Aerobic granular sludge – state of the art

M.K. de Kreuk*, N. Kishida** and M.C.M. van Loosdrecht*

*Department of Biotechnology, Delft University of Technology, Julianalaan 67, 2628BC, Delft, The Netherlands (E-mail: m.dekreuk@tnw.tudelft.nl; m.c.m.vanloosdrecht@tnw.tudelft.nl)

**Department of Chemical Engineering, Waseda University, 3-4-1, Ohkubo, Shinjuku-ku, Tokyo, Japan

Abstract In September 2006, preliminary to the IWA biofilm conference, a second workshop about aerobic granular sludge was held in Delft, The Netherlands, of which a summary of the discussion outcomes is given in this paper. The definition of aerobic granular sludge was discussed and complemented with a few additional demands. Further topics were formation and morphology of aerobic granular sludge, modelling and use of the aerobic granular sludge in practice.

Keywords Aerobic granular sludge; EPS; modelling; morphology; pilot plant; self-aggregation

Introduction

At the end of the 1990s, research on biofilm structure and formation (van Loosdrecht et al., 1995, 1997a) and on the role of storage polymers (van Loosdrecht et al., 1997b; Krishna and van Loosdrecht, 1999) resulted in the idea of growing aerobic granules without carrier material on readily biodegradable substrates in a Sequencing Batch Reactor (SBR) (Morgenroth et al., 1997; Beun et al., 1999; Dangcong et al., 1999). The conversion of readily biodegradable COD into a substrate yielding a lower maximal growth rate facilitated granule formation. In these aerobic reactors, it was proven to be possible to grow stable granular sludge (Figure 1) with integrated COD and nitrogen removal. In 1998, an international patent was submitted and granted (Heijnen and van Loosdrecht, 1998). An extension of this first patent was submitted, including the description of anaerobic feeding (van Loosdrecht and De Kreuk, 2004).

From the year 2000, aerobic granule formation has been excessively studied worldwide (Figure 2). Many theories about aerobic granule formation made their way into different studies. Analysis of a cross section of literature published in the last decade shows that type of substrate, COD and N-load, superficial gas velocity or shear stress and oxygen concentration are important parameters. An important secondary parameter for the formation and maintenance of dense granules is the growth rate of the organisms that is influenced by cycle configuration or loading rates (De Kreuk et al., 2005a). An extensive overview of parameters that are important for anaerobic and aerobic granule formation has been given by Liu and Tay (2004).

In the year 2004, the first workshop on aerobic granular sludge was organised in Munich, Germany (Bathe et al., 2005). The conclusion that could be made from the aerobic granular sludge workshop 2004 was: “all participants agreed that up till then a lot of basic knowledge was gained on aerobic granule formation and that the pioneering stage was finished. Researchers should continue with specific research topics (e.g. factors influencing granule strength and formation, microbial diversity, conversion processes, pathogen removal, pilot- and demonstration-scale studies) and that the technology should be put into practice. Within a couple of years, this workshop should be repeated to see how research went from there; hopefully with new insights and with new applications.”
Therefore, in September 2006, preliminary to the IWA biofilm conference, a second workshop about aerobic granular sludge was held in Delft, The Netherlands, of which a summary of the discussion outcomes is given below.

**Definition of aerobic granular sludge**

Despite the long-time application of anaerobic granular sludge in wastewater treatment, aerobic granular sludge is a new observation. Therefore a definition of aerobic granular sludge was made during the first aerobic granular sludge workshop, 2004 (De Kreuk et al., 2005b):

*Granules making up aerobic granular activated sludge are to be understood as aggregates of microbial origin, which do not coagulate under reduced hydrodynamic shear, and which settle significantly faster than activated sludge flocs.*

The explanation of the different parts of the statement was discussed and analysed during the 2006 workshop and delegates agreed on the statement, when the following explanation is used:

1. Aggregates of microbial origin: speaking of granular activated sludge in the statement implies that aerobic granules need to contain active microorganisms and cannot only consist of components of microbial origin (as proteins, EPS, etc.). The microbial population in aerobic granular sludge are to be expected more or less similar to the ones in activated sludge and or biofilms, thus there is no need to describe specific groups of microorganisms in the definition. Furthermore, this part implies that no carrier material is intentionally involved or added; the aggregate is formed without the dosage of such carrier material.

**Figure 1** Activated sludge from a wastewater treatment plant (a) and aerobic granular sludge cultivated in a laboratory scale reactor (b) and in a pilot plant (c)

**Figure 2** Number of publications about aerobic granular sludge per year (Source: Web of Science)
(2) No coagulation under reduced hydrodynamic shear: this part describes the difference in behaviour between activated sludge and aerobic granular sludge. Activated sludge flocs tend to coagulate when they settle (when liquid-sludge mixture is not aerated or stirred), whilst granules do not coagulate and settle as separate units.

(3) Which settle significantly faster than activated sludge flocs: this means that SVI10 (SVI after 10 min of settling) in combination with SVI30 should be used for characterising the settleability of granular activated sludge as was suggested by Schwarzenbeck et al. (2004). The difference between the SVI10 and SVI30 value gives an excellent indication about the granule formation and indicates the extent of thickening after settling.

(4) The minimum size of the granules should be as such that the biomass still fulfils point three. This minimum size was set to 0.2 mm, which was decided based on measurements in the past. This limit could be adjusted per case/granule type, as long as the other demands of the definition hold.

(5) Sieving is considered a proper method to harvest granules from activated sludge tanks or from aerobic granule reactors, which also determines certain strength of the required biomass matrix.

When an aggregate fulfils all characteristics as described above, it can be called aerobic granular sludge. This simplifies the interpretation of experimental results and clarifies when to speak about aerobic granular sludge, activated sludge or biofilms.

**Formation and morphology of aerobic granular sludge**

Many factors are held responsible for the formation and stability of aerobic granular sludge, but it is undecided among scientists which factor is the dominant one. Discussions focused on this topic and a few parameters were highlighted: a) use or appearance of specific self-aggregating cultures; b) selection by settling velocity; c) applied shear stress; d) growth rate of the organisms; e) substrate gradients inside the granules; f) formation of extra-cellular polymeric substances (EPS).

Formation of aerobic granular sludge in laboratory experiments mostly occurs with a strict selection regime for well settling sludge by applying short settling times. The aggregate forming organisms will be maintained in the reactor, while other organisms are washed out with the effluent. To enhance the start-up of an aerobic granular sludge reactor, the use of specific self-aggregating cultures was suggested by the researchers of Nanyang University (Singapore). Non-pathogenic cultures with self-aggregating abilities were selected and added to a reactor during start-up. This shortened the start-up time considerably (3 days instead of 9 days without specific inoculum). The selection for specific organisms to enhance granule formation and stability has also been applied by other researchers, e.g. the use of phosphate or glycogen accumulating organisms (Dulekgurgen et al., 2003; De Kreuk and van Loosdrecht, 2004) or nitrifying organisms (Liu et al., 2004). However, the attending researchers agree that, based on published research, substrate gradients inside the granules are very important as well and that sharp decreasing substrate gradients inside the granules should be avoided by using the ability of converting readily biodegradable substrates into storage polymers and/or using organisms with low actual growth rates. EPS production by slow growing organisms enhance the granule formation (Liu et al., 2004b; McSwain et al., 2005, presented research results of National Taiwan University) and is seen as an important aspect by the attending researchers as well.

Applied shear stress and settling time has been an important topic for studies in the past and still leads to discussions. At the aerobic granular sludge workshop 2006, several researchers showed their results about this topic (Northwestern University, USA; University of Beijing, China; Istanbul Technical University, Turkey). Shear stress is difficult to
quantify and is often related to superficial gas velocity. However, factors such as stirring should not be underestimated and reported as well.

There was general agreement on the positive effects of hydraulic selection pressure on granule formation and stability. When aerobic granular sludge is used with a membrane to filter the effluent, the selection pressure for aggregates by settling totally disappears. Even then, very dense and large flocs were obtained, having a positive effect on the membrane fouling. The high density flocs did only meet the definition of aerobic granular sludge during short periods of the total experiment, especially during the periods that autotrophic organisms were present (results presented by INSA, France).

**Modelling of aerobic granular sludge**

Mathematical modelling has proven very useful to study complex processes, such as the aerobic granular sludge systems (Beun et al., 2001; Lübken et al., 2005). Biological processes in the granules are determined by concentration gradients of oxygen and diverse substrates. The concentration profiles are the result of many factors, e.g. diffusion coefficients, conversions rates, granule size, biomass spatial distribution and density. All of these factors tightly influence each other, thus the effect of separate factors cannot be studied experimentally. Moreover, experiments in granular sludge reactors take many weeks to reach steady state. A good computational model for the granular sludge process provides significant insight in the most important factors that affect the nutrient removal rates and in the distribution of different microbial populations inside the granules. Further, models could also be used for process optimisation and for the scale-up and design (e.g. hydraulics) of a full-scale reactor.

Aerobic granular sludge systems can be modelled in different ways, using different modelling tools depending on the fields of interest. When the overall reactor behaviour is described (substrate removal or sludge production), traditional biofilm modelling can be used, as in AQUASIM (Reichert, 1998). Such models have already been developed by the groups in Munich and Delft. The effect of process parameters on the nutrient removal rates could be reliably evaluated with such models. Influence of oxygen concentration, temperature, granule diameter, sludge loading rate and cycle configuration have been analysed. Oxygen penetration depth in combination with the position of the autotrophic biomass played a crucial role in the conversion rates of the different components and thus on overall nutrient removal efficiencies (de Kreuk et al., Accepted).

When a more detailed insight in microbial or EPS distribution inside the granule is desired or to study factors influencing granule shape (presence of filamentous outgrowth, biofilm structure modelling), individually based modelling can be used (Kreft et al., 2001). Such a model for aerobic granular sludge was presented by TU Delft and Universidade Nova de Lisboa, Portugal, to describe an aerobic granular sludge SBR (Xavier et al., Submitted). This multiscale model described the granular sludge reactor in detail, from the metabolism of microbial groups, through the spatial structure of granules to the dynamics of the whole reactor. However, simulations were computationally demanding and use will be restricted to a description of observed trends. With the model a preferential distribution of species along radial distances was shown, which were more heterogeneous than in strict layers. This heterogeneous structure and growth in microcolonies underlined the difficulty of representative micro-electrode measurements. Also, the accumulation of inert material in the cores of the granules was shown (Figure 3). Mainly the outer layers of the granule will be eroded, which contain less inert material. Therefore, aerobic granular sludge is expected to contain more inert material resulting from biomass decay than activated sludge.
Waseda University (Japan) and TU Delft developed a similar multi-scale model with nitrifying granules. This model showed that within the nitrifying granules, EPS producing heterotrophic organisms will grow on products from cell-lyses. These heterotrophic organisms denitrify part of the produced nitrate and grow mainly inside of the granules. At this position, they excrete EPS, which strengthens the structure of the granule and enhances the growth of the total granule. The nitrifying organisms, growing mainly in the outer layers, will detach more which leads to a smaller sludge residence time for these species.

Different effects of shear stress were not taken into account in the models as presented during the workshop. Dr. Ivanov, Nanyang University, Singapore pointed out that mechanical compaction of granules by shear stress caused by particle-particle collisions, air-bubbles or mechanical stirring might affect the distribution of microorganisms, detachment and porosity of the granular sludge. Changing porosity can affect the diffusion in the pores of the granule and detached parts from the granules can start new granules, both leading to a different microbial composition of the granule then described with current models.

**Aerobic granular sludge in practice**

In wastewater treatment, the activated sludge process is the dominant system. Biology of these systems has been optimised and the limits of the system have been reached. However, sludge settleability and washout from clarifiers will remain a point of attention. Major concerns of water authorities are: minimising costs of wastewater treatment; meeting effluent requirements; being prepared for future developments in effluent demands; area availability; environmental aspects as smell and noise; energy consumption. Aerobic granular sludge could (partly) meet these concerns.

Following successful pilot-plant studies at two sewage treatment plants in The Netherlands by DHV, a first full-scale municipal wastewater treatment plant in The Netherlands, based on aerobic granular sludge is planned (presented by waterboard “Hollandse Delta”). As end-users, waterboards in The Netherlands see it as their (public) responsibility to cooperate with consultancies and universities to develop this innovation and invest in full-scale applications.

Several wastewaters from industrial and municipal origin were used to cultivate aerobic granules and to study the treatment process and the results were presented during the workshop; a pilot plant study (two reactors of 1.5 m³ each) with the Nereda™ system treating sewage at a Dutch wastewater treatment plant (presented by DHV Water, The Netherlands); a demonstration scale sequencing batch biofilter granular reactor (SBBGR, 3.1 m³) treating sewage at a Italian wastewater treatment plant (presented by IRSA, [image]
Italy); laboratory scale experiments using livestock wastewater, containing high concentrations of nitrogen and phosphate (presented by Waseda University, Japan); using anaerobically pretreated abattoir effluent (presented by AWMC, Australia) and using low strength sewage (presented by Sumitomo Heavy Industries Ltd., Japan).

The main conclusion from all presentations was that the different aerobic granular sludge technologies were well applicable on tested wastewater; granules could be formed with good stability and conversion processes were satisfying. However, the more TSS in the wastewater and the more diluted the sewage, the less suitable tested systems were. It should be pointed out that in all experiments alternating anaerobic and aerobic periods were used, except for the SBBGR experiment and the experiment with diluted sewage, in which a pulse feed followed by aeration was used.

Conclusions

Research in aerobic granular sludge developed from mostly laboratory scale experimental research to modelling studies and studies at pilot and practical scale. The recommendation of the workshop 2004 was that the pioneering stage of aerobic granular sludge research was finished. Researchers should continue with specific research topics (e.g. factors influencing granule strength and formation, microbial diversity, conversion processes, pathogen removal, pilot- and demonstration scale studies) and that the technology should be put into practice. Partly this recommendation has been fulfilled; however, still many specific aspects about aerobic granular sludge formation, stability, diversity and process optimisation are unrevealed. One of the main concerns now for laboratory scale research is that a clear and standardised method for measuring EPS should be defined in the future, as only then the function of EPS in granule formation can be studied.

For applications of aerobic granular sludge in sewage treatment or industry, the end-users need to make the first steps now towards this new technology and to allow research to obtain more specific insights in self aggregation of microorganisms and all related aspects.

Acknowledgements

We thank the participants from the aerobic granular sludge workshop 2006 for their enthusiastic and active involvement and IWA for supporting this workshop.

References


AQUASIM 2.0 - Computerprogram for the Identification and Simulation of Aquatic Systems 2.0. EAWAG, Dubendorf.


