# FORMATION OF AEROBIC GRANULES IN SEQUENCING BATCH REACTOR SBR TREATING DAIRY INDUSTRY WASTEWATER

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### Abstract

Many recent studies in the field of wastewater treatment and environmental protection have focused their attention on the possibility of obtaining aerobic granular sludge in order to develop new innovative wastewater treatment technologies. Compared to conventional activated sludge wastewater treatment plants, aerobic granular technology represent a novel alternative offering numerous advantages such as high biomass retention, good settling ability and simultaneous removal of organic load and nutrients. The main focus of research was to evaluate granules formation and evolution of treatment performances. Two lab scale sequencing batch bioreactors were used in the experiment. The first bioreactor (D) was inoculated with conventional activated sludge while the other one (GM) was inoculated with crushed aerobic granular sludge. Both bioreactors were fed with dairy industry wastewater with high organic and nutrients load (CODCr=1723 – 3550 mg  $O_2/L$ ,  $BOD_5 = 492 - 1806 mg O_2/L$ ;  $NH_4^+ = 64, 6 - 114 mg/L$ , P tot = 5, 04 - 21, 5 mg/L). The first granular structures were observed after 5 days (10 treatment tegranules in D bioreactor reached 2 mm in diameter while the granules in GM bioreactor. By the end of the experiment the granules in D bioreactor reached 2 mm in diameter while the granules in GM bioreactor reached up to 4 mm in diameter. Treatment performances increased along with the growth of granules size.

Key words: aerobic granular sludge, dairy wastewater, SBR.

## INTRODUCTION

Granular sludge technology is one of the great achievements in environmental biotechnology of the twentieth century, and was first observed in a anaerobic upflow sludge blanket (UASB) reactor designed to treat industrial wastewater at the end of the 1970's (Lettinga et al., 1980). However, the concept of aerobic granular sludge appeared later on - in the 1990's when Mishima et al. (1991) reported the first aerobic granular sludge in an aerobic upflow sludge blanket reactor treating municipal wastewater. Later researches on biofilm structure and on the role of storage polymers (extracellular polymeric substances - EPS) on biofilm formation lead to the idea of growing aerobic granules without carrier material on readily biodegradable substrates in Sequencing Batch

Reactor (SBR) (van Loosdrecht, 1997). 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 that time, SBR has been intensively used by researchers worldwide to develop and understand the concept and mechanism of aerobic granulation (Liu et al., 2004) and to evaluate the performances and practical potential application of this technology. Microbial granules can be considered as dense microbial aggregates. 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. Almost all aerobic granular sludge has been obtained and cultivated using sequencing batch reactors (SBRs) (Li et al., 2008, Jang et al., 2003) and has been used to treat high-strength wastewaters containing organics, nitrogen, phosphorous and toxic substances (Adav et al., 2008, Jiang et al., 2004) The granulation process can be affected by a number of parameters, such as seed sludge, substrate composition, organic loading rate, feeding strategy, reactor design, settling time, exchange ratio. and aeration intensity (hydrodynamic shear force).

## MATERIALS AND METHODS

The experiments were conducted in two identical column type SBR reactors with a height to diameter ratio of 10 and a total working volume of 8 L in order to evaluate the possibility of forming aerobic granules starting from different inoculum and to evaluate the evolution of treatment performances during startup and steady state conditions. Each of the SBR reactors, as it can be seen in the shematic representation of the AGSBR (figure 1) consisted of: influent vessel (60 L), feeding (Heidolph, PUMPDRIVE pump 5001, peristaltic pump), effluent vessel (60 L) and effluent withdrawal pump (Heidolph, PUMPDRIVE 5001, peristaltic pump). The cyclic operation of the SBR systems was ensured by a Programable Logic Controller (PLC) which controlled the feeding pumps and air inlet and effluent outlet electrovalves. Both bioreactors had identical operational time sequence: anaerobic feeding (45 min.), aerobic reaction (11 h), settling (5min.) and effluent withdrawal (10 min.). During aerobic reaction stage, an air compressor supplied each column at an airflow of 4 L/min. As settling time is an important hydrodynamic selection pressure operational parameter on the microbial community in the bioreactor, a short settling time was preferred and used to allow the selection and growth of fast settling bacteria and the wash out of the sludge with poor settleability. At startup, the two bioreactors used in the experiment were inoculated as follows: 1<sup>st</sup> bioreactor (D) was inoculated with 5 g/L of conventional activated sludge sampled from a municipal wastewater treatment plant while the 2<sup>nd</sup> bioreactor (GM) was inoculated with crushed and sieved (0.5 mm) aerobic sludge granules. The granules used were sampled from another lab scale working AGSBR. The idea was to evaluate how fast they recover the granular structure and treatment performances. Both bioreactors were with dairv industry fed wastewater characterized by high organic and nutrients load as shown in table 1.

Table 1. Main quality parameters of the influent

Parameter	Concentration range
CODCr mg O2/L	1723 - 3550
BOD <sub>5</sub> mg O <sub>2</sub> /L	492 - 1806
NH4 <sup>+</sup> mg/L	64.6 - 114
Ntot mg/L	64 - 162
P tot mg/L	5.04 - 21.5

Treatment performances were evaluated based on COD, NH4<sup>+</sup>, NO2<sup>-</sup>, NO3<sup>-</sup> and PO4<sup>3-</sup>. COD analyzed volumetrically based on was potassium dichromate method according to the ISO standard (SR ISO 6060:1996) and using KI16, Gerhardt, heating mantle (Model Germany).  $NH_4^+$ ,  $NO_2^$ and NO<sub>3</sub> were determined according to the SR EN ISO 14911:2003 and SR EN ISO 10304/1:2009 standards (for the last two indicators). respectively, using ion chromatography system ICS-3000 (Dionex, USA). The granules formation and growth evolution were monitored by particle size analyses carried out using Malvern, Mastersizer S2600 and by microscopic investigation (trinocular Optech microscope and trinocular Motic stereomicroscope with built-in cameras).



Figure 1. Schematic representation of the aerobic granular sludge SBR

### **RESULTS AND DISCUSSIONS**

In case of the 1<sup>st</sup> bioreactor (D) inoculated with conventional activated sludge, the first granular structures were observed after 5 days (10 treatment cycles) and continuously increased up to 2 mm in diameter after 22 days (figure 2, and 3).

Microscopic investigations emphasized the tendency of flocs to adhere to each other to form granules and implicitly to grow in diameter. Thus, in figure 2 (a) representing the inoculum we can observe dispersed activated sludge flocs while in 2 (b) and 2 (c) we can observe that the flocs are more compacted and granules are being formed so that after 26 days, the sludge in the bioreactor is under the form of granules with diameter of up to 2 mm (figure 3).



Figure 2. The evolution in time of aerobic sludge granules (microscopic images 4X)



Figure 3. Stereomicroscopic images (10X): A – inoculum –conventional sludge; B – granules 0,1-2 mm (after 26 days)

In case of the second bioreactor (GM) which was inoculated with crushed and sieved granules, compared to the granules obtained in bioreactor D (round shaped and smooth surface) within the same period of time and under the same operational conditions, the granules formed in bioreactor GM had irregular shape variable size but compact structure. (figure 4)



Figure 4. Stereomicroscopic images: A – inoculum – crushed aerobic granular sludge (40X); B – sludge granules (10X) ~0,5-1,6 mm (after 26 days)

Once with the increase of size granules the sludge settling speed and biomass concentration in the bioreactors increased leading to good treatment performances considering the nutrient and organic load of the influent, the total hidraulic retention time of 12 hours and the fact that it is only a one step process (aerobic). The treatment performances obtained in the two experimental SRB reactors, D and GM, are presented comparatively in table 2.

Table 2. Comparative presentation of the treatment performances in both reactors

Parameter	Bioreactor D	Bioreactor GM
CODCr	91 – 95 %	70 - 91 %
BOD <sub>5</sub>	93 – 97 %	75 - 93 %
$\mathrm{NH_4}^+$	94 – 99 %	82 - 94 %
P tot	65 – 93 %	65 – 90 %
Ntotal	48 - 81 %	50 - 80 %

Treatment efficiency was higher in D bioreactor than in GM bioreactor. This can be explained by lower specific surface area of the granules and lower diffusion gradients of nutrients within the granules.

## CONCLUSIONS

The focus of the research was to evaluate the granules formation and performances evolution during startup and steady state conditions. In both cases the first granules formation were

observed after 5 days of inoculation. Even though the two bioreactors used in the experiment underwent the same operational conditions GM bioreactor, inoculated with crushed granular sludge, has shown slightly lower treatment performances during start-up compared to D bioreactor, inoculated with conventional activated sludge. The aerobic granular sludge proved to be stable and adaptable to high nutrients concentrations succeding to efficiently remove the organic load and nutrients from the influent wastewater.

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