BUDAPEST UNIVERSITY OF THECHNOLOGY AND ECONOMICS
FACULTY OF CHEMICAL TECHNOLOGY AND BIOTECHNOLOGY
GYÖRGY OLÁH DOCTORAL SCHOOL

Effects of N and P deficiency, redefining the role of Glycogen Accumulating Organisms in activated sludge wastewater treatment systems in respect of the bioreactor arrangement

Thesis book

Author: **Bernadett Lemaire**, biochemical engineer, M. Sc.
(maiden name: Bernadett Kiss)

Supervisor: **Dr. Andrea Jobbágy**, H. C. Professor

BUTE, Department of Applied Biotechnology and Food Science
Research group of Wastewater Treatment Biotechnologies

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1. INTRODUCTION, AIMS AND SCOPE

While in domestic wastewater treatment shortage of carbon source has been more and more likely to experience (*Tardy et al., 2012*), shortage of nutrients, i.e. low N and/or P availability may be the characteristic of the industrial, especially of food industrial influents. Winery wastewater may derive from a number of technological steps including cleaning of the basins, the equipment and the floor; rinsing the transfer lines, washing the barrels, bottling facilities and filtration units, etc. Both the volume and the pollution load of winery effluents vary greatly in relation to the operational period (i.e. vintage, racking, filtering and bottling) and the kind of wine produced. The amount of wastewater generated in a winery ranges from 0.7 to 1.2 times of the wine produced (*Andreottola et al., 2009*), which draws an increased attention to the winery wastewater treatment. Several processes are currently available for treating winery wastewater. However, it is still the biological treatment, mainly the Conventional Activated Sludge (CAS) treatment, being most commonly used in full-scale as it is considered to be the most environmental-friendly and cost-effective solution, although its control needs adequate engineering expertise.

The nutrients – mainly the nitrogen and phosphorus – usually play an important role in the wastewater treatment, as their presence is fundamental for the bacterial growth, reproduction and energy transport processes. The influent wastewater of the sewage treatment plants usually contains considerable amount of nitrogen and phosphorus. Whereof the relative amount of nitrogen is showing a particularly increasing tendency in the inlet water of the Hungarian municipal wastewater treatment plants. While in food industrial wastewaters it may be in deficit. It is generally presumed that microorganisms need these nutrients in an optimal ratio relative to the available organic matter (mostly given in COD- chemical oxygen demand or in BOD_5 – biochemical oxygen demand).

Marginal nutrient availability may cause enhanced proliferation of filamentous microorganisms (*Wanner and Jobbágy, 2014*), whereas serious nutrient deficiency may lead to overproduction of extracellular polysaccharides (glycocalyx), resulting in adherent, bulking biomass in fully aerated CAS systems. This viscous bulking just like the dominance of filaments, i.e. filamentous bulking deteriorates activated sludge floc structure and leads to poor separability and hindered settleability (*Jenkins et al., 2004*).

The traditional way of eliminating any kinds of nutrient deficiency is dosing external nitrogen and/or phosphorus sources (*Wanner and Jobbágy, 2014*). Application of this method is not only increasing the treatment costs but it also involves difficulties of determining the optimal dose which thence has to be continuously adjusted to the influent organic load to avoid underdosing. While in case of nutrient overdose, the surplus of nutrients has to be removed from the treated water. Determining the adequate dosing point is also of great importance, as the dosed nutrients may be precipitated by other chemicals that are dosed for pH adjustment or to enhance the settling.

Existence of Glycogen Accumulating Organisms (GAOs) was first reported about 20 years ago, by *Liu et al.* (1996), as a group of microorganisms that are able to take up and store carbon sources anaerobically.

Previously Phosphorous Accumulating Organisms (PAOs) were thought to be unique to be able to exist under alternating anaerobic-aerobic conditions, while performing Enhanced Biological Phosphorous Removal (EBPR). Since their similar metabolic characteristics to PAOs, GAOs are believed to be responsible for failures of EBPR systems, thus most of the scientific studies have been carried out aiming to find ways to efficiently suppress their growth.

The Research group of Wastewater Treatment Biotechnologies at the Department of Applied Biotechnology and Food Science was the first to develop and verify in lab-scale a cost-effective solution to treat nutrient deficient wastewater. According to this newly developed Enhanced Biological Carbon Removal (EBCR) technology by the implementation of an appropriate reactor arrangement, i.e. by the installation of an anaerobic selector, Glycogen Accumulating Organisms can be enriched in nutrient deficient wastewaters. As GAOs are able to take up excess amount of carbon in their cells by obtaining energy from their intracellular glycogen pool, their controlled proliferation may decrease the relative nutrient deficiency, thus the cause of viscous bulking (*Jobbágy et al. 2002*).

My PhD work aimed to elucidate the theoretical basics of this novel method and to verify its full-scale applicability in the treatment of food industrial wastewaters. My goal was to explore the problems appearing in the full-scale application and to prove that this solution is more cost-effective and more secure than the conventional methods.

Accordingly, my objectives were the followings:

- to demonstrate the possibly occurring inconvenient consequences of employing the conventionally used methods (mainly the nutrient dosing) in the treatment of nutrient deficient wastewaters;
- to investigate the effect of nitrogen and phosphorus deficiency on the Enhanced Biological Carbon Removal and to identify its role in the deterioration of the biomass structure and the treatment efficiency;
- to prove that the developed Enhanced Biological Carbon Removal technology is adequately working in full-scale and it is the consequence of the GAO activity.

Andreottola, G., Foladori, P., Ziglio, G., (2009) Biological treatment of winery wastewater: an overwiew. Water *Science and Technology*. 60(5):1117-1125.

Jenkins, D., Richard, M.G., Daigger, G.T. (2004): Manual on the Causes and Control of Activated Sludge Bulking and Foaming. 3rd edition., CRC Press LLC, Boca Raton, Florida, US

Jobbágy, A., Literáthy, B., Tardy, G. (2002) Implementation of Glycogen Accumulating Bacteria in Treating Nutrient-deficient Wastewater. *Water Science and Technology*. 46(1-2), 185-190.

Liu, W.-T., Mino, T., Nakamura, K. and Matsuo, T. (1996) Glycogen accumulating population and its anaerobic substrate uptake in anaerobic-aerobic activated sludge without biological phosphorous removal. *Water Research*. 30(1), 75-82.

Tardy, G.M., Bakos, V., Jobbágy, A. (2012): Conditions and technologies of biological wastewater treatment in Hungary. *Water Science and Technology*. 65, 1676-1683.

Wanner, J. and Jobbágy, A., 2014. Activated sludge solids separations, Chapter 10 in Activated sludge – 100 years and counting,Eds. Jenkins, D. and Wanner, J., 2014 IWA Publishing, Glasgow, pp. 171-173. ISBN 9781780404936

2. METHODOLOGY

Aiming to reveal the difficulties of the traditional way of overcoming nutrient deficiency through applying external nutrient dosing two 2-staged continuous-flow lab-scale activated sludge systems were operated simultaneously. In one of the experimental systems the aerobic main reactor was preceded by an aerated selector while in the other it was preceded by an unaerated selector. The influent synthetic feed in both cases was at the border of nutrient deficiency, similarly to the cases when supplementary nutrients are dosed to a nutrient deficient wastewater in a commonly used ratio relative to the carbon source.

In order to examine the effect of phosphorus deficiency a continuous-flow onsite comparative pilot-scale experiment was carried out to examine the applicability of chemically enhanced primary clarification containing systems with staged bioreactors in the biological nitrogen and phosphorus removal.

With the purpose of investigating the consequences of N and P insufficiency, the effect of different grades of nitrogen deficiency at both marginal availability and severe deficiency of P was examined in continuous-flow model experimental systems on the performance (on the biomass structure, on the Enhanced Biological Phosphorus Removal (EBPR) and on the growth of GAOs) of the activated sludge systems treating winery wastewater. Thus, two continuous-flow anaerobic-aerobic experimental systems were operated simultaneously, one with marginal nitrogen availability and the other receiving severely nitrogen deficient influent wastewater. At first with P in excess just as in the EBPR systems and subsequently under P deficient conditions, simulating the winery wastewater.

To investigate the applicability of the obtained lab-scale results in practice, the operation of a pretreatment system of a cannery wastewater and the consecutive domestic sewage treatment plant was examined through the analysis of the previously collected operational data and the results of the on-site profile measurements. The research revealed the reasons for the low performance and the sludge settling problems and a reasonable upgrading solution was proposed for the pretreatment system.

The eventual experiments of this PhD study were carried out at the Balatonboglár Winery's sewage treatment plant with the first full-scale implementation of the EBCR technology, where the influent is characteristically nutrient deficient. To investigate the efficiency of the Enhanced Biological Carbon Removal technology in full-scale on-site profile measurements were carried out. Supplementary batch tests were also performed and in cooperation with the National University of Singapore, through utilization of Fluorescence In Situ Hybridization (FISH) and Terminal Restriction Fragment Length Polymorphism (TRFLP) microbiological techniques direct proof has been obtained in order to prove the presence of GAOs in the biomass.

3. RESULTS

Results related to the research to demonstrate the possibly occurring inconvenient consequences of employing the conventionally used supplementary nutrient dosing methods in the treatment of nutrient deficient wastewaters:

It has been verified in lab-scale that the common practice of external nutrient dosing may not be successfully used to treat nutrient deficient food industrial wastewaters. As food industrial wastewaters typically contain high amount of readily biodegradable carbon sources the biomass yield may be higher than the commonly used values. Thus, the minimum required N, P doses do not by all means correspond to the commonly used ratios whose application may lead to the underestimation of the external nutrient need.

The experimental results proved that reduced nutrient dosing often leads to marginal nutrient availability that causes more difficulties in the operation of an activated sludge system, than the complete lack of nitrogen and phosphorus which can be reduced or compensated by the directed proliferation of GAOs in the biomass.

In continuous-flow model experiment it has been observed that the marginal nutrient availability caused by the reduced nutrient dosing leads to the overproduction of extracellular polysaccharides in fully-aerobic activated sludge systems. While in unaerated selector containing systems low-DO conditions may occur in the selector that facilitates the proliferation of filamentous bacteria. Both of these events lead to biomass separability problems.

Results related to the research aiming to investigate the effect of nitrogen and phosphorus deficiency on the Enhanced Biological Carbon Removal and to identify its role in the deterioration of the biomass structure and the treatment efficiency:

In a continuous-flow on-site model experiment it has been proven that through utilization of primary clarification or chemically enhanced primary clarification the organic load can be decreased, that represents an advantage in the nitrification process. However, the influent phosphorous content may be partly removed in the clarification basin, where the organic matter is extracted, which is limiting the possibility of the biological phosphorus removal. And the denitrification might be incomplete as part of the carbon source may also be eliminated in the primaries, thus less necessary denitrifiable remains in the wastewater.

According to the experimental results obtained from a continuous lab-scale comparative experiment it can be assumed that compared to PAOs, GAOs are less sensitive to nitrogen deficient conditions therefore not only the P deficient conditions but severe N deficiency may also lead to the proliferation of GAOs thus the failure of the EBPR systems.

In laboratory-scale continuous-flow comparative experiment it has been experienced that withdrawal of the P dosing (COD/P=100/0.03) immediately cut the biological phosphorous removal, just like in EBPR systems where chemicals for reducing phosphorus content are overdosed.

Results related to the research to prove that the developed Enhanced Biological Carbon Removal technology is adequately working in full-scale:

The results of the research verified that the vegetable processing wastewaters are produced in highly fluctuating wastewater quality and quantity. Nevertheless, the fluctuations of the influent nutrient, mainly nitrogen content of food industrial wastewaters, can be tolerated by the establishment of a highly flexible system. This system should include an unaerated first stage, where efficient denitrification can be achieved, in case the influent sewage contains nitrogen in excess. During production campaigns resulting in N-deficient wastewater, the unaerated basin can be operated under anaerobic conditions which may enhance the growth of glycogen accumulating organisms, and thereby help to avoid viscous bulking.

On-site profile measurements verified that the Balatonboglár Winery's wastewater treatment plant, containing consecutive unaerated and aerated activated sludge basins, tolerated extremely high influent C/N and C/P ratios as well as low influent pH without deteriorating its excellent carbon removal efficiency. Results of supplementary batch tests carried out by taking grab-samples from the plant showed that the biomass of the Winery's wastewater treatment plant consumed carbon source under unaerated conditions, a phenomenon that could neither be related to denitrification, nor to eventual metabolism of polyphosphate accumulating organisms. Despite the severe nutrient deficiency, the sludge structure and the settling characteristics were adequate. These results, together with the high intracellular carbohydrate content of the biomass suggested the presence of glycogen accumulating organisms. Microscopic observations as well as the executed DNA-based analysis (FISH, T-RFLP) verified the high abundance of GAOs in the biomass, which performed the role of excess biological carbon removal in this novel technology developed by our Research group.

4. NOVEL FINDINGS

1. At the wastewater treatment plants the common practice of N and P dosing with the aim of compensating the shortage of nutrients may lead to marginal nutrient deficiency. At limited nitrogen and phosphorus availability, under low-DO conditions unfavourable biomass structure will develop, which can be characterised by the excess growth of filamentous microorganisms. It has been experimented in lab-scale that in these cases application of a selector containing system cannot improve the biomass structure and thus the settling ability; as under these conditions at high aeration rate the proliferation of filamentous microorganisms, in more severe cases overproduction of extracellular polysaccharides may occur that leads to instable operation of the system. [Publications I., II.]

2. Continuous-flow onsite comparative pilot-scale experiment revealed that chemicals dosed to enhance the primary settling efficiency may partly remove the influent phosphorous content, that is limiting the possibility of the biological phosphorus removal, moreover the denitrification might be incomplete as part of the carbon source may also be eliminated. [Publications V., VII.]

3. It has been demonstrated in model experimental systems that withdrawal of phosphorus availability (COD/P=100/0.03) is enough to immediately cut the enhanced biological phosphorous removal. This event may easily occur in Enhanced Biological Phosphorus Removal (EBPR) systems where chemicals for reducing phosphorus content are overdosed. [Publications II., V.]

4. In lab-scale experiment it has been observed that sole pronounced deficiency of N relative to the available readily biodegradable carbon sources leads to the proliferation of GAOs in the biomass, thus the failure of the EBPR systems. [Publication II.]

5. It has been verified in lab-scale that the GAO-activity based Enhanced Biological Carbon Removal (EBCR) system ensures stable pollutant removal performance even at joint deficiency of both N and P; and the settling ability of the biomass remains adequate even after the complete withdrawal of the phosphorus dosing. [Publication II.]

6. Full-scale profile measurements of pollutants' concentration revealed that through the aerobic treatment of the highly fluctuating food industrial wastewaters, the low performance and the biomass settling problems are deriving from the occasionally too high or too low influent N/C ratios. Implementation of an unaerated/aerated reactor arrangement containing system was suggested as a unique combination of the pre-denitrification and the EBCR technologies, where in case of high influent N/C ratios efficient denitrification can be achieved by operating the unaerated first stage anoxically. While low influent N/C ratios could be tolerated by operating the unaerated basin under anaerobic conditions which enhances the growth of glycogen accumulating organisms. [Publication III.]

7. The profile measurements carried out at the Balatonboglár Winery's wastewater treatment plant as well as the supplementary batch tests and the DNA-based microbiological analysis' confirmed in full-scale that the Enhanced Biological Carbon Removal based on the GAO activity can be successfully applied for the treatment of severely nutrient deficient wastewaters even at low influent pH. [Publication IV. and VI.]

5. APPLICABILITY IN PRACTICE

The GAO-activity based Enhanced Biological Carbon Removal (EBCR) technology ─ that has been implemented at the Balatonboglár Winery's sewage treatment plant where its applicability has been confirmed in full-scale ─ is the most beneficial for the treatment of N and/or P deficient food industrial wastewaters produced in seasonally fluctuating quality and quantity. As the conventional activated sludge (CAS) technology is the most commonly used, at an operating food industrial (or other non-toxic, nutrient deficient, increased readily biodegradable carbon source containing) sewage treatment plant it can be implemented by the installation of a wall. This EBCR technology permits to economise the conventional techniques' extra cost of chemicals, and avoids the risk of the instable operation occurring as a consequence of insufficient dosing, or the denitrification costs due to overdosing. Moreover, in case of any variations in the influent quality (e.g. increased N content) the deterioration of the treatment efficiency can be prevented by the anoxic operation of the unaerated basin.

6. PUBLICATIONS

Basic publications of the thesis

Peer reviewed scientific papers in English

I. Bakos, V., Kiss, B., Jobbágy, A. (2016) Problems and causes of marginal nutrient availability in winery wastewater treatment. *Acta Alimentaria,* 45(4), 532–541. IF: 0.357 (2016)

II. Jobbágy, A., Kiss, B., Bakos, V. (2017) Conditions Favoring Proliferation of Glycogen Accumulating Organisms for Excess Biological Carbon Removal in Treating Nutrient Deficient Wastewater. *Periodica Polytechnica Ser. Chem. Eng.* 61(3), 149-155. IF: 0.557 (2016)

III. Jobbágy, A., Kiss, B., Bakos, V., Tardy, G. (2009) Activated sludge nuisances in a vegetable processing wastewater pretreatment plant. *Acta Alimentaria,* 38 (3) 393-404. IF: 0.505 (2009)

IV. Kiss, B., Bakos, V.,Liu, W. T., Jobbágy, A. (2011) Full-Scale Use of Glycogen-Accumulating Organisms for Excess Biological Carbon Removal. *Water Environment Research*, 83 (9) 855-864. IF: 0.883 (2011)

Presentations published in conference proceedings in English

V. Kiss, B., Bakos, V., Szabó, A., Jobbágy, A. (2007) Comparative experimental studies for upgrading the Northpest Wastewater Treatement Plant. 10th IWA Specialised Conference on „Design, Operation and Economics of Large Wastewater Treatment Plants" 9-13 September 2007, Vienna, Austria (poster-presentation) Conference Proceedings pp. 177-180

VI. Kiss B., Bakos V., Jobbágy A. (2017) Utilization of Glycogen Accumulating Organisms for Excess Biological Carbon Removal in Activated Sludge Systems. 9th IWA Eastern European Young Water Professionals Conference on „Uniting Europe for Clean Water: Cross-Border Cooperation of Old, New and Candidate Countries of EU for identifying problems, finding causes and solutions", 24-27 May 2017, Budapest, Hungary, Conference Proceedings pp. 441-447 ISBN: 978-963-313-256-2

Scientific paper in Hungarian

VII. Jobbágy, A., Kiss, B. (2006) A Biológiai Nitrogéneltávolítás Problémái és Lehetőségei. *Vízmű Panoráma,* 14 (1) 7-11.

Platform presentations related to the scope of the doctoral thesis in Hungarian

Kiss, B. (2007) Glikogénakkumuláló mikroorganizmusok nagyüzemi alkalmazása biológiai többletszén eltávolításra boripari szennyvíz tisztításakor. Oláh György Doktori Iskola 4. Doktoráns Konferencia, Budapest, február 7.

Kiss, B. (2008) A glikogénakkumuláló mikroorganizmusok szerepének újraértelmezése az eleveniszapos szennyvíztisztításban. Oláh György Doktori Iskola 5. Doktoráns Konferencia, Budapest, február 8.

Poster presentations related to the scope of the doctoral thesis

Kiss, B., Literáthy, B., Jobbágy, A. (2007) Kommunális és tápanyaghiányos szennyvizet tisztító biomasszák anaerob/anoxikus metabolízisének vizsgálata. „10 éves a MASZESZ" Jubileumi Konferencia, Lajosmizse, 2007. május 22-23.

Bakos, V., Kiss, B., Jobbágy, A. (2007) A tápanyag hiány hatása az eleveniszapos szennyvíztisztításban. IV. Szennyvízágazati Konferencia: Kutatás – Innováció – Alkalmazás, Budapest, 2007. november 29-30.

Other poster presentation in English in the field of wastewater treatment, not directly related to the scope of the doctoral thesis

Kiss, B., Bakos, V., Tardy, G. M., Jobbágy, A. (2007) A Délpesti Szennyvízitisztító Telep kombinált biológiai tisztító rendszerének optimalizálása. „10 éves a MASZESZ" Jubileumi Konferencia, Lajosmizse, 2007. május 22-23.

Other scientific papers in English in the field of wastewater treatment, not directly related to the scope of the doctoral thesis

Gyüre, Cs., Kiss, B. (2009) Vigyázz, kész, rajt!...- A Budapesti Központi Szennyvíztisztító Telep a próbaüzem küszöbén. *Vízmű Panoráma*. 17(4) 13-15.

Other scientific papers in English not directly related to the scope of the doctoral thesis

Kiss, B., Bajnóczy, G., Pálmai Gy. (2005) Green Electricity from Wood Benefits and Limits in Hungary. *Periodica Polytechnica Ser. Chem. Eng.* 48 (2) 119–128.

Other poster presentations in English not directly related to the scope of the doctoral thesis

Kiss, B., Bajnóczy, G., Gagyi-Pálffy E. (2005) Green Electricity from Wood Benefits and Limits in Hungary.14th European Biomass Conference and Exhibition: Biomass for Energy Industry and Climate Protection, Paris

Printed or electronic lecture note

Bajnóczy, G., Kiss B.: Környezetkémia és technológia (electronic note)