Aerobic Granular Sludge Cultivation in a Sequencing Batch Reactor (SBR) Using Activated Sludge as Inoculum

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Granular sludge technology represents a novel alternative to the conventional activated sludge wastewater treatment plants. Granules were cultured under aerobic conditions in a laboratory scale sequencing batch reactor (SBR). In order to enhance the growth of granular sludge, the SBR was operated with very short sedimentation and draw phases resulting in the washout of slow settling biomass and development of high density sludge granules (anaerobic feeding - 45 min, aerobic reaction - 11 h, settling - 5 min and effluent withdrawal - 10 min). The reactor was inoculated with conventional activated sludge, sampled from a wastewater treatment plant, and fed with dairy industry wastewater with high organic and nutrient load. Treatment performances increased along with the growth of granules size. Microscopic investigations were performed to assess the diversity and dynamics of the aerobic granular sludge biocenosis community and granules formation.

Keywords: aerobic granular sludge, SBR, granule formation, biocenosis

With an ever increasing population, biological wastewater treatment process has a crucial importance to the modern world [1, 2]. Granular sludge technology is one of the great achievements in environmental biotechnology of the twentieth century, used for different wastewater treatment applications [3, 4] as an alternative to physico-chemical methods [5]. According to Liu et al. (2004), aerobic granular sludge can be defined as *an enormous metropolis of microbes containing millions of individual bacteria due to microbial granulation.* The granulation process was first studied in an upflow anaerob reactor (UASB) [6, 7].

Aerobic granules can be successfully cultivated in a SBR [8-10] without carrier material using readily bio-degradable substrates [11]. The term SBR is used as a synonym for variable volume, periodic process, suspended growth, biological wastewater treatment technology [12]. The light and dispersed flocs are washed out gradually, while the denser sludge particles are retained and accumulated through a repetitive selection in SRB operations, leading to the formation of compact granules. In these aerobic reactors, it was proven to be possible to grow stable granular sludge with integrated simultaneous COD and nitrogen removal capacity. Since then, SBR has been intensively used by researchers worldwide to develop and understand the concept and mechanism of aerobic granulation [13] and to evaluate the performances and potential of practical application of this technology. The type of inoculum used, feeding composition and SBR operational parameters (pH, temperature, cycling time, etc.) affect the granulation process [13].

Experimental part

Material and method

The experiments were conducted in a SBR reactor with a height to diameter ratio of 10 and a total working volume of 8 L. The schematic representation of the SBR (fig. 1) consists of:

- influent vessel (60 L);
- feeding pump (Heidolph, PUMPDRIVE 5001, peristaltic pump);
- column type reactor
- effluent vessel (60 L);
- effluent withdrawal pump (Heidolph, PUMPDRIVE 5001, peristaltic pump).

A Programable Logic Controller (PLC) ensured the cyclic operation of the SBR. A short settling time was preferred in order to allow the selection and growth of the fast settling bacteria and the wash out of the sludge with poor settleability.

The operational time sequence was as following: anaerobic feeding (45 min), aerobic reaction (11 h), settling (5 min) and effluent withdrawal (10 min). During the aerobic reaction stage, an air compressor supplied the column an airflow of 4 L/min.

The inoculum used in these experiments was represented by 5g/L of conventional activated sludge sampled from a municipal wastewater treatment plant. The bioreactor was fed with dairy industry wastewater characterized by high organic and nutrients load (table 1).

Treatment performances were evaluated based on analytical determinations of COD, NH_4^+, NO_2, NO_3^- and PO_4^{3-}. COD was analyzed volumetrically based on potassium dichromate method according to the ISO standard (SR ISO 6060:1996) and using heating mantle (Model KI16, Gerhardt, Germany). NH_4^+, NO_2^- and NO_3^- were determined using ion chromatography system ICS-3000 (Dionex, USA) according to the SR EN ISO 14911:2003 and SR EN ISO 10304/1:2009 standards (for the last two indicators).

Microscopic investigations (trinocular Optech microscope and trinocular Motic stereomicroscope with

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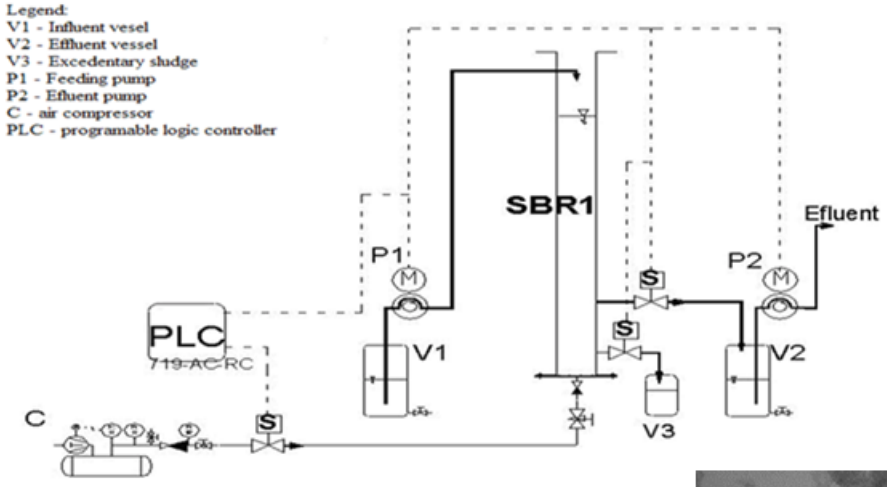


Fig. 1. Schematic representation of the SBR

Table 1
MAIN QUALITY PARAMETERS OF THE INFLUENT

CODCr mg O_2/L	1689 – 4610
BOD_5 mg O_2/L	492 – 1806
NH_4^+ mg/L	23 – 114
Ntot mg/L	53 – 162
P tot mg/L	10.4 – 50

built-in cameras) were performed to determine the diversity and dynamics of the aerobic granular sludge biocenosis community and granules formation.

Results and discussions

The microscopic investigation of the granular sludge biocenosis, which was monitored throughout the experiment, was characterized by the appearance and disappearance alternation of some species as a result of the operational parameters (aerobic conditions, HRT, etc.), influent quality and granules formation. At start up the inoculum was characterized by dispersed activated sludge flocs (fig. 2).

In the first step of granules formations both the number of each taxonomic colonies (especially colonial stalked ciliates *Opercularia* sp.) and also their percentage was higher than in the early phase of stability and sludge maturation. The first granular structures were observed only after 5 days.

The influent characteristic had a significant impact on the granular sludge biocenosis diversity. The overall effect was caused by the continuous entrance of spiral bacteria, flagellates and free swimming ciliates in the bioreactor (from the influent) as a result of high organic load. With the passing of the first stage of granules formation *Opercularia* sp. began to be gradually replaced with *Epistylis* sp. Also a decrease of the filamentous bacteria abundance

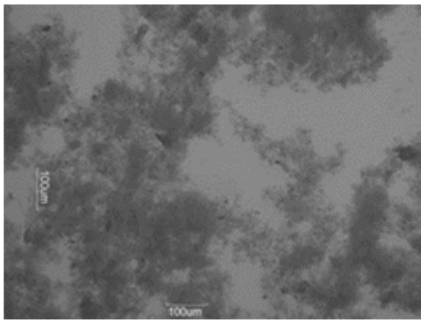


Fig. 2. Inoculum – conventional activated sludge (stereomicroscopic images 10X)

was recorded as a result of their incorporation in the granular structure (figs. 3a and 3b).

Throughout the experiment *Vorticella* species were reported, the most frequent being *Vorticella microstoma* (fig.4). *Vorticella convallaria* appearance in the bioreactor was the result of the trophic network adaptation and treatment conditions, for these species are known as bioindicators of a good wastewater treatment performance, but unfortunately failed to dominate the *Vorticella microstoma* species due to influent fluctuations in nutrients and organic matter content.

Initially the dense structure of the granules did not favor the development of stalked ciliates on the granules outer surface. This aspect can be caused by the following reasons:

- the granules outer surface did not facilitate the adherence of stalked ciliates either as a result of metabolites accumulation resulting from the granular core, either by the fact that the high density substances excreted by microorganisms have facilitated cohesion with other particles, which give the granules a complex structure by the continuous addition of new particles/organic and inorganic compounds, aspect that would prevent the stability of the stalked ciliates;
- a decrease in the water current strength from/within the granules as a result of the granular channels shrinking, which would imply that a lower number of microorganisms would be able to cross the granules outer surface.

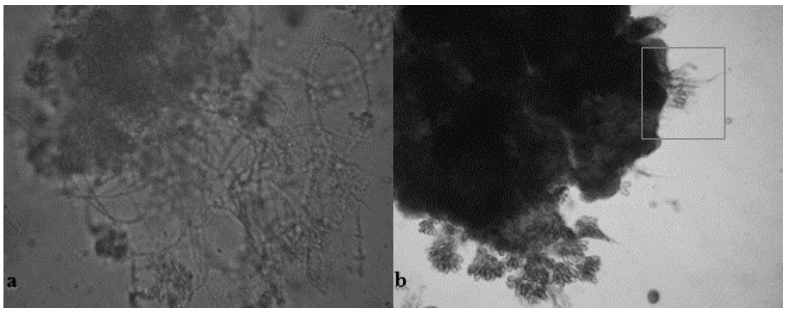


Fig. 3. Microscopic images: a – filamentous bacteria during the first phase of granules formation (20X); b – filamtous bacteria caught in the granules structural network during the stability phase (4X)

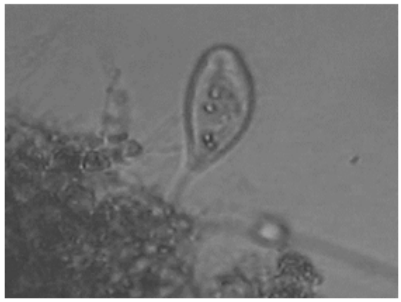
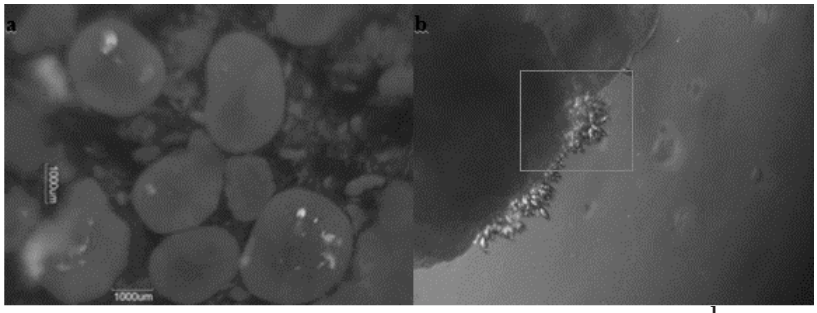


Fig. 4. *Vorticella microstoma* (microscopic image 10 X)



a b

Fig. 5a. obtained granules (stereomicroscopic image); b - *Opercularia sp.* on the granule surface layer

Table 2
TREATMENT PERFORMANCES

Effluent quality	Removal efficiencies
CODCr = 92 – 374 mg O_2/L	CODCr = 68 - 96 %
BOD_5 = 28.5 – 112 mg O_2/L	BOD_5 = 84.41 – 97.2 %
NH_4^+ = <0.05 – 23 mg/L	NH_4^+ = 68 – 99.85 %
Ntot = 3.4 – 42.9 mg/L	Ntot = 36.6 – 79.4 %
P tot = 4.42 – 18.08 mg/L	P tot = 11.54 – 72.24 %

After 26 days, the sludge in the bioreactor was in the form of granules with a diameter of up to 2 mm (fig. 5a). The granular sludge structure completion, characterized by a smooth, regular, round shape and a clear outer surface has later determined the development of colonial stalked ciliates (fig. 5b).

Treatment performances increased along with the growth of the granules size, taking into consideration the high nutrient and organic load of the influent and the total hidraulic retention time of 12 h. COD was removed with efficiencies that varied in the range of 68 - 96 %, while the BOD removal efficiencies ranged between 84 – 97 %. The obtained treatment performances are shown in table 2.

Nitrogen removal efficiencies varied during the experiment between 36 and 79 %, while phosphate removal efficiencies varied in the range of 11 and 72 %. This variations can be correlated with the influent nitrogen and phosphate load that ranged between 53 and 162 mg/L for nitrogen and between 10 and 50 mg/L for phosphate. High efficiencies for both organic and nitrogen load and even phosphorus load removal were possible due to diffusion gradients of substrate, nutrients and oxygen within the granules.

Conclusions

The experimental results conducted the following conclusions for industrial applications:

- the first granular structures were observed after only 5 days and increased significantly reaching up to 2 mm in size, after 26 days;
- the granular sludge biocenosis was characterized by testaceous rhizopoda (*Arcella* sp.), nude rhizopoda (*Amoeba* sp.), solitary stalked ciliates (*Vorticella* sp) and colonial stalked ciliates (*Epistylis* sp.);
- a significant impact on the granular sludge biocenosis community was given by the specific characteristics (high organic and nutrients load) of dairy industry wastewater used as influent. The overall impact was caused by the continuous entrance of spiral bacteria, flagellates and free swimming ciliates found in the influent in the bioreactor;
- the appearance of *Vorticella convallaria* indicated good treatment conditions as these species are known as bioindicators of good wastewater treatment performance;
- filamentous bacteria abundance began to decrease as a result of their incorporation in the granular structure;
- the obtained granular sludge had a smooth, regular, round shape and a clear outer surface;
- treatment performances increased along with the growth of granules size

Acknowledgements: The work was supported by European Social Fund, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/S/132765 and Project SOP HRD - PERFORM/159/1.5/S/138963 and Programme Nucleu 13N/2009, Project PN 09/13.03.10

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Manuscript received: 28.08.2015