR with a certain amount of seeding particles is placed on the glass plate. Prepared sample is covered with a cover plate. Following, probe is analysed with a microscope.

As explained in the introduction part, in order to obtain effective results biocompatibility must be guaranteed. Thus, appropriate seeding particles as well as light source must be implemented. The intensity of implemented illumination must not exceed certain level acceptable by the microorganisms. Otherwise, the viability of protozoa is drastically reduced (Petermeier et al., 2007). In the present work light built in the microscope is used as the light source. Laser being often used in the Particle Image Velocimetry investigations is inapplicable here. Moreover, seeding particles applied to trace the flow should fulfil the biocompatibility requirement. Artificial tracers, e.g. polystyrene are instantaneously detected by microorganisms and rejected as not being nutrients. Thus, as shown in the previous works (Kowalczyk et al., 2007, Petermeier et al., 2007, Zima et al. 2007) effective studies can be obtained only with appropriate seeding biotracers like yeast cells (*Saccharomyces cerevisiae*, dimension approx. 3 - 10 μm) and milk (fat and proteins, dimension 0.3 - 3 μm). However, studies of Petermeier et al. (2007) and Kowalczyk et al. (2007) indicate that yeast cells as seeding particles enable obtaining correct flow pattern only for a relatively small 10-fold optical magnification. Investigations with 20-fold as well as 50-fold optical magnification indicate strong limitation of experiments with yeast cells as seeding particles. Insufficient applicable concentration and large dimensions of tracer particles compared to the size of flow structures cause appearance of many visualisation artefacts and spurious velocity vectors from PIV evaluation. In the present work milk enabling investigations with high optical magnifications (50-fold) is proved to be an appropriate tracing substance.

Images are recorded by a high speed CCD camera (Mikrotron GmbH) with 65 frames/seconds. Images have resolution of 860x1024 pixels (323 μ m x 385 μ m for 50-fold optical magnification). Figure 1 depicts the used μ -PIV system.



Figure 1 Microscope with CCD camera

The calculation of the fluid velocity is carried out with help of software PIVview2C (PIVTEC GmbH), developed by Raffel et al. (1998). In order to extract particle displacement the cross correlation mode is used. Multiple-pass interrogation algorithm increases the data yield due to higher amount of matched particles and reduces the bias error (Westerweel et al., 1997). In the current work the interrogation window size is chosen as 32x32 pixels, the grid size is 20x20 pixels. Sub-pixel displacement of the correlation peak is obtained by 3-Point Gauss Fit. It selects the four closest neighbours of a correlation maximum and fits a 3-point Gaussian curve each of the major axis (Willert and Gharib, 1991). Further, velocities data from PIVview2C are processed with Tecplot (Amtec Engineering).

Results and Discussion

Analysing flow induced by ciliates, a characteristic micro flow pattern with two counter rotating vortices generated by cilia beats can be observed (see Figure 2).

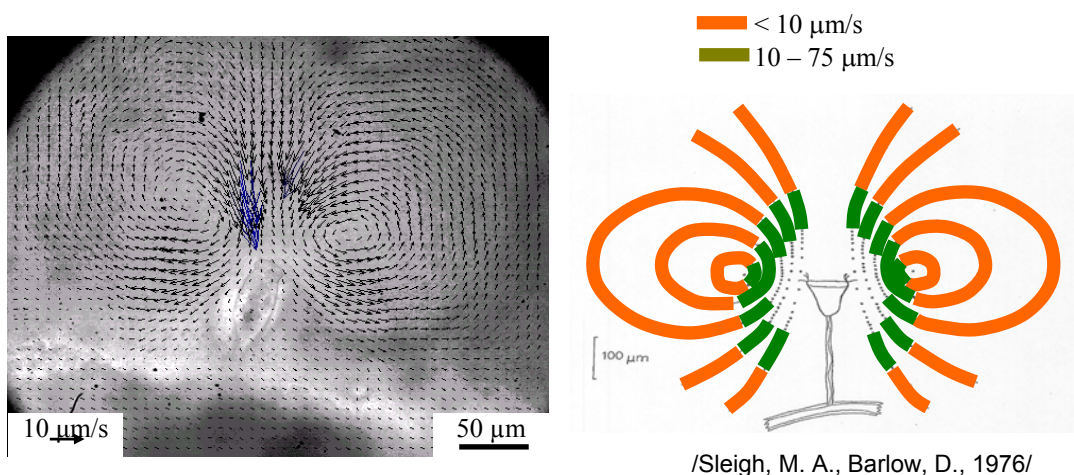


Figure 2 Characteristic flow pattern at 50 fold optical magnification

Present investigations confirm the first studies with flow patterns induced by *Vorticella* carried out by Sleigh and Barlow (1976).

Experiments with different seeding concentrations reveal crucial differences. As example flow induced by one ciliate for different milk to water proportions of 1:2 and 1:4 is shown in Figure 3. An increasing velocity magnitude with increasing dilution of seeding milk is observed, e.g. for the higher milk concentration (1:2) velocity has lower value of $u_{\max} = 71 \mu\text{m/s}$ while for lower concentration (1:4) velocity reaches higher value of $u_{\max} = 132 \mu\text{m/s}$. Above comparison indicates that liquid velocity is almost two times higher for lower concentration.

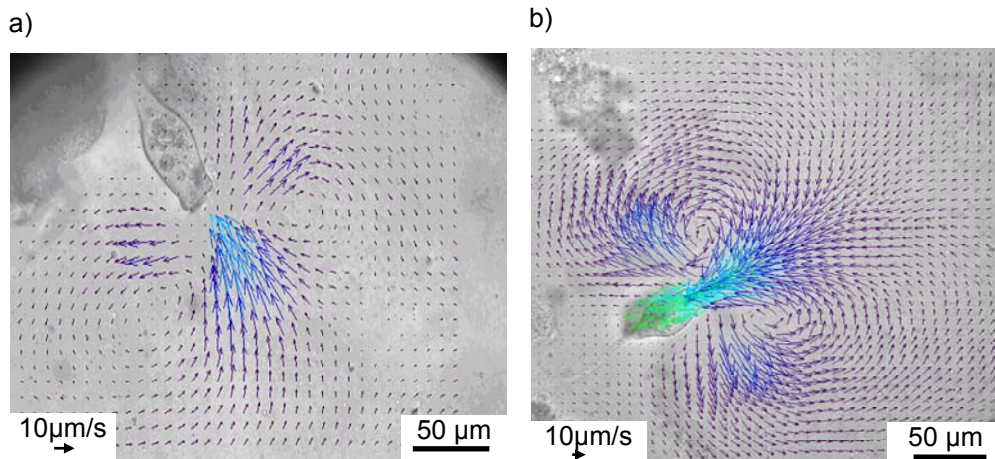


Figure 3 Velocity distribution observed by 50-fold optical magnification with different milk concentration a) 1:2, c) 1:4

Moreover, the number of ciliates influences significantly the analysed flow pattern. As example a comparison of velocity distribution for one ciliate and colony with milk to water concentration of 1:1 is done (see Figure 4). Figure 4 shows an increasing tendency of velocity with increasing ciliates number. Maximal velocity equal to $u_{\max} = 26 \mu\text{m/s}$ is significantly lower (four times) for the single ciliate than for the colony where $u_{\max} = 114 \mu\text{m/s}$. Above comparison shows that cooperative colony work influences the flow velocity displaying bio-synergetic effect. The characteristic flow pattern with two vortices can be seen for the first case with single organism. In the second one, instead of typical flow every ciliate produces one vortex. Additionally, synergetic vortex belonging partially to two different ciliates is recognized. This agrees excellently with the theoretical prediction of Hartmann et al. (2007).

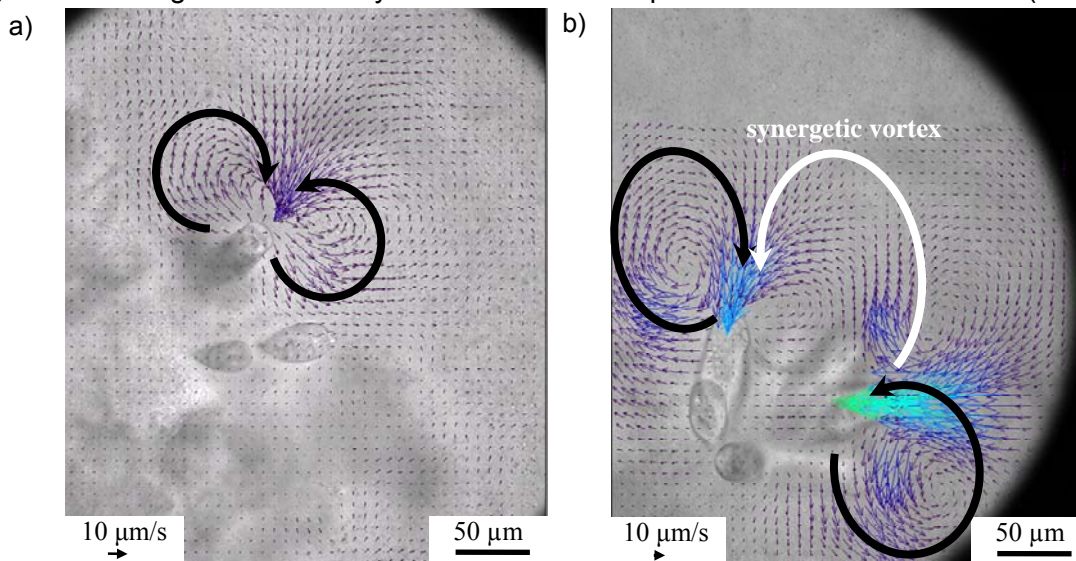


Figure 4 Velocity field observed for a) single ciliate, b) two ciliates and c) colony

Moreover, kinetic energy investigations show its increasing tendency with rising seeding particles dilution (see Figure 5).

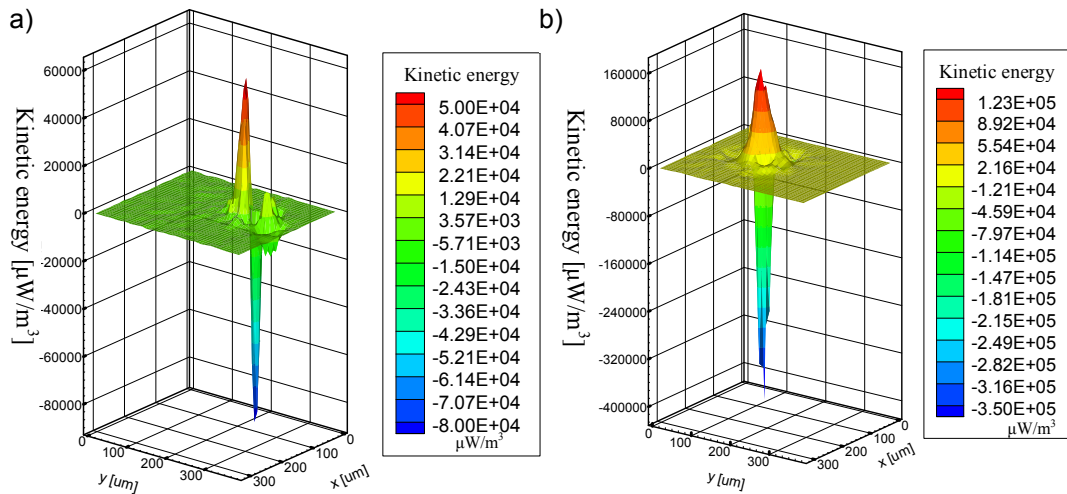


Figure 5 Kinetic energy produced by one ciliate with different milk proportions: (a) 1:2, (b) 1:4

Herein, the maximal kinetic energy of the fluid is equal to $E_{kinmax} = 50000 \mu W/m^3$ for higher seeding substance concentration (1:2), while for concentration of 1:4 the kinetic energy reaches value of $E_{kinmax} = 123000 \mu W/m^3$. Accordingly the kinetic energy is more than two times higher for lower milk (seeding) concentration (1:4).

Furthermore, μ -PIV studies reveal that the cooperative fluid transport induced by ciliates living in colonies or groups is more efficient than in case of a single organism. That is confirmed by the investigation of the convective kinetic energy produced by living protozoa. As shown in Figure 6 ciliates living in colony produce more kinetic energy per single organism than a living alone ciliate. The synergy factor amounts approximately 1.7.

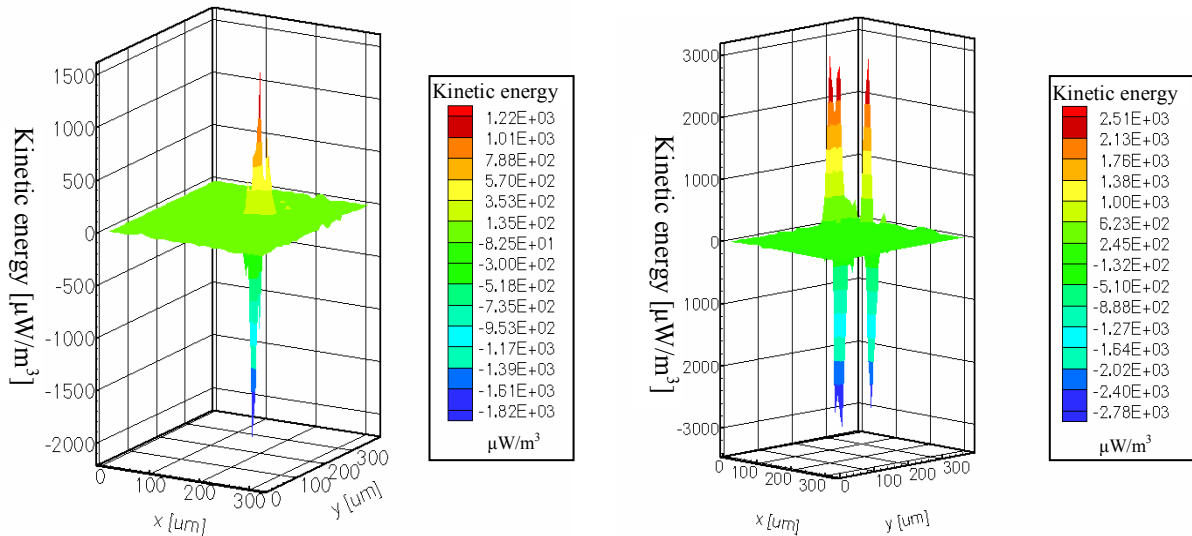


Figure 6: Kinetic energy generated by single ciliate (left) and a colony (right)

In order to prove the mixing intensity, normal and shear rate investigations for various seeding particles concentration and different ciliates number are carried out. Normal and shear strain rate investigations with different milk concentrations show its increasing tendency with higher milk dilution. As example normal strain rate investigations are given (see Figure 7).

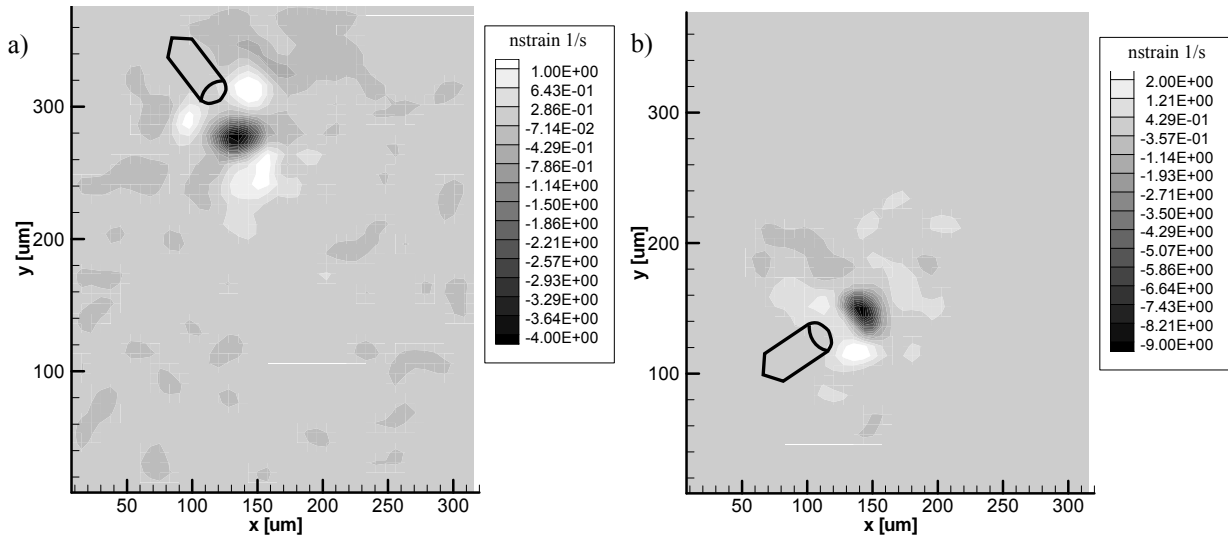


Figure 7 Normal strain rate generated by single cilium for different seeding substance concentrations a) 1:2, b) 1:4

Herein, normal strain rate for higher seeding particles dilution (1:4) lies between -9.0 1/s and 2.0 1/s while for the lower milk dilution (1:2) is in range from -4.0 1/s to 1.0 1/s. It indicates that normal strain rate is two times higher for 1:4 concentration.

Increasing tendency of shear and normal strain rate with increasing ciliates number (one cilium and colony) is observed. In this case, as example, shear strain rate studies are presented. As shown in Figure 8 shear strain rate alters between -2.50 1/s and 3.50 1/s for the colony while for single cilium shear strain rate changes between -1.0 1/s and 1.2 1/s. Here synergy factor for ciliates in colony referred to single cilium has value of 2.73. It indicates that colony work is more efficient in mixing process than single cilium.

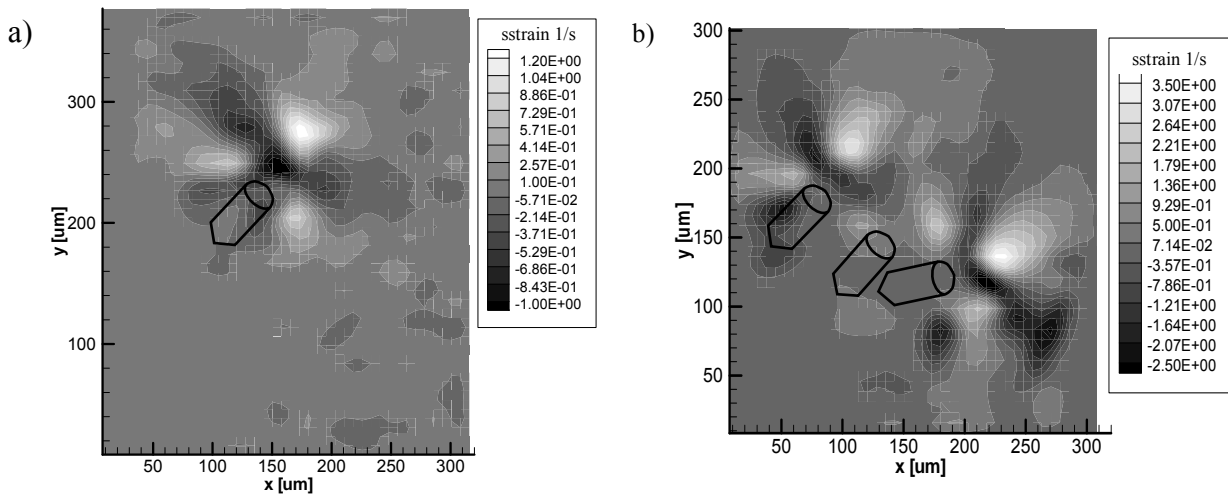


Figure 8 Shear strain rate generated by a) single cilium b) colony

Conclusions

Presented investigations indicate crucial role of ciliates for granulation and mixing process. Flow induced by ciliates can be treated as efficient nutrient transport by minimum energy requirement. Different seeding particles concentration influence significantly liquid velocity, kinetic energy as well as normal and shear strain rate. Increasing tendency of liquid

velocity, kinetic energy, strain rates appears with higher milk dilution. Moreover, ciliates number affect the analyzed flow pattern, e.g. maximal velocity is four times lower for the single ciliate than for the colony. Cooperative colony work influences the flow displaying bio-synergetic effect. Additionally, kinetic energy investigations reveal that cooperative fluid transport induced by ciliates living in colony is more efficient than for single *Opercularia asymmetrica*. Ciliates living in colony produce more kinetic energy per single organism than alone one. Moreover, normal and shear strain rate studies indicate more efficient work of colony than single ciliate in mixing process.

Literature

- Delgado A., Petermeier H., Kowalczyk W., 2007: Micro-PIV in Life Science, Particle Image Velocimetry, 2nd Edition, Ed.: M. Raffel, C. Willert, S. Wereley, J. Kompenhans, Springer-Verlag Berlin and Heidelberg GmbH & Co. K
- Etterer, T., Wilderer, P.A., 2001: Generation and properties of aerobic granular sludge. Water Science Technology, Vol. 43, pp.19-26
- Hartmann C., Özmutlu Ö., Petermeier H., Fried J., Delgado A., 2007. Analysis of the flow field induced by the sessile peritrichous ciliate *Opercularia asymmetrica*. Journal of Biomechanics, Vol. 40, pp. 137–148
- Kowalczyk W., Zima B.E., Delgado A., 2007: A biocompatible seeding particle approach for μ -PIV measurements of a fluid flow provoked by microorganisms. Experiments in Fluids, Vol. 43, 147-150.
- Petermeier H., Kowalczyk W., Delgado A., Denz C., Holtmann F., 2007: Detection of microorganismic flows by linear and nonlinear optical methods and automatic correction of erroneous images artefacts and moving boundaries in image generating methods by a neuronumerical hybrid implementing the Taylor's hypothesis as a priori knowledge, Experiments in Fluids, Vol. 42, pp. 611-623
- Raffel M., Willert Ch. E., Kompenhans J., 1998: Particle Image Velocimetry. A Practical Guide. Springer-Verlag, Berlin Heidelberg
- Sleigh M.A., Barlow D., 1976: Collection of food by *Vorticella*. Transactions of the American Microscopic Society Vol. 95, pp. 482-486
- Vopel K., Reick C.H., Arlt G., Pöhn M., Ott J.A., 2002: Flow microenvironment of two marine ciliates with ectobiotic chemoautotrophic bacteria. Aquatic Microbial Ecology, Vol. 29, pp. 19-28
- Weber S., Ludwig W., Schleifer K.H., Fried J., 2007: Microbial composition and structure of aerobic granular sewage biofilms, Applied and Environmental Microbiology, Vol. 73, pp. 6233-6240
- Westerweel J., Dabiri D., Gharib M., 1997: The effect of a discrete window offset on the accuracy of cross-correlation analysis of digital particle image velocimetry. Experiments in Fluids, Vol. 23, pp. 20-28
- Willert C., Gharib M., 1991: Digital Particle Image Velocimetry. Experiments in Fluids, Vol. 10, pp. 181-193
- Zima-Kulisiewicz B.E., Díez L., Kowalczyk W., Hartmann C., Delgado A., 2008: Biofluid mechanical investigations in Sequencing Batch Reactor (SBR). Chemical Engineering Science, Vol. 63, pp. 599-608