ISMARLEY LAGE HORTA MORAIS

TRATAMENTO BIOLÓGICO DE EFLUENTES DE FÁBRICAS DE POLPA CELULÓSICA E PAPEL COM LODO AERÓBIO GRANULAR

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Engenharia Civil, para obtenção do título de Doctor Scientiae.

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APROVADA: 15 de dezembro de 2016.

Ana Augusta Passos Rezende	André Pereira Rosa
Ann Honor Mounteer (Coorientadora)	Cláudio Arcanjo de Sousa

Cláudio Mudadu Silva
(Orientador)

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BIOGRAFIA

Ismarley Lage Horta Morais, filho de Ismar de Assis Morais e Isabel Lage Moreira, nasceu na cidade de Timóteo, Minas Gerais, em 8 de novembro de 1984.

Em março de 2004 iniciou o curso de Engenharia Ambiental na Universidade Federal de Viçosa, Minas Gerais, graduando-se em janeiro de 2009.

Em março de 2009, iniciou o curso de pós-graduação em Ciência Florestal, na área de ambiência, na Universidade Federal de Viçosa desenvolvendo trabalhos na área de tratamento aeróbio granular de efluentes de máquina de papel e obteve o título de Magister Scientiae em fevereiro de 2011.

Começou a lecionar disciplinas de matemática e física para os cursos de engenharia da Unipac de Conselheiro Lafaiete em abril de 2011 e, em novembro de 2012, começou a trabalhar como engenheiro ambiental na Construtora Século XXI.

Em março de 2014 retornou a Viçosa para iniciar o curso de pós-graduação em engenharia civil, na área de saneamento e meio ambiente e obteve o título de Doctor Scientiae em dezembro de 2016.

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RESUMO

MORAIS, Ismarley Lage Horta, D.Sc., Universidade Federal de Viçosa, dezembro de 2016. **Tratamento biológico de efluentes de fábrica de polpa celulósica e papel com lodo aeróbio granular.** Orientador: Cláudio Mudadu Silva. Coorientadora: Ann Honor Mounteer.

O tratamento de águas residuárias com lodo aeróbio granular apresenta muitas vantagens em comparação ao processo convencional de lodos ativados com lodo floculento. Os grânulos são agregados microbianos densos e compactos que possibilitam uma maior retenção de biomassa no reator biológico e uma elevada capacidade de sedimentação, favorecendo a remoção biológica de matéria orgânica, nutrientes, compostos tóxicos e clarificação final do efluente devido à estrutura e propriedade de sedimentação do lodo. Estes benefícios resultaram em um aumento do interesse de implantação do processo de tratamento com lodo aeróbio granular e a busca de maiores informações à respeito da formação, estabilidade e a influência dos parâmetros operacionais sobre a granulação. Assim, este trabalho apresenta uma revisão bibliográfica com a compilação das informações recentes sobre o lodo aeróbio granular incluindo a possibilidade de utilização dos grânulos aeróbios em biorreatores a membrana, em elevadas temperaturas e as aplicações em plantas de tratamento de larga escala. Foi realizada, ainda, a avaliação da adição de 100 $mg.L^{-1}$ e $200mg.L^{-1}$ de cálcio na estabilidade, resistência mecânica e diâmetro dos grânulos formados em reatores em batelada sequencial alimentados com efluente de uma fábrica de polpa celulósica kraft. Os reatores apresentaram eficiências similares de remoção de matéria orgânica e o diâmetro médio dos grânulos foi de cerca de 11 mm em todos os reatores, embora os grânulos formados no reator que recebeu 100 $mg.L^{-1}$ de Ca^{2+} apresentou velocidade de sedimentação 36% superior aos demais e maior resistência mecânica. A melhoria da granulação pode ser obtida ainda pela seleção de microrganismos que contribuem para a formação dos agregados. A produção de substâncias poliméricas extracelulares (SPE) pelas bactérias é um dos fatores que influencia a agregação celular, uma vez que as SPE agem como agente cimentante e atuam na adesão entre as células. A produção de SPE de dezenove isolados microbianos, obtidos de grânulos aeróbios formados no tratamento de efluente de fábrica de papel reciclado foi avaliada e seis isolados dos gêneros Staphylococcus, Agrobacterium, Enterobacter e Rhodococcus melhoraram a granulação biológica. A ausência destes isolados nos testes de co-agregação reduziu a relação entre proteínas e polissacarídeos (relação PN/PS) e diminuiu a formação de agregados.

ABSTRACT

MORAIS, Ismarley Lage Horta, D.Sc., Universidade Federal de Viçosa, December, 2016. **Biological treatment of pulp and paper mill effluents with aerobic granular sludge.** Adviser: Claudio Mudadu Silva. Co-adviser: Ann Honor Mounteer.

Aerobic granular sludge wastewater treatment has many advantages over the conventional activated sludge process. The granules are dense and compact microbial aggregates that allow a higher biomass retention in the biological reactor and a high settling velocity, favoring the biological removal of organic matter, nutrients, toxic substances and improves wastewater clarification. Due to the sludge structure and settleability, these benefits have attracted considerable interest in the implementation of the aerobic granular sludge process and givenrise to the need for better understanding of the formation, stability and influence of the operational parameters on the granulation. Thus, this work was divided into three chapters. Chapter 1 presents a review of recent developments on aerobic granular sludge including the possibility of using aerobic granules in membrane bioreactors, at high temperatures and for a full-scale implementation. The addition of divalent cations in the reactors can enhance granulation and granule stability. In Chapter 2, the effect of the addition of 100 $mg.L^{-1}$ and 200 $mg.L^{-1}$ of calcium in the stability, mechanical strength and diameter of the granules formed in sequential batch reactors (SBR) fed with pulp mill effluent was evaluated. The reactors showed similar organic matter removal efficiencies and granule size was approximately 11 mm in all SBR, although the granules formed in the reactor with addition of 100 $mg.L^{-1}$ of Ca^{2+} had a settling velocity 36% higher and greater mechanical resistance than the others. Granulation can also be enhanced by the selection of microorganisms that contribute to the aggregates formation. Bacterial extracellular polymeric substances (EPS) production is one factor that contributes to cell aggregation, since EPS acts as an intercellular cement that may reinforce cohesion inside the bacterial clusters. In Chapter 3, EPS production of nineteen microbial isolates obtained from aerobic granules formed in the recycled paper wastewater treatment was evaluated and six isolates of the genera Staphylococcus, Agrobacterium, Enterobacter and Rhodococcus contributed to biological granulation. The absence of these isolates in the co-aggregation tests reduced the protein-polysaccharide ratio (PN / PS ratio) and reduced the aggregates formation.

I. Introdução geral

O sistema de lodos ativados é um dos processos de tratamento biológico de efluentes mais utilizados mundialmente em fábricas de polpa celulósica e papel. No processo, microrganismos são responsáveis pela oxidação da matéria orgânica do efluente em reatores aeróbios e, em seguida, o lodo biológico é separado do efluente tratado por um processo de sedimentação em decantadores secundários. O lodo sedimentado nos decantadores retorna aos reatores de forma a manter uma alta concentração de microrganismos capazes de realizar a degradação da matéria orgânica.

A eficiência do processo de lodos ativados depende da capacidade de sedimentação do lodo biológico. Os flocos biológicos de baixa densidade, que possuem reduzida velocidade de sedimentação, não são retidos pelo decantador e deterioram a qualidade do efluente tratado, mesmo que a oxidação da matéria orgânica tenha sido realizada eficientemente no reator.

O arraste de lodo junto ao efluente tratado no decantador secundário de processos de lodos ativados é um problema típico nas estações de tratamento de efluentes (ETE). O indesejado arraste de lodo pode ter causas diversas. A ocorrência de variações na quantidade e qualidade do efluente de alimentação que podem provocar choques de carga orgânica e de vazão; a presença de compostos tóxicos à microbiota; a variação de pH e temperatura; a falta de oxigênio e nutrientes são fatores que podem afetar negativamente a formação dos flocos e provocar a perda de lodo no decantador secundário. As propriedades de sedimentação de lodo são frequentemente consideradas como limitantes da eficiência do processo (YE et al., 2016).

A mudança de morfologia dos agregados microbianos de flocos (lodo floculento convencional) para grânulos (lodo aeróbio granular) nos reatores aeróbios ocorre a partir da alteração de alguns parâmetros de operação dos processos de lodos ativados tais como a redução do tempo de decantação e o aumento da aeração. Os grânulos são agregados densos e de tamanho maior do que os flocos. Grânulos aeróbios apresentam uma maior velocidade de sedimentação e uma maior resistência a compostos tóxicos do que os flocos convencionais. Esse fato tem sido explicado pela sua estrutura compacta e à matriz de substâncias poliméricas extracelulares (SPE) presentes nos grânulos (WAN et al., 2015).

A utilização de biorreatores aeróbios com lodo granular é incipiente no mundo. Desde a sua descoberta em 1991 (LEE et al., 2010; SARMA; TAY; CHU, 2016), esse tema tem sido objeto de várias pesquisas que buscam viabilizar o processo uma vez que

possui muitas vantagens sobre o processo convencional de lodo floculento. A literatura tem apresentado trabalhos conduzidos majoritariamente em laboratório e com efluentes sintéticos. Existe um número reduzido de trabalhos com efluentes reais, sobretudo provenientes da produção de polpa celulósica e papel (HONGLEI et al., 2013; LIU; NGUYEN; PAICE, 2010; MORAIS; SILVA; BORGES, 2016).

A busca de maiores conhecimentos nessa área tem sido intensa por se tratar de um tema inovador e a compilação desses conhecimentos em forma de uma revisão bibliográfica é desejável.

A formação e a estabilidade dos grânulos formados são fatores importantes para a aplicação do tratamento de efluentes utilizando o lodo aeróbio granular. A presença de pelo menos uma fase com pouca disponibilidade de substrato, um reduzido tempo de sedimentação e a presença de cátions metálicos divalentes são responsáveis pelo êxito da granulação aeróbia (HUANG et al., 2012)

A adição de cátions como o cálcio neutraliza a carga negativa presente na superfície da célula bacteriana e aumenta a agregação do lodo. Os cátions provocam um aumento da força iônica e reduzem a espessura da camada dupla ao redor da célula, levando a diminuição da força repulsiva (DING et al., 2015). Além disso, precipitados de cálcio incorporados ao lodo como sais de carbonato e fosfato também favorecem a sedimentação (YE et al., 2016). Não se sabe ainda em que concentrações o cálcio beneficia ou prejudica a formação do lodo aeróbio granular. A maioria dos trabalhos, com concentrações entre 100 e 200 $mg.L^{-1}$, mostrou efeito positivo da adição do cálcio, enquanto aquelas acima de 300 $mg.L^{-1}$ formaram precipitados de cálcio e causaram problemas na granulação biológica (YU; TAY; FANG, 2001). Além disso, muitos estudos avaliaram o crescimento de microrganismos de cultura pura (HAO et al., 2016). É necessário examinar a influência dos íons metálicos sobre a fixação microbiana e formação dos grânulos aeróbios, sobretudo para efluentes industriais.

As SPE são polímeros complexos de elevado peso molecular e constituem os principais componentes dos bioflocos e biofilmes (WANG et al., 2015). As SPE contêm grande quantidade de compostos como proteínas, polissacarídeos, ácidos húmicos, ácidos nucléicos e fosfolipídeos, responsáveis pelo armazenamento de energia e nutrientes, além da resistência ao ataque de fungos e substâncias tóxicas (YAN et al., 2015). São responsáveis, ainda, por mudanças na sedimentabilidade, na hidrofobicidade da superfície do lodo e influenciam na formação do lodo granular (YAN et al., 2015).

A formação de grânulos pode ser obtida pela seleção de culturas microbianas que possibilitem a diminuição do tempo de formação dos grânulos, o aumento da densidade e a manutenção da capacidade de degradação dos mesmos (IVANOV et al., 2006). Grânulos mais densos são formados devido a interações específicas envolvendo as SPE da matriz do grânulo (CAUDAN et al., 2014).

A primeira parte da tese trata-se de uma revisão bibliográfica sobre granulação aeróbia com foco nos fatores que influenciam a formação de grânulos, o seu desenvolvimento e sua estabilidade. São também apresentadas as possibilidades de utilização de grânulos aeróbios em biorreatores a membrana (BRM), em elevadas temperaturas ou para a remoção de nutrientes e compostos tóxicos, além de aplicações do lodo aeróbio granular em reatores de larga escala. A segunda parte é a avaliação da influência da adição de cálcio na granulação em relação à eficiência de remoção de matéria orgânica, ao aumento da velocidade de sedimentação e resistência mecânica dos grânulos e a composição das SPE. A terceira parte é a avaliação do isolamento de microrganismos que contribuem ou que prejudicam a formação dos grânulos e a composição das SPE produzidas por consórcios de co-agregação.

CAPÍTULO 1

Recent developments on aerobic sludge granulation technology

Abstract

Aerobic granules are large, dense biological aggregates that can be utilized in wastewater treatment plants. The positive characteristics of this process such as high sedimentation velocity, biomass retention, and resistance to toxic substances has attracted great interest from researchers. Despite this intense research, the mechanisms responsible for aerobic granulation and the effects of different operational and environmental factors are not well understood. This review attempts to address recent developments in sludge aerobic granulation based on the literature, focusing on factors that influence granule formation, development, and stability. The possibilities of using aerobic granules in membrane bioreactors (MBR), at high temperatures to remove nutrients and toxicity are also presented. Finally, applications of granular aerobic sludge in full-scale treatment plants are addressed.

Keywords: aerobic granular sludge, granule structure, granule formation, MBR with granular sludge, full scale

1. Introduction

Granular sludge was first described in strict anaerobic systems in the late 1970s, however the formation and application of aerobic granules was only observed in 1991 (ADAV et al., 2008; SARMA; TAY; CHU, 2016). Granulation is a gradual process that transforms flocculent sludge into granular sludge by simultaneous densification and selection of aggregates through settling (CAUDAN et al., 2014). The higher settling velocity allows the reduction of secondary clarifiers and reduces by up to one-fifth, the area required by conventional wastewater treatment plants (SARMA; TAY; CHU, 2016).

Anaerobic granulation exhibited several drawbacks that include the long formation period required, the unsuitability for low organic load wastewater, and low efficiency for the removal of nutrients (N and P) from wastewaters (ADAV et al., 2008). In order to overcome these deficiencies in the anaerobic granulation process, research efforts were devoted to aerobic granulation for wastewater treatment (QIN; LIU; TAY, 2004), making it a popular topic of discussion in the field of wastewater treatment (ADAV et al. , 2008).

The morphology of aerobic biogranules is completely different from any other aggregate, including the flocs of conventional activated sludge. Biogranules are spherical or rounded (MORAIS et al., 2016) with a regular, compact structure. They have an excellent settling ability, and a strong resistance to high organic loads. Aerobic biogranules are composed of a diverse microbial community that enables high metabolic activity and greater tolerance to toxicity (ADAV et al., 2008; CAUDAN et al., 2014; KOCATURK; ERGUDER, 2016; SAJJAD; Al. 2013). In addition, aerobic granulation has been recommended for the treatment of high strength organic wastewaters containing nitrogen, phosphorus, toxic substances, and xenobiotics (ADAV et al., 2008; CAUDAN et al., 2014, CHEN et al., 2016, KOCATURK, ERGUDER, 2016).

Granulation is influenced by several parameters and operatinal conditions. Type of seed sludge used, substrate composition, organic load rate, food to microorganism ratio, feeding strategy, reactor design and hydrodynamics, settling time, exchange ratio, presence of divalent cations, and aeration intensity are the most important among these (ADAV et al., 2008; LIU, YU; WANG; TAY, 2005; SAJJAD KIM, 2015b).

The two major granulation selection pressures in a batch reactor are the settling time and the volume exchange ratio. Granules can be selected according to their settling velocity. Settling velocities lower than 5 $m.h^{-1}$ and exchange ratios greater than 60% increase the fraction of granules in the biological sludge (LIU, YU; WANG; TAY, 2005). Granular sludge formation is encouraged by slow bacterial growth and the presence of high shear forces in the bioreactor (CAUDAN et al., 2014).

Currently, most aerobic granulation research is still restricted to the treatment of laboratory synthetic wastewaters. The results of these studies have many limitations for practical applications. Thus, the benefits of treatment with granular aerobic sludge must still be demonstrated with real and complex wastewaters (LONG et al., 2014).

Nevertheless, the quantity of research into wastewater treatment utilizing aerobic granular sludge has grown rapidly around the world (AWANG; SHAABAN, 2016; WEI et al., 2014). Granular aerobic sludge research has been extensively undertaken for the treatment of various types of wastewaters, including effluents containing nitrogen, phosphorus, and toxic substances. Due to these advantages, granular aerobic sludge may be considered one of the most promising biological treatment technologies of the 21st century (LI, KAI et al., 2015).

This study presents a broad literature review regarding recent scientific developments on aerobic sludge granulation. The aim of this review is to provide an understanding of granule formation, its applications in nutrient removal systems, and in membrane bioreactors under mesophilic and thermophilic conditions. Furthermore, recent information on technological applications that use granular aerobic sludge on a full scale are presented.

2. Formation of granular aerobic sludge

Granular aerobic sludge can be obtained under certain operating conditions. Progress has been made in understanding how granulation occurs and how the process can be accelerated, although granulation mechanisms are still not well understood (ZHANG, QUANGUO; HU; LEE, 2016).

The process of aerobic granule formation is a crucial step for its applicability in wastewater treatment plants given that a conventional activated sludge can take several weeks to form biogranules (IVANOV et al., 2006; MALIK, A et al., 2003). Aerobic granules can grow from different carbon sources and are formed under different organic loads (LIU, YU et al., 2004).

Aerobic granules are widely investigated for the treatment of domestic and industrial wastewater despite instability problems. The success of the application of this process demands a better understanding of the composition of the wastewater and its effects. An important parameter is the carbon / nitrogen (C / N) or chemical oxygen demand / nitrogen (COD / N) ratio present in the effluent. The control of the COD / N ratio can be carried out to exert microbial selection pressures to favor heterotrophic bacteria (aerobic or anaerobic) or nitrifying species, contributing to granulation and the efficiency of the system (WU et al., 2012).

The specific growth rate of aerobic granules is lower than that of flocculent sludge, i.e., competition harms the formation and growth of aerobic granules and eventually causes granular aerobic sludge eradication if the flocculent sludge is not effectively drained from the bioreactor (LIU, YU; WANG; TAY, 2005).

The most significant factors that favor slow organism growth and granule production in an aerobic reactor are the presence of feast and famine conditions, high shear force, and short settling time. Thus, granular aerobic sludge has been produced almost exclusively in sequential batch reactors (SBR), and may take from one week to

several months to develop mature granules with stable COD removal efficiency (SARMA; TAY; CHU, 2016).

The SBR operating cycle consists of feeding, aeration, sedimentation, and effluent removal. Increasing the cycle from 1.5 to 8 hours reduced the specific growth rate of the biomass, while granules grown in cycles of 1.5 hours presented a larger size and those cultivated in cycles of 4 hours were the most compact (LIU, YONG QIANG; TAY, 2007).

Fast-settling granules are dense spherical aggregates, while slowly settling particles are small, low density, and irregular in shape, which allows the selection of granules by the settling velocity (NI; YU, 2010). The short settling time causes the washout of poorly settling suspended biomass, only retaining well settling granules (ADAV; LEE; LAI, 2008).

The relationship between settling velocity (v_{sett}) and the ratio of biomass of aerobic granules to total biomass indicates that the fraction of aerobic granules in the reactor increases with the increase in v_{sett}. When v_{sett} values are lower than 1 $m.h^{-1}$, only suspended bioflocs are cultivated and no aerobic granules are developed. As v_{sett} increases above 1 $m.h^{-1}$, a blanket of aerobic granular sludge starts to form and prevails over the suspended flocs at vs_{ett} values above 4 $m.h^{-1}$ (LIU, YU; WANG; TAY, 2005). This indicates that if the SBR is operated at values below the settling velocity of suspended flocs (3 to 5 $m.h^{-1}$), the flocculent sludge will not be effectively washed out of the reactor.

Improved granulation can be achieved by manipulating operating conditions (LIU, YONG QIANG; TAY, 2015). Granules were formed in only 7 days using a 5-minute settling time from the reactor start up rather than a gradual reduction of settling time (QIN; LIU; TAY, 2004; QIN; TAY; LIU, 2004).

The increase in the organic load stimulates the secretion of extracellular polymeric substances (SPE) and enhances aerobic granulation (LIU; LIU; TAY, 2004). The application of a high hydraulic selection pressure such as a short cycle time (30 min), a short settling time (1 min), and a high organic load (24 kg $COD.m^{-3}.d^{-1}$) resulted in the formation of granules after 7 hours (ZHANG, XING et al., 2013). However, the granules formed by this method only lasted 12 days in the reactor. The high organic loading rate implies the formation of granules with low resistance and compromises the operation. The long-term stability of the granules formed using the rapid formation strategy can be obtained by reducing the organic loading rate after reaching the steady state (LIU, YONG QIANG; TAY, 2015).

The granulation mechanism comprises four steps, which are cell-to-cell contact, micro-aggregate formation, excess production of EPS, and hydrodynamic compacting of the granule matrix (Figure 1) (LIU, YONG QIANG; TAY, 2015). The use of granular activated carbon (CAG) as a support medium increases the aggregation rate by favoring the micro-aggregate formation step (ZHANG, QUANGUO; HU; LEE, 2016). The presence of divalent and trivalent cations, such as Ca^{2+}, Mg^{2+}, Fe^{2+} and Fe^{3+} can bind to negatively charged cells to form microbial nuclei (SOBECK; HIGGINS, 2002; WAN et al., 2015).

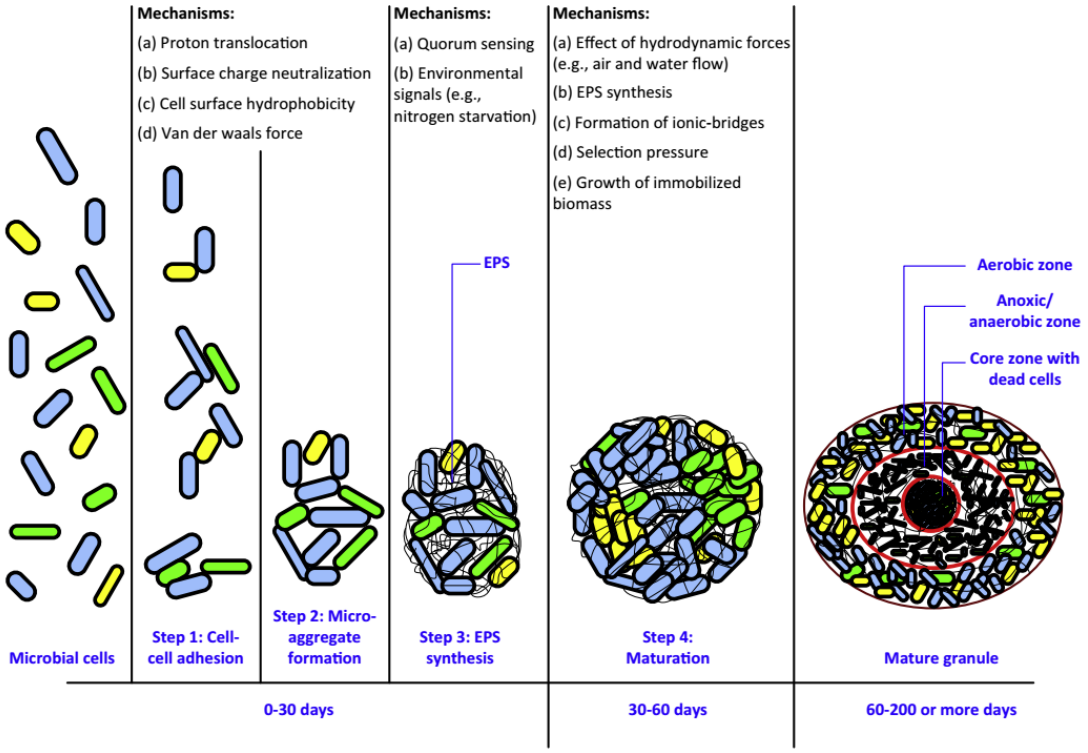


Figure 1 –Mechanisms of formation of aerobic granules. Source: (SARMA; TAY; CHU, 2016)**.**

The bacterial community present in the activated sludge influences the aerobic granulation process. Hydrophilic bacteria have a lower tendency to attach to sludge flocs than their hydrophobic counterparts do. Thus, the greater the number of hydrophobic bacteria in the sludge the faster aerobic granulation occurs (ADAV et al., 2008). The role of seeding sludge in aerobic granulation shows that the temperature at which the sludge was produced influences the process. Granulation did not occur in some systems in which sludge samples were collected at temperatures below 30 °C due to the absence of Brevundimonas sp. (CHEN, LEE, 2015).

Biological reaction processes in aerobic granules are determined by oxygen concentration gradients and by the types of substrate used. The substrate and dissolved oxygen (DO) concentration profiles are a result of many factors, e.g., diffusion

coefficient, conversions rate, granule size, biomass spatial distribution and density (NI, YU, 2010). All of these factors strongly influence each other and the effect of individual factors therefore cannot be studied in isolation.

The aeration period of a SBR operation consists of two phases: a degradation phase in which the substrate is depleted to a minimum, followed by an aerobic starvation phase. The absence of carbonaceous organic matter contributes to increased hydrophobicity, although it is not a prerequisite for granulation (ADAV et al., 2008).

Strong shear forces with higher aeration rates do not only compact aggregates into granules, but also provide enough oxygen to suppress filamentous growth, contributing to the long-term operational stability of a bioreactor (ADAV et al., 2008). Three SBR with different aeration rates (1-3 $L.min^{-1}$) fed with wastewater containing phenols were compared. No granules formed at low aeration rates (1 $L.min^{-1}$), while at high aeration rates (3 $L.min^{-1}$) stable and mature granules (1 - 1.5 mm) with a compact interior formed. At an intermediate rate (2 $L.min^{-1}$), large granules (3 - 3.5 mm) with overgrowth of filaments were formed. An intermediate rate of aeration was not sufficient to meet oxygen requirements or to inhibit filamentous growth, leading to bioreactor failure (ADAV; LEE; LAI, 2007).

The substrate also influences the formation of granular aerobic sludge. The use of propionate as a source of carbon delayed the granulation process compared to that with acetate. However, the use of propionate yielded stronger granules (ZHANG, QUANGUO, HU, LEE, 2016).

The increase in temperature contributes to granule formation. Reactors operated at temperatures below 20 °C led to the formation of irregular shaped granules with filamentous bulking, causing the loss of biomass and unstable operations. In addition, denitrification capacity and nutrient removal rate of the granules at low temperatures were reduced (ADAV et al., 2008). Temperatures above 40 °C hamper granule formation (EBRAHIMI et al., 2010; LÓPEZ-PALAU et al., 2013).

2.1. Extracellular polymeric substances

Extracellular polymeric substances (EPS) are important for microbial aggregate formation, since bacteria are dispersed in an EPS matrix. These substances have a structural function and serve as a protective barrier and affect porosity, density, hydrophobicity, mechanical stability, and other properties of biofilms (LIU, YONG QIANG; TAY, 2007).

Bacterial EPS are associated with the formation of aggregates and cell-to-cell bonds (REN; XIE; XING, 2009). EPS expelled by microorganisms during growth are crucial for granule formation and maintenance. Proteins and polysaccharides are the main components of EPS containing large amounts of functional groups such as hydroxyl and carboxyl (CAUDAN et al., 2014; KOCATURK; ERGUDER, 2016; SARMA; TAY; CHU, 2016). These functional groups have a high binding capacity and are closely associated with the stability of microbial aggregates (LIU, YONGJUN et al., 2014; SAJJAD; KIM, 2015b).

The main characteristics of aerobic biogranules depend on their EPS composition. Hydrophobic proteins are the major components of EPS and therefore an increase in EPS production results in increased hydrophobicity, density, and settleability of the granules (CORSINO, SANTO FABIO et al., 2016). EPS contribute to cell adhesion and formation of a microbial matrix of the granules, improving their stability and structural integrity (LIU, YONGJUN et al., 2014). Although EPS are essential for aerobic granulation, excessive levels reduce granule stability (CORSINO, SANTO FABIO et al., 2016). Excess EPS production transforms aerobic granules into a gelatinous structure, characterized by a dense, viscous surface layer that limits oxygen and nutrient transfer, which in turn reduces bacterial metabolic activity. The reduction of the porosity of the granules also limits the transport of nutrients to the inner layers of the granules, causing bacterial death and rupturing of the granules over the long term (CORSINO, SANTO FABIO et al., 2016).

The composition of EPS in terms of its main components, proteins and carbohydrates, influences the characteristics of the granules formed. A low polysaccharide/protein (PS/PN) ratio results in the deterioration of strength and settleability and disintegration of the granules. Ratios less than 0.6 may cause structural instability and granular disintegration (KOCATURK; ERGUDER, 2016).

The production and consumption of EPS increases according to the cycle time of of the SBR. Bacteria use these products as a source of carbon and energy when subjected to long periods without feeding, decreasing the amount of EPS and proteins as the cycle time increases (CORSINO, SANTO FABIO et al., 2016).

Proteins and polysaccharides are the major constituents of EPS and can account for 75 to 90% of EPS mass, while humic substances, uronic acids, and nucleic acids are present in smaller amounts (BASUVARAJ; FEIN; LISS, 2015 YAN, LILONG et al., 2015).

Proteins are an important component for changes in the structure of the flocs, contributing to the increase of settling velocity and favoring the formation of aerobic granules (BASUVARAJ; FEIN; LISS, 2015; SAJJAD; KIM; KIM, 2016). The proteins and their amino acid composition contribute to the hydrophobic character of the flocs. The high content of negatively charged amino acids allows a greater amount of electrostatic bonds of multivalent cations with proteins than with polysaccharides, increasing the stabilization of the aggregate structure (BASUVARAJ; FEISS, LISS, 2015; CAUDAN et al., 2014). Protein functions include the aggregation of bacterial cells and the formation of an active gel-like matrix that maintains cell cohesion (BASUVARAJ; FEIN; LISS, 2015).

Polysaccharides are related to the formation of a gel-like layer more favorable to the formation of biofilms than granules (BASUVARAJ; FEIN; LISS, 2015). Polysaccharides are hydrophilic polymers that absorb and contribute to a high retention of water or biological fluids, resulting in poor settling and poor dewatering of the sludge (BASUVARAJ; FEIN; LISS, 2015; SAJJAD; KIM, 2015b). For the granules, a high or excessive polysaccharide content is detrimental to the settling and dewatering properties due to a higher water content associated with polysaccharide-rich EPS (BASUVARAJ; FEIN; LISS, 2015).

The amount of EPS, especially proteins, increases significantly during the transition from flocculent to granular sludge (CAUDAN et al., 2014). The use of flocculent sludge with higher polysaccharide content (ratio PN/PS = 0.5) showed a change in EPS composition during granule formation. The granular aerobic sludge presented a higher protein content (ratio PN/PS = 1.6) in its total EPS fraction (BASUVARAJ; FEIN; LISS, 2015). The proteins were located predominantly in the core region of the granules where the cells were tightly packed (BASUVARAJ; FEIN; LISS, 2015; CAUDAN et al., 2014).

The PN content and PN/PS ratio show a positive correlation with the particle size of the granular sludge. The protein content gradually increases during the formation of the granular sludge while the polysaccharide content remains practically constant (YAN, LILONG et al., 2015).

The EPS content may also vary depending on the presence of toxic substances. The addition of 20 $mg.L^{-1}$ of 2,6-dichlorophenol in a synthetic effluent increased PN concentration by about 30 fold while the PS concentration remained more or less constant (LI, KAI et al., 2016).

2.2. Divalent cations addition

The presence of divalent cations improves the physical (particle size, settleability, filtrability etc.) and chemical (EPS) characteristics of the granules. Several authors observed a shorter time for granule formation in systems with added calcium (LIU, YONGJUN et al., 2014, SAJJAD, KIM, 2015b, SOBECK; HIGGINS, 2002). The positive charge of divalent cations neutralizes the negative charge of the microbial biomass surface and EPS molecules thereby enhancing granulation (SAJJAD; KIM, 2015a, b; SOBECK; HIGGINS, 2002). These cations can stimulate granulation by neutralizing the negative charges present on bacterial surfaces, implying strong van der Waals attractive forces, or functioning as cationic bridges between bacteria given that most microorganisms are negatively charged at the typical pH of bioreactors (LIU, YU et al., 2004).

Added calcium improves settleability, and increases the amount of EPS produced by the granules. Magnesium, on the other hand, also shows a positive correlation with granule formation, but the effects of Mg^{2+} are lower than those of Ca^{2+}. In the monitoring of parallel systems, the addition of calcium caused a greater increase in particle size and reduction of the sludge volume index (SVI) when compared to systems that received added magnesium. This was due to the increase of polysaccharides synthesis in EPS in the presence of Ca^{2+} (SAJJAD; KIM, 2015b). Added calcium increases the strength of the granules and the addition of magnesium increases granule microbial diversity (CAUDAN et al., 2014). The addition of Mg^{2+} favors nucleation of the granules and Ca^{2+} addition favors growth and maintenance of a more rigid structure. The simultaneous addition of Mg^{2+} during nucleation and Ca^{2+} in the granule-growth stage accelerates the granulation process more than the addition of either cation alone (CAO et al., 2014).

Metal ions accelerate granulation by forming an initial nuclei for bacterial attachment (LIU, YONG QIANG; TAY, 2015). Denitrification is indicated as beneficial to the process due to the formation of $CaCO_3$ (ZHANG, QUANGUO; HU; LEE, 2016). Calcium carbonate accumulates inside the granule forming a rigid structure, thereby increasing granule strength (CHEN et al., 2016).

Cellular polysaccharides are adhesives that facilitate cell cohesion and adhesion, causing an increase in the granule diameter (SAJJAD; KIM, 2015b). The cohesion of the granules depends on the establishment of calcium bridges between anionic proteins that increase the size and compactness of the aggregates. Calcium bridges are more specific

for anionic proteins than for anionic polysaccharides, and are more frequent in the presence of divalent cations than monovalent cations (CAUDAN et al., 2014; SARMA; TAY; CHU, 2016).

Analysis of the interactions between the divalent cations and the EPS constituents revealed that calcium binds preferentially to hydroxyl groups (OH) bound to the polysaccharide carbons than to nitrogen (N) of amide group of proteins. Magnesium, on the other hand, exhibits greater interaction with N of amide groups of proteins than with polysaccharides. This behavior can be explained by (i) the specific position of the functional groups present in protein and polysaccharide molecules and (ii) the larger size of Ca^{2+} in relation to Mg^{2+} (SAJJAD; KIM, 2015b).

Divalent cations retain small particles and increase resistance of the three-dimensional matrix of the exopolymers, causing an increase in granule size and dewaterability. Added calcium leads to an increase in the production of polysaccharides by the microorganisms in the granules, while the addition of magnesium has a greater effect on protein production. Proteins are the hydrophobic constituents of EPS that facilitate the dewatering of sludge. It is observed that the use of different divalent metals changes the composition of EPS, although their total amounts are almost similar. While Ca^{2+} mainly affects the size and settleability of the granules, Mg^{2+} has a greater effect on dewatering (SAJJAD; KIM, 2015b).

Protein amide groups are surrounded by carbon atoms, which are attached to high molecular weight alkyl and aryl groups. In addition, these groups are in opposite positions (trans position) to the amide group. The larger size of the Ca^{2+} ions makes it difficult to bind to amide N or O of the amide groups due to steric hindrance, whereas it is easier for calcium to interact with the readily available polysaccharides hydroxyl groups. Magnesium, because it is much smaller than calcium, encounters no steric hindrance in binding with the N of protein amide groups and fits well within the structure of the polymer, implying a higher protein retention in the presence of Mg^{2+} (SAJJAD; KIM, 2015b).

Surface charge neutralization and aggregation enhancement can also be achieved by the addition of trivalent cations, such as aluminum. The effect of poly aluminum chloride (PAC) on the chemical properties of the granular sludge showed an increase in hydrophobicity and a reduction in the zeta potential of granular sludge, by lowering electronegativity of the suspended solids surface charge and increased EPS production.

Microorganisms secrete EPS to protect themselves against osmotic pressure in the presence of cations (LIU, YONGJUN et al., 2014).

PAC addition increases the re-formation of aerobic granular sludge that disintegrates spontaneously after a long period of batch reactor operation. Granules are formed in less time and are stronger than granules formed without the addition of PAC (LIU, YONGJUN et al., 2014).

The efficiency of divalent cation addition is influenced by the presence of chelating agents, which may impair the granulation process. Chelating agents dissolve calcium precipitates and destabilize the granules. Addition of phosphate ions (PO_4^{3-}) reduces formation of EPS ionic bridges (CORSINO, SANTO FABIO et al., 2016; SARMA; TAY; CHU, 2016). Phosphate solubilization by acids present in the wastewater or by microorganisms during biological removal of phosphorus causes aerobic granule destabilization (SARMA; TAY; CHU, 2016).

Addition of 1 to 5 $mg.L^{-1}$ Cu^{2+} does not affect structural integrity, but makes aerobic granules less compact. However, granules disintegrate with the addition of 10 $mg.L^{-1}$ of Cu^{2+}due to the reaction of the metal ions with the -NH2, -OH, -COOH and C-N functional groups. The increase in Cu^{2+} concentration from 0.0 to 10.0 $mg.L^{-1}$ leads to the decrease in weight percentages of essential elements Fe, Ca, Na, and K in the granules while that with copper increases correspondingly. Cu^{2+} is exchanged with the essential metals and chelated by the nitrogen functional groups of the protein, reducing the structural stability of the sludge (ZHENG et al., 2013).

The presence of chelating agents may also improve granular aerobic sludge formation. Aerobic granulation was greater in three SBR that were fed with added nitrilotriacetic acid (NTA) compared to the reactor fed with acetate in the absence of NTA. NTA contributed to an increase in selective pressure for the enrichment of the granules. Acetate is easily degradable and the presence of NTA reduced the growth rate of the granules and allowed the enrichment of slow growing microorganisms in the reactors fed with NTA. The chelating agent may have exerted a strong detachment force on the granule surface. This effect resembles that of the shear force and contributes to the formation of denser granules (NANCHARAIAH et al., 2008).

Chelating agents reduce the formation of biofilms at high concentrations. The addition of 50 mM of ethylenediamine tetraacetic acid (EDTA) reduced the number of biofilm-associated cells by more than 99% (BANIN; BRADY; GREENBERG, 2006).

However, it likely increases biogranulation at lower concentrations. Granules formed in the presence of 0.26 mM, 0.52 mM and 1.05 mM of nitrilotriacetic acid (NTA) were smoother, denser, more compact, and showed better settling characteristics than those formed in its absence (NANCHARAIAH et al., 2008). The role of chelating agents in the formation of granular aerobic sludge requires further investigation.

2.3. Microbial coaggregation

The formation of granules can be obtained by selecting microbial cultures. When microorganisms are inoculated into the sludge, they can remain in the reactor for a long period of time, helping to form aggregates and maintaining the degradative capacity of these organisms (IVANOV et al., 2006).

The addition of a pure culture with high self-aggregative capacity to an activated sludge resulted in the formation in only eight days of aerobic granules with a mean diameter of 446 μm (IVANOV et al., 2006). A rapid reactor start-up for solvent recovery was achieved by the inoculation of a mature granular sludge and the implementation of an aerobic biological selector (LONG et al. 2016).

Coaggregation offers several advantages for bacteria. It avoids cell washout and protozoan predation, resistance to toxins, better transfer of chemical signals; exchange of genetic information and better provisions for carbon and energy sources (MALIK, A et al., 2003; MALIK, ANUSHREE, KAKII, 2008; NAGAOKA et al., 2008). Bioaugmentation with specific pure cultures accelerates aerobic granulation, although it is a complex and costly process for practical application (LIU, YONG QIANG; TAY, 2015).

Physical interactions between aggregated bacteria facilitate metabolic interactions, such as oxygen protection, cell-cell communication, and genetic exchange between cells. Aggregation usually depends on highly specific lectin-carbohydrate interactions occurring between the aggregating partners and can be defined as interspecific (coaggregation) or intraspecific (self-aggregation) (LEDDER et al., 2008; RICKARD; MCBAIN et al., 2003; RICKARD; GILBERT; HANDLEY, 2004).

Coaggregation interactions contribute to the development of biofilms through two routes. The first route is via single cells in suspension specifically recognizing and adhering to genetically distinct cells in the developing biofilm. The second route is by the prior coaggregation in suspension of secondary colonizers followed by the subsequent

adhesion of this coaggregate to the developing biofilm (RICKARD & GILBERT et al., 2003). However, the mechanisms that control interactions in biofilms containing several species has yet to be determined (SIMÕES; SIMÕES; VIEIRA, 2007).

From the standpoint of mass transfer and substrate utilization, it is more advantageous for the bacteria to remain dispersed than to form aggregates. However, physico-chemical properties of the cell surface have great effects on the formation of biofilms. The hydrophobicity induced by conditions of the medium can function as a factor that triggers aerobic granulation. As the cell becomes more hydrophobic, cell adhesion is observed, that is, cell surface hydrophobicity should influence the ability of cells to aggregate. On the other hand, a change in the chemical composition of the wastewater can cause the loss of the established granular sludge (LIU, YU et al., 2004).

Cell hydrophobicity can be induced by environmental conditions and enable cell-cell aggregation, which is crucial for the formation of biological granules (EKMEKCI; ASLIM; OZTURK, 2009; MALIK, ANUSHREE; KAKII, 2008; PHUONG; KAKII; NIKATA , 2009). Thermodynamically, microbial aggregation is controlled by the reduction of free energy. The increase in the hydrophobicity of cell surfaces reduces Gibbs energy from the surface, which promotes cell-to-cell interaction and subsequently leads to aggregate formation (QIN; TAY; LIU, 2004).

The association among bacteria is highly specific and primary colonizers may aggregate with each other, but do not readily aggregate with secondary colonizers. However, some species of secondary colonizers may aggregate with both primary and secondary colonizers, acting as bridge organisms (BUSWELL et al., 1997; MALIK et al., 2003; NAGAOKA et al., 2008; RICKARD GILBERT et al., 2003). In the absence of this type of organism other secondary colonizers cannot be part of the biofilm (RICKARD; GILBERT et al., 2003).

2.4. Important microorganisms in granulation processes

Several microorganisms that act in the formation of aerobic granules have been reported in the literature. Factors such as temperature, nutrients, and salinity are crucial for the selection of microorganisms favorable to granulation.

The bacterium Blastomonas natatoria 2.1 coaggregated specifically with all 18 other bacteria isolated from an original freshwater community. B. natatoria 2.1 might have a role as a bridging organism in the development of the biofilm community

mediating adhesion between primary and secondary colonizers (RICKARD; GILBERT et al., 2003).

The sampling of biological sludge at different temperatures throughout the year in the same wastewater treatment plant showed different behavior in relation to the formation of granules. Sludge collected at lower temperatures did not form granules. A comparison of the sludge over the year showed that no granules were formed in the samples lacking Brevundimonas sp., an effective producer of EPS that has an optimal growth temperature of 30 ℃ (CHEN, LEE, 2015). This indicated the importance of this EPS producer in granulation.

The microbial composition of granules varied as a function of temperature in a biotreatment aimed at the removal of phosphorus. Filamentous bacteria Leptothrix spp. were more abundant at 20 °C than at higher temperatures (30 °C and 35 °C). Removal of phosphorus was insignificant and there was a greater abundance of Gammaproteobacteria at 30 and 35 °C. Removal of phosphorus at 20 °C was related to the presence of Rhodocyclaceae. The presence of Sphingomonadaceae at all temperatures indicated that this family played an important role in the maintenance and stability of aerobic granules (EBRAHIMI et al., 2010).

The evolution of the bacterial population during formation of granular sludge for removal of phosphorus and denitrification indicates an increase in microbial diversity and richness during the granulation process, followed by a stabilization of these parameters upon the presence of granules. Betaproteobacteria, Gamaproteobacteria and Deltaproteobacteria are indicated as groups that contribute to the increase in mechanical resistance and formation of granular aerobic sludge. Chloroflexi excel contributes to carbohydrate biodegradation, nitrification, and denitrification and Actinobacteria are important for COD removal (HE et al., 2016). Proteobacteria and Bacteroidetes phyla are the predominant bacteria and have the best adaptation to changes in growth environment (SONG et al., 2009; ZHAO et al., 2015).

The study of partial nitrification in wastewater with high concentrations of ammonia indicates that Nitrosomonas europaea and Nitrosomonas sp. are the bacteria responsible for ammonia oxidation, whereas Nitrobacter sp. and Nitrospira cf. Moscoviensis oxidize nitrite. Reduction of treatment temperature and carbon source concentration decreases the nitrification performance and there is a predominance of ammonia-oxidizing bacteria (AOB) in the microbial community of the granules. At the same time, increasing the temperature favors nitrite-oxidizing bacteria (NOB), allowing

them to compete with the AOB in the granules (WAN et al., 2014). Nitrosomonas were the major ammonia-oxidizing bacteria in the sludge used as inoculum, but after the formation of the granules, Nitrosomonas and Nitrosospira were present in equal amounts (WINKLER et al., 2013). The variation of reactor operating temperature indicated that ammonia was oxidized by Accumulibacter sp., Thauera sp., Tetrasphaera-PAO and Azoarcus-Thauera, regardless of temperature (20 and 25 °C) favored the occurrence of denitrifying bacteria such as Comamonadaceae, Curvibacter sp., Azoarcus cluster, Rhodobacter sp., Roseobacter sp. and Acidovorax spp. (MIECZKOWSKI et al., 2016).

The salinity effect on the microbial community present in aerobic granules was evaluated and the main classes identified in low salinities were Alphaproteobacteria, Cytophagia and Betaproteobacteria, while the classes Alphaproteobacteria, Flavobacteria and Actinobacteria prevailed in high salinity. The presence of Alphaproteobacteria under different conditions is related to the metabolic ability to adapt and survive under stress conditions. Bacterial strains of EPS producing-bacteria were found in both aerobic granules at low and high salinity conditions, indicating that EPS production is important for aerobic granulation at high salinity (RAMOS; SUÁREZ-OJEDA; CARRERA, 2015).

In a study of the microbiological composition of the granules, it was found that mature granules have an anaerobic spherical core with Rhodocyclaceae covered by an outer spherical shell with both aerobic and anaerobic strains (LV et al., 2014). The development of granules indicated an increase in the abundance of the anaerobic species Flavobacteriaceae, Xanthomo nadaceae, Rhodobacteraceae and Microbacteriaceae, due to the formation of an anaerobic core (LV et al., 2014). Strains of Sphingomonas sp., Paracoccus sp., Sinorhizobium americanum, and Flavobacterium sp. present in the core promoted the production of EPS and improved the formation of granules (WAN et al., 2015).

The presence of filamentous bacteria in aerobic granules at low levels improves stability and mineral capture by the granules, contributing, for example, to enhancing phosphorus removal (LI, YONGMEI et al., 2014). However, the excess of these microorganisms may impair treatment with granular aerobic sludge. The predominance of filamentous bacteria in aerobic granules modifies their structure, making them loose, fluffy, and irregular and leads to the disintegration of granules (CORSINO, S. F. et al., 2016). The outgrowth of filamentous bacteria on the granule surface reduces the settling velocity of the granules and results in sludge wash out (LIU, HONGBO et al., 2014). The

increase in temperature in the bioreactor from 30 to 50 °C causes the elimination of filamentous bacteria in aerobic granules (AB HALIM et al., 2015).

2.5. Granule stability

The loss of structural stability and bioactivity after long-term operations are the main challenges to the application of aerobic granular processes (ZHANG, QUANGUO; HU; LEE, 2016). The mechanical strength of granules depends on their growth conditions and the substrate (CAUDAN et al., 2014). The formation of granules is stimulated by slow-growing bacteria and the presence of high shear forces (CAUDAN et al., 2014; LIU, YONG QIANG; TAY, 2015). Rapid-growth bacteria lead to instability of the aerobic granules (LIU, YONG QIANG; TAY, 2015). The presence of a recalcitrant substrate leads to a lower growth rate of heterotrophic bacteria, with the selection of slow-growing bacteria prevailing, increasing the long-term stability of the aerobic granules (CAUDAN et al., 2014; LIU, YONG QIANG; TAY, 2015).

One of the most serious barriers to practical applications of aerobic granules is the loss of granule stability over long-term operation, caused by granule break-up or filamentous bacteria overgrowth. In the first case, the granules deteriorate into small pieces and are washed out. In the second case, the growth of filamentous bacteria produces low density granules that are easily washed out (ADAV et al., 2008).

Filamentous bacteria overgrowth, reduction of nutrient transfer into the granules, the formation of anaerobic zones in the aggregates, and EPS properties may result in granule disruption (KOCATURK; ERGUDER, 2016). Maintenance of alkaline conditions avoids excessive growth of filamentous bacteria that cause granule disintegration and biomass washout (ZHANG, QUANGUO; HU; LEE, 2016). The increase in the duration of the feeding phase is related to the loss of stability of aerobic granules due to the excessive growth of filamentous bacteria (CORSINO, SANTO FABIO et al., 2016).

Aerobic granules can also disintegrate under high organic load. An increase in organic loading rate can increase granule size and enhance the growth of anaerobic cores inside the granules. The dead cells within the granules cause the collapse of the granular structure (ZHANG, QUANGUO; HU; LEE, 2016). In systems with chemical oxygen demands (COD) greater than 3000 $mg.L^{-1}$, the isolates lost their capacity for self-aggregation and the production of proteins and polysaccharides. The reduced protein

quantities excreted by the isolates were associated with the low integrity of the granules under high organic loadings (ADAV; LEE; LAI, 2010).

Aerobic granule stability may be associated with one or more characteristics of the granules, such as morphology, settleability, density, strength, surface hydrophobicity, specific oxygen uptake, microbiological structure and diversity and EPS content (LEE et al., 2010). Granule rupture is caused by mineralization of their core, as well as the clogging of their pores, caused by excess EPS that limits the flow of nutrients and oxygen from the bulk into the inner layers (CORSINO, SANTO FABIO et al., 2016).

A strategy to achieve granule stability is the selection of flocculating microorganisms through a short settling time and a high height/diameter ratio of the cylindrical bioreactor. The application of intermittent feeding or the occurrence of a low feed availability period in the bioreactor cycle increases the hydrophobicity of the cell and increases granulation. A high aeration rate provides sufficient dissolved oxygen and benefits cell aggregation. Low pH and temperatures should be avoided to prevent fungal growth. The presence of divalent cations helps cell granulation. Also, high shear forces contributes to the formation of a compact core (LEE et al., 2010). Microbial selection pressure is not a prerequisite for granulation. The microbial community is similar to that of the sludge used as an inoculum, and the bioflocs detach, collide, and aggregate randomly and continuously with granules (ZHANG, QUANGUO; HU; LEE, 2016).

Among the factors involved in the formation and disintegration of granules, granule size is considered essential (LIU, YONGJUN et al., 2014), because granule size plays an important role in limiting mass transport and diffusion. The increase in size and age of the granule reduce the porosity of these structures (YONGJUN et al., 2014). Aerobic granules become unstable after they reach a certain dimension. Indeed, once this occurs, these granules breakdown and most of them are washed out, while others aggregate again forming new granules. Over the long term, granulation is a dynamic process in which granulation and degranulation continuously take place (CORSINO, SANTO FABIO et al., 2016).

EPS excreted by micoorganisms during cell growth and lysis play an important role in granule formation and maintenance (LIU, YONGJUN et al., 2014). Due to the compact structure and large size, few substrates can penetrate into the interior of the aerobic granule, causing EPS located at the core of the granule to be used as energy source and the core biomass undergoes microbial decay and lysis (LEE et al., 2010). These

changes may eventually cause the structure to weaken and result in the disintegration of the aerobic granules by hydraulic shear forces.

2.6. Nutrient removal

One of the major problems related to granular aerobic sludge technology is to obtain uniform conditions for nutrient removal. The bacteria responsible for the removal of nitrogen are slow-growing microorganisms and the efficiency of nitrogen removal depends on granule maturation and the adaptation of the autotrophic bacteria, reaching efficiencies above 90% (CORSINO, SANTO FABIO et al., 2016)

Nitrogen removal efficiency depends on the availability of a carbon source for denitrification (CORSINO, SANTO FABIO et al., 2016). The dense spherical structure of the granules causes a limitation of oxygen transfer, resulting in an oxygen gradient in the sludge granules and allowing denitrification (LI, YONGMEI et al., 2014). The increase in the famine phase in an SBR can reduce the availability of carbon, creating a very slow denitrification rate (CORSINO, SANTO FABIO et al., 2016).

Aerobic granulation can be obtained with COD/N ratios between 1 and 20 (KOCATURK; ERGUDER, 2016). The amount and activity of heterotrophs increases with respect to nitrifiers at high COD/N values, for example equal to 20. Nitrifiers are favored at lower COD/N ratios such as lower than 3.3 (KOCATURK, ERGUDER, 2016). However, COD/N ratios lower than 2 may negatively affect the stability of the granules, resulting in granule disruption at a COD/N equal to 1. The disintegration is due to the degradation of the EPS matrix and microbial community change (LUO et al., 2014).

Bioreactors operating with a COD/N ratio higher than 10 are recommended for a greater COD removal and with a ratio lower than 5 when further nitrogen removal is desired. COD/N ratios ranging from 7.5 to 30 result in high COD removal efficiency (92%) and reduced nitrogen removal (33%) due to a greater growth of heterotrophs resulting in large, white, fluffy granules. Under these conditions, maintenance of high treatment efficiency and granular stability is difficult due to significant heterotroph growth rates. COD/N ratios between 2 and 5 result in a complete nitrification and in lower COD removal (60%). The granules formed under these conditions are small, dense, orange granules enriched in nitrifiers with slow-growing but stable characteristics. The optimum value of the COD/N ratio in terms of removal of COD (75-79%) and nitrogen (> 90%) is equal to 7.5 (KOCATURK, ERGUDER, 2016).

Aerobic granules were used for the treatment of high-strength ammonium wastewaters and it was found that granular adsorption and nitrification followed by denitrification corresponded to 9% and 76%, respectively, of the total nitrogen removal (YU et al., 2014). Application of granules in nitrification and denitrification processes has been used in order to save energy and substrate (ZHANG, QUANGUO; HU; LEE, 2016).

Granules can be applied for simultaneous removal of nitrogen and phosphorus (WEI et al., 2014). Aerobic granules grown at 50 ℃ had a mean diameter of 3.36 mm and COD, ammonia and phosphate removal efficiency of 98.17%, 94.45%, and 72.46%, respectively (AB HALIM et al. 2015). Phosphorus accumulated in the aerobic granules due to chemical precipitation in the presence of the filamentous bacteria (genus Thiotrix) (LI, YONGMEI et al., 2014; ZHANG, QUANGUO; HU; LEE, 2016). Older sludge age of the granular system and the growth of filamentous bacteria increases the capture and involvement of minerals in the granules (LI, YONGMEI et al., 2014).

Use of aerobic granular sludge for the treatment of wastewater with high concentrations of ammonia and phosphorus presents many problems. The low growth rate of nitrifying bacteria increases the time required to form granules and ammonia can prevent the formation of granular sludge by inhibiting microorganism metabolism (WEI et al., 2014). Rapid formation of granules is desirable, however it is important to maintain a favorable condition for the growth of nitrifying bacteria to obtain a high nutrient removal capacity (LOCHMATTER; HOLLIGER, 2014). In addition, the reduction of nitrate to nitrogen gas during the anoxic phase in conventional biological nutrient removal systems may inhibit phosphate release (WEI et al., 2014).

Phosphate removal in systems with granular aerobic sludge may occur chemically or biologically. Biological phosphate removal is promoted by phosphate accumulating organisms (PAO) and chemical removal can occur by dosing metal salts or by biologically induced phosphate precipitation. Phosphate precipitation products such as struvite and hydroxyapatite are especially interesting because they are common products used in agriculture (STUBBÉ, 2016).

Phosphate accumulating microorganisms are important for the removal of phosphorus, but precipitation is the main mechanism acting on systems with aerobic granules. Increased pH during treatment and longer sludge retention time (SRT) contribute to the accumulation of phosphorus in the aerobic granular sludge (LI, YONGMEI et al., 2014). Accumulation of phosphorus in the granules was 150.7 ± 28.5

mg.gSS^{-1} of P (milligram of phosphorus per gram of suspended solids) in a reactor with a high phosphorus concentration in the influent (35 mg.L^{-1} of P). In addition, the addition of calcium (40 mg.L^{-1} Ca) increased the phosphate precipitation, augmenting the phosphorus content in the sludge from 2.5% to 10% in 25 days (LI, YONGMEI et al., 2014).

2.7. Biodegradation, bioaccumulation and biosorption

The removal mechanisms of toxic and harmful organic pollutants involve adsorption, absorption, concentration, biotransformation, or microbiological degradation (YAN, QING et al., 2016). The most promising feature of aerobic granules in treating contaminants of emerging concern is the ability to treat pollutants simultaneously through three environmentally friendly methods that may vary depending on the type of pollutant: biodegradation, bioaccumulation, and biosorption. Granules are capable of providing the advantages of aerobic and anaerobic biodegradation since they are composed of both aerobic and anoxic/anaerobic zones (SARMA; TAY; CHU, 2016).

Microorganisms in aerobic granules may share biodegradation pathways similar to any other mixed microbial cultures, where a degradation product generated by one organism may be used by others to achieve complete degradation. Activated sludge systems, on the other hand, do not completely degrade the contaminants of emerging concern that are often found in effluents such as pharmaceutical drug residues and endocrine disrupting substances (SARMA; TAY; CHU, 2016).

EPS is generally the first microbial cell barrier that interacts with toxic substances and is closely related to the stability of aerobic granules (LI, KAI et al., 2015). Comparison of the responses of nitrifying granules (NG) and conventional granules (CG) to tetracycline (TC) indicated that more EPS were produced with higher protein content than polysaccharides to protect against TC stress (SHI et al. 2013). Many organic pollutants, such as benzene and phenanthrene dyes, can be absorbed by hydrophobic EPS. Besides, EPS also contains active enzymes that may contribute to the removal of organic contaminants through biological transformation (LI, KAI et al., 2015).

Persistent organic pollutants with high octanol-water partition coefficients (K_{OW}) tend to adsorb on the surfaces of the granules and can subsequently be removed. Aerobic granulation has been considered a promising technology for the removal of heavy metals, such as nickel, cadmium, copper, and zinc (DENG; SU, 2016; SARMA; TAY; CHU, 2016).

Aerobic granules were used to treat wastewater containing phenol, o-cresol and p-nitrophenol under high salinity conditions and the biogranules reached the complete biodegradation of aromatic compounds (RAMOS; SUÁREZ-OJEDA; CARRERA, 2015). Treatment of organic pollution by pharmaceuticals and personal care products indicated that biogranules promoted the growth of Proteobacteria, Bacteroidetes, Beta Proteobacteria and Zooglea to remove some but not all of the PPCP tested (ZHAO et al., 2015).

The residence time and concentration of RP4 (antibiotic resistance plasmid) in the sludge decreases as the size of the aggregates increases. Smaller aggregates are more vulnerable to changes and alien species, while larger aggregates with a diameter greater than 0.9 mm maintain a relatively stable microbial composition and are immune to the hazards of newly introduced bacteria. The granules have a compact formation and a high density of microorganisms, and are considered an independent microbial ecological system, which hampers invasion of the RP4 plasmid. Therefore, the use of the granular sludge system can reduce the transmissions of ARG and reduce potential ecological threats, thus improving the ecological safety of wastewater treatment processes (ZOU et al., 2015).

The addition of 40 $mg.L^{-1}$ of Bisphenol A (BPA), a highly toxic yet important industrial chemical, to synthetic wastewater caused an increase in the amount of EPS, especially proteins, produced by aerobic granular sludge (LI, KAI et al., 2015). A system with aerobic granules showed an efficiency equal to 91.8% while a conventional activated sludge removed 71.3% of the BPA present in the municipal wastewater from Bari, Italy. These results may be explained by the very old sludge age (about 160 d) of the aerobic granule system which offers a greater chance of removal of endocrine disruptors (BALEST, LYDIA et al., 2008). The relatively high stability of these compounds in municipal effluents is mainly due to the insufficient retention time of the sludge from conventional biological systems (BALEST, L et al., 2008).

3. Membrane bioreactor with granular sludge

Since granules are dense aggregates larger than flocs they were found to be a possible alternative to minimize the fouling problem in an MBR. It is possible to maintain higher flow with granular sludge than flocculent sludge, due to the reduction in membrane fouling, one of the main challenges for operation of MBR, causing a flux reduction and increasing the costs of cleaning and regeneration of the membrane modules (LI, WEN

WEI et al., 2012; LI, XIUFEN et al., 2005; MORAIS; SILVA; BORGES, 2016; TU et al., 2010; WANG et al., 2013).

Many factors affect membrane fouling, including sludge particles and structures that can deposit and accumulate on the membrane surface to form a cake layer and large soluble molecules plugging and narrowing the pores of the membrane. Aerobic granules may contribute to delaying membrane fouling as they do not easily adhere to membrane pores due to their large particle size (WANG et al., 2013).

Different fouling mechanisms, such as adsorption of macromolecules, pore blocking or biofilm formation, can be established on the surface of the membranes. Physiological characteristics of the sludge, such as the concentration of solids and the presence of EPS, change according to the operating conditions of the bioreactor. Thus, fouling processes in membrane bioreactors are unpredictable and difficult to control (LE CLECH et al., 2003).

Mechanisms of membrane fouling in MBR differ according to the type of sludge. Resistance due to the gel layer is the main factor for membrane fouling in systems operated with flocculent sludge, while pore blocking is mainly responsible for the fouling caused by granular aerobic sludge (WANG et al., 2013). The smaller particles in the flocculent sludge accumulate rapidly on the surface of the membranes to produce a cake under high flux due to the transmembrane pressure and high compressibility of the biological flocs (WANG et al., 2013).

The size of the granular sludge particles is generally larger than the pore size of the microfiltration membranes. Particles larger than 100 μm corresponded to 20% and 90% of the flocculent and granular sludge, respectively (WANG et al., 2013). The presence of EPS contributes to the production of granules larger than the pore size of membranes in MBR. This characteristic, coupled with low compressibility of the granular sludge, can lead to a significant decrease in membrane fouling. Thus, the main form of membrane fouling caused by the sludge can be eliminated by replacing flocculent sludge with granular sludge, and the permeability throughout the operation would be conditioned only by the solutes and colloids present in the medium (MENG et al., 2009; WANG et al., 2013).

Flux is also an important factor that influences membrane fouling. A flux lower than 20 $L.m^{-2}.h^{-1}$ produced 75 times more water than a flux of 40 $L.m^{-2}.h^{-1}$ in a flat sheet membrane module (JOHIR et al., 2012). Under high flux operations there is a faster

sludge deposition on the membrane surface and the gel layer is formed more compactly than when operated with smaller fluxes (LI, XIUFEN et al., 2005). The granules have a lower tendency for membrane fouling than flocculent sludge and the use of granular aerobic sludge allows the operation of the reactor with a higher flux (LI, WEN WEI et al., 2012; TAY et al., 2007; ZHOU et al. Al., 2007).

Studies with aerobic granular sludge in MBR are carried out in batch or short run continuous flux trials (CORSINO, S. F. et al., 2016; WANG et al., 2013). Aerobic granules lose their stability faster in a continuous flux reactor than in SBR, due to the absence of selection pressure in the continuous flux reactors as the high settling velocity (CORSINO, SF et al., 2016). In almost all studies, the granular aerobic sludge was produced in an SBR and inoculated in an MBR (SAJJAD; KIM; KIM, 2016).

4. Aerobic granular sludge at high temperatures

There is still little known about the formation of granular aerobic sludge at high temperatures. Detailed knowledge regarding the effects of high temperatures on aerobic granulation is still limited, although some studies on granular aerobic sludge have been carried out at high temperatures (AB HALIM et al., 2015).

Thermophilic aerobic effluent treatment can be recommended for high-temperature wastewater. The advantages of this treatment include high biodegradation rates, low sludge production, deactivation of pathogenic organisms, and elimination of pre-treatment cooling requirements. However, solid-liquid separation is a problem and results in high solid concentrations in effluents (GUVEN, 2004).

Aerobic biological wastewater treatment at thermophilic temperatures (about 55 °C) produces sludge with poor flocculation. However, granules were formed at 55 °C with diameters between 1 and 7 mm (GUVEN, 2004) or with a diameter between 1.2 and 1.9 mm and granule resistance to disintegration was comparable to aerobic mesophilic granules (ZITOMER et al., 2007). The species Anoxybacillus flavathermus and Pseudoxanthomonas taiwanensis, which are alkaliphilic or alkalitolerant, were isolated from these granules indicating that high alkalinity may favor thermophilic granule-forming populations (ZITOMER et al., 2007).

The effect of temperature on the granulation process was evaluated in an SBR to achieve partial nitrification. The granules showed no significant changes to their morphological characteristics with a temperature between 28 and 37 °C. The diameter of

more than 80% of the granules was greater than 6 mm when the temperature was raised to 39 °C, but the density decreased from 28 to 19 $g.L^{-1}$ and the sludge volume index (SVI) increased from 33 to 80 $mL.g^{-1}$. The system rapidly destabilized and nitrification ceased when the temperature reached 41 °C (LÓPEZ-PALAU et al., 2013). The increase in temperature causes an increase in the size and ash content of the granules (AB HALIM et al., 2015).

Biological phosphate removal was higher in the system at 50 °C than at 30 °C and 40 °C, with efficiencies of 72%, 68%, and 67%, respectively. On the other hand, the highest ammonia removal efficiency (97.5%) was observed at 30 °C, against 94.6% and 94.4% at 40 °C and 50 °C, respectively (AB HALIM et al., 2015). Phosphorus removal reduced to insignificant values when the system temperature containing granules formed at 20 °C was raised to 35 °C (EBRAHIMI et al., 2010).

5. Full-scale aerobic granular sludge systems

Aerobic granular sludge was developed in the 1990s and used initially in lab-scale systems. Granular sludge was applied by Royal-HaskoningDHV in the development of Nereda® technology in the early 2000s that allowed the deployment of full scale applications. Nereda® technology was initially adapted for industrial application and then expanded for domestic sewage treatment. The Nereda® process uses an optimized sequential batch reactor cycle where the 4 steps of a typical RBS cycle are reduced to 3 steps. The first stage consists of the simultaneous fill and draw, in which the wastewater is pumped into the reactor at the same time as the effluent is drawn. The second stage consists of the aeration phase, in which the biological degradation of the organic matter occurs. The cycle is finalized with the sedimentation phase where the separation of clear effluent from biological sludge occurs. The Nereda® process can achieve nutrient removal. Nitrifying bacteria accumulate in the outer layer of the granules and produce nitrate, which will be consumed in the denitrification inside the granules, where anoxic conditions prevail (GIESEN; THOMPSON, 2013).

The first full scale installation was implemented in the city of Epe, in the Netherlands, in 2011 (PRONK et al., 2015, STUBBÉ, 2016). The reactors were designed for average daily flow of 8,000 $m^3.d^{-1}$ and a maximum flow of 36,000 $m^3.d^{-1}$ (GIESEN; THOMPSON, 2013). A granular sludge blanket was formed after a starting period of 5 months and consisted of more than 80% granules with a diameter greater than 0.2 mm and more than 60% greater than 1 mm. The quality required for the effluent (7 mg $.L^{-1}$ of

N and 1 mg.L^{-1} of P) is easily achieved throughout the year. Energy consumption is 58% lower than the conventional activated sludge process due mainly to the absence of pumps for recycling sludge return, and mixers used in conventional nutrient removal plants. In addition, the specific volume required for granular aerobic sludge systems is 33% lower than the volume of conventional activated sludge plants (PRONK et al., 2015).

The technology used relies on a sequencing fed-batch process with a constant working volume. This is possible due to simultaneous feeding and effluent discharge, that relies on a plug-flow pattern for displacement of effluent from the reactor (bottom feed and discard at the top of the reactor) (PRONK et al., 2015).

The Netherlands currently has other full-scale aerobic granular sludge treatment plants, such as those in the cities of Utrecht and Garmerwolde. The Garmerwolde granular aerobic sludge installation went into operation in June 2013 with a maximum flow of 4200 $m^3.h^{-1}$ (PRONK et al. 2015) and phosphate removal was frequently used until May 2015, after which time only occasional dosing was applied, when the effluent phosphate levels exceeded requirements (STUBBÉ, 2016).

The Utrecht and Garmerwolde installations are operated with cycles of about 6 hours, one hour feeding with simultaneous effluent withdrawal, 15 minutes of excess sludge discharge, 4 hours of aeration, and one hour for settling. The cycle is reduced to a minimum of 3 hours during rainy weather conditions. At both treatment sites, the aerobic granular sludge system is operated together with a conventional activated sludge treatment (STUBBÉ, 2016).

The success of Nereda® technology has increased interest in using granular sludge at full-scale wastewater treatment plants and even larger plants are being designed and built, including a plant in the city of Dublin and one in Utrecht with populations of 2,400,000 and 500,000, respectively. There are more than 16 plants operational worldwide, including Kingaroy in Australia with an equivalent population of 12,500 inhabitants, operating since May 2016, that has achieved excellent effluent results to date (AQUATEC MAXCON, 2016).

6. References

AB HALIM, M. H.; NOR ANUAR, A.; AZMI, S. I.; et al. Aerobic sludge granulation at high temperatures for domestic wastewater treatment. **Bioresource Technology**, v. 185, p. 445–449, 2015.

ADAV, S. S.; LEE, D. J.; LAI, J. Y. Effects of aeration intensity on formation of phenol-fed aerobic granules and extracellular polymeric substances. **Applied Microbiology and Biotechnology**, v. 77, n. 1, p. 175–182, 2007.

ADAV, S. S.; LEE, D. J.; LAI, J. Y. Intergeneric coaggregation of strains isolated from phenol-degrading aerobic granules. **Applied Microbiology and Biotechnology**, v. 79, n. 4, p. 657–661, 2008.

ADAV, S. S.; LEE, D. J.; SHOW, K. Y.; TAY, J. H. Aerobic granular sludge: Recent advances. **Biotechnology Advances**, v. 26, n. 5, p. 411–423, 2008.

ADAV, S. S.; LEE, D.-J.; LAI, J.-Y. Potential cause of aerobic granular sludge breakdown at high organic loading rates. **Applied Microbiology and Biotechnology**, v. 85, n. 5, p. 1601–1610, 2010.

AQUATEC MAXCON. Nereda® Aerobic Granular Biomass. Disponível em: <http://www.aquatecmaxcon.com.au/technologies/sewage-treatment/nereda-aerobic-granular-biomass#description>. Acesso em: 1/11/2016.

AWANG, N. A.; SHAABAN, M. G. Effect of reactor height/diameter ratio and organic loading rate on formation of aerobic granular sludge in sewage treatment. **International Biodeterioration and Biodegradation**, v. 112, p. 1–11, 2016.

BALEST, L.; LOPEZ, A.; MASCOLO, G.; DI IACONI, C. Removal of endocrine disrupter compounds from municipal wastewater using an aerobic granular biomass reactor. **Biochemical Engineering Journal**, v. 41, n. 3, p. 288–294, 2008.

BALEST, L.; MASCOLO, G.; DI IACONI, C.; LOPEZ, A. Removal of endocrine disrupter compounds from municipal wastewater by an innovative biological technology. **Water Science and Technology**, v. 58, n. 4, p. 953–956, 2008.

BASUVARAJ, M.; FEIN, J.; LISS, S. N. Protein and polysaccharide content of tightly and loosely bound extracellular polymeric substances and the development of a granular activated sludge floc. **Water research**, v. 82, p. 104–117, 2015.

BUSWELL, C. M.; HERLIHY, Y. M.; MARSH, P. D.; KEEVIL, C. W.; LEACH, S. A. Coaggregation amongst aquatic biofilm bacteria. **Journal of Applied Microbiology**, v.

83, n. 4, p. 477–484, 1997.

CAO, X.; SHENG, Y.; CAO, H.; ZHANG, Y. Comparison of Mg2+- and Ca2+-enhancing anaerobic granulation in an expanded granular sludge-bed reactor. **Science China Chemistry**, v. 57, n. 11, p. 1596–1601, 2014.

CAUDAN, C.; FILALI, A.; SPÉRANDIO, M.; GIRBAL-NEUHAUSER, E. Multiple EPS interactions involved in the cohesion and structure of aerobic granules. **Chemosphere**, v. 117, n. 1, p. 262–270, 2014.

CHEN, Y.-Y.; LEE, D.-J. Effective aerobic granulation: Role of seed sludge. **Journal of the Taiwan Institute of Chemical Engineers**, v. 0, p. 1–2, 2015.

CHEN, Y.-Y.; PAN, X.; LI, J.; LEE, D.-J. Strengthening aerobic granule by salt precipitation. **Bioresource Technology**, v. 218, n. 16, p. 1253–1256, 2016.

LE CLECH, P.; JEFFERSON, B.; CHANG, I. S.; JUDD, S. J. Critical flux determination by the flux-step method in a submerged membrane bioreactor. **Journal of Membrane Science**, v. 227, n. 1–2, p. 81–93, 2003.

CORSINO, S. F.; CAMPO, R.; DI BELLA, G.; TORREGROSSA, M.; VIVIANI, G. Study of aerobic granular sludge stability in a continuous-flow membrane bioreactor. **Bioresource Technology**, v. 200, p. 1055–1059, 2016.

CORSINO, S. F.; CAPODICI, M.; TORREGROSSA, M.; VIVIANI, G. Fate of aerobic granular sludge in the long-term: The role of EPSs on the clogging of granular sludge porosity. **Journal of Environmental Management**, v. 183, n. 3, p. 1–10, 2016.

DENG, S.; WANG, L.; SU, H. Role and influence of extracellular polymeric substances on the preparation of aerobic granular sludge. **Journal of Environmental Management**, v. 173, p. 49–54, 2016.

EBRAHIMI, S.; GABUS, S.; ROHRBACH-BRANDT, E.; et al. Performance and microbial community composition dynamics of aerobic granular sludge from sequencing batch bubble column reactors operated at 20 °c, 30 °c, and 35 °c. **Applied Microbiology and Biotechnology**, v. 87, n. 4, p. 1555–1568, 2010.

EKMEKCI, H.; ASLIM, B.; OZTURK, S. Characterization of vaginal lactobacilli coaggregation ability with Escherichia coli. **Microbiology and Immunology**, v. 53, n. 2, p. 59–65, 2009.

GIESEN, A.; THOMPSON, A. **The Nereda ® Process – Wastewater Treatment with Aerobic Granular Biomass**. 2013.

GUVEN, E. **Granulation in thermophilic aerobic wastewater treatment**, 2004. Marquette University.

HE, Q.; ZHOU, J.; WANG, H.; ZHANG, J.; WEI, L. Microbial population dynamics during sludge granulation in an A/O/A sequencing batch reactor. **Bioresource Technology**, v. 214, p. 1–8, 2016.

IVANOV, V.; WANG, X. H.; TAY, S. T. L.; TAY, J. H. Bioaugmentation and enhanced formation of microbial granules used in aerobic wastewater treatment. **Applied Microbiology and Biotechnology**, v. 70, n. 3, p. 374–381, 2006.

JOHIR, M. A H.; GEORGE, J.; VIGNESWARAN, S.; et al. Effect of imposed flux on fouling behavior in high rate membrane bioreactor. **Bioresource Technology**, v. 122, p. 42–49, 2012.

KOCATURK, I.; ERGUDER, T. H. Influent COD/TAN ratio affects the carbon and nitrogen removal efficiency and stability of aerobic granules. **Ecological Engineering**, v. 90, p. 12–24, 2016.

LEDDER, R. G.; TIMPERLEY, A. S.; FRISWELL, M. K.; MACFARLANE, S.; MCBAIN, A. J. Coaggregation between and among human intestinal and oral bacteria. **FEMS Microbiology Ecology**, v. 66, n. 3, p. 630–636, 2008.

LEE, D. J.; CHEN, Y. Y.; SHOW, K. Y.; WHITELEY, C. G.; TAY, J. H. Advances in aerobic granule formation and granule stability in the course of storage and reactor operation. **Biotechnology Advances**, v. 28, n. 6, p. 919–934, 2010.

LI, K.; WEI, D.; YAN, T.; DU, B.; WEI, Q. Responses of soluble microbial products and extracellular polymeric substances to the presence of toxic 2,6-dichlorophenol in aerobic granular sludge system. **Journal of Environmental Management**, v. 183, p. 594–600, 2016.

LI, K.; WEI, D.; ZHANG, G.; et al. Toxicity of bisphenol A to aerobic granular sludge in sequencing batch reactors. **Journal of Molecular Liquids**, v. 209, p. 284–288, 2015.

LI, W. W.; WANG, Y. K.; SHENG, G. P.; et al. Integration of aerobic granular sludge and mesh filter membrane bioreactor for cost-effective wastewater treatment. **Bioresource Technology**, v. 122, p. 22–26, 2012.

LI, X.; GAO, F.; HUA, Z.; DU, G.; CHEN, J. Treatment of synthetic wastewater by a novel MBR with granular sludge developed for controlling membrane fouling. **Separation and Purification Technology**, v. 46, n. 1–2, p. 19–25, 2005.

LI, Y.; ZOU, J.; ZHANG, L.; SUN, J. Aerobic granular sludge for simultaneous accumulation of mineral phosphorus and removal of nitrogen via nitrite in wastewater. **Bioresource Technology**, v. 154, n. 2, p. 178–184, 2014.

LIU, H.; XIAO, H.; HUANG, S.; MA, H.; LIU, H. Aerobic granules cultivated and operated in continuous-flow bioreactor under particle-size selective pressure. **Journal of Environmental Sciences (China)**, v. 26, n. 11, p. 2215–2221, 2014.

LIU, Y.; LIU, Z.; WANG, F.; et al. Regulation of aerobic granular sludge reformulation after granular sludge broken: Effect of poly aluminum chloride (PAC). **Bioresource Technology**, v. 158, p. 201–208, 2014.

LIU, Y. Q.; LIU, Y.; TAY, J. H. The effects of extracellular polymeric substances on the formation and stability of biogranules. **Applied Microbiology and Biotechnology**, v. 65, p. 143–148, 2004.

LIU, Y. Q.; TAY, J. H. Influence of cycle time on kinetic behaviors of steady-state aerobic granules in sequencing batch reactors. **Enzyme and Microbial Technology**, v. 41, n. 4, p. 516–522, 2007.

LIU, Y. Q.; TAY, J. H. Fast formation of aerobic granules by combining strong hydraulic selection pressure with overstressed organic loading rate. **Water Research**, v. 80, p. 256–266, 2015.

LIU, Y.; WANG, Z.-W.; TAY, J.-H. A unified theory for upscaling aerobic granular sludge sequencing batch reactors. **Biotechnology advances**, v. 23, n. 5, p. 335–344, 2005.

LIU, Y.; YANG, S. F.; TAY, J. H.; et al. Cell hydrophobicity is a triggering force of biogranulation. **Enzyme and Microbial Technology**, v. 34, n. 5, p. 371–379, 2004.

LOCHMATTER, S.; HOLLIGER, C. Optimization of operation conditions for the startup of aerobic granular sludge reactors biologically removing carbon, nitrogen, and phosphorous. **Water Research**, v. 59, p. 58–70, 2014.

LONG, B.; YANG, C. Z.; PU, W. H.; et al. Rapid cultivation of aerobic granule for the treatment of solvent recovery raffinate in a bench scale sequencing batch reactor. **Separation and Purification Technology**, v. 160, p. 1–10, 2016.

LONG, B.; YANG, C.-Z.; PU, W.-H.; et al. Rapid cultivation of aerobic granular sludge in a pilot scale sequencing batch reactor. **Bioresource technology**, v. 166, n. 4, p. 57–63, 2014.

LÓPEZ-PALAU, S.; SANCHO, I.; PINTO, A.; DOSTA, J.; MATA-ÁLVAREZ, J.

Influence of temperature on the partial nitritation of reject water in a Granular Sequencing Batch Reactor. **Environmental Technology**, v. 34, n. 18, p. 2625–2632, 2013.

LUO, J.; HAO, T.; WEI, L.; et al. Impact of influent COD/N ratio on disintegration of aerobic granular sludge. **Water Research**, v. 62, p. 127–135, 2014.

LV, Y.; WAN, C.; LEE, D. J.; LIU, X.; TAY, J. H. Microbial communities of aerobic granules: Granulation mechanisms. **Bioresource Technology**, v. 169, p. 344–351, 2014.

MALIK, A.; KAKII, K. Novel coaggregating microbial consortium: Testing strength for field applications. **Bioresource Technology**, v. 99, n. 11, p. 4627–4634, 2008.

MALIK, A; SAKAMOTO, M.; HANAZAKI, S.; et al. Coaggregation among nonfloculating bacteria isolated from activated sludge. **Applied and Environmental Microbiology**, v. 69, n. 10, p. 6056–6063, 2003.

MENG, F.; CHAE, S. R.; DREWS, A.; et al. Recent advances in membrane bioreactors (MBRs): Membrane fouling and membrane material. **Water Research**, v. 43, n. 6, p. 1489–1512, 2009.

MIECZKOWSKI, D.; CYDZIK-KWIATKOWSKA, A.; RUSANOWSKA, P.; ŚWIĄTCZAK, P. Temperature-induced changes in treatment efficiency and microbial structure of aerobic granules treating landfill leachate. **World Journal of Microbiology and Biotechnology**, v. 32, n. 6, 2016.

MORAIS, I. L. H.; SILVA, C. M.; BORGES, C. P. Aerobic granular sludge to treat paper mill effluent: organic matter removal and sludge filterability. **Desalination and Water Treatment**, v. 57, n. 18, p. 8119–8126, 2016.

MORAIS, I. L. H.; SILVA, C. M.; NASCIMENTO, A. G. DO; FILHO, N. P.; DIAS, J. C. T. Aerobic granular sludge to treat paper mill effluent : selection of ideal strains that contribute to the formation of strong aggregates. **Desalination and Water Treatment**, v. 57, n. 59, p. 28537–28550, 2016.

NAGAOKA, S.; HOJO, K.; MURATA, S.; et al. Interactions between salivary Bifidobacterium adolescentis and other oral bacteria: In vitro coaggregation and coadhesion assays. **FEMS Microbiology Letters**, v. 281, n. 2, p. 183–189, 2008.

NANCHARAIAH, Y. V.; JOSHI, H. M.; KRISHNA MOHAN, T. V.; VENUGOPALAN, V. P.; NARASIMHAN, S. V. Formation of aerobic granules in the presence of a synthetic chelating agent. **Environmental Pollution**, v. 153, n. 1, p. 37–43, 2008.

NI, B.-J.; YU, H.-Q. Mathematical modeling of aerobic granular sludge: a review. **Biotechnology advances**, v. 28, n. 6, p. 895–909, 2010.

PHUONG, K.; KAKII, K.; NIKATA, T. Intergeneric coaggregation of non-flocculating Acinetobacter spp. isolates with other sludge-constituting bacteria. **Journal of Bioscience and Bioengineering**, v. 107, n. 4, p. 394–400, 2009.

PRONK, M.; DE KREUK, M. K.; DE BRUIN, B.; et al. Full scale performance of the aerobic granular sludge process for sewage treatment. **Water Research**, v. 84, p. 207–217, 2015.

QIN, L.; LIU, Y.; TAY, J. Effect of settling time on aerobic granulation in sequencing batch reactor. **Biochemical Engineering Journal**, v. 21, p. 47–52, 2004.

QIN, L.; TAY, J.; LIU, Y. Selection pressure is a driving force of aerobic granulation in sequencing batch reactors. **Process Biochemistry**, v. 39, p. 579–584, 2004.

RAMOS, C.; SUÁREZ-OJEDA, M. E.; CARRERA, J. Long-term impact of salinity on the performance and microbial population of an aerobic granular reactor treating a high-strength aromatic wastewater. **Bioresource Technology**, v. 198, p. 844–851, 2015.

REN, N.; XIE, T.; XING, D. Composition of extracellular polymeric substances influences the autoaggregation capability of hydrogen-producing bacterium Ethanoligenens harbinense. **Bioresource Technology**, v. 100, n. 21, p. 5109–5113, 2009.

RICKARD, A. H.; GILBERT, P.; HIGH, N. J.; KOLENBRANDER, P. E.; HANDLEY, P. S. Bacterial coaggregation: an integral process in the development of multi-species biofilms. **Trends in Microbiology**, v. 11, n. 2, p. 94–100, 2003.

RICKARD, A. H.; GILBERT, P.; HANDLEY, P. S. Influence of growth environment on coaggregation between freshwater biofilm bacteria. **Journal of Applied Microbiology**, v. 96, n. 6, p. 1367–1373, 2004.

RICKARD, A. H.; MCBAIN, A. J.; LEDDER, R. G.; HANDLEY, P. S.; GILBERT, P. Coaggregation between freshwater bacteria within biofilm and planktonic communities. **FEMS Microbiology Letters**, v. 220, n. 1, p. 133–140, 2003.

SAJJAD, M.; KIM, I. S.; KIM, K. S. Development of a novel process to mitigate membrane fouling in a continuous sludge system by seeding aerobic granules at pilot plant. **Journal of Membrane Science**, v. 497, p. 90–98, 2016.

SAJJAD, M.; KIM, K. S. Studies on the interactions of Ca2+ and Mg2+ with EPS and their role in determining the physicochemical characteristics of granular sludges in SBR

system. **Process Biochemistry**, v. 50, n. 6, p. 966–972, 2015a.

SAJJAD, M.; KIM, K. S. Influence of Mg2+ catalyzed granular sludge on flux sustainability in a sequencing batch membrane bioreactor system. **Chemical Engineering Journal**, v. 281, p. 404–410, 2015b.

SARMA, S. J.; TAY, J. H.; CHU, A. Finding Knowledge Gaps in Aerobic Granulation Technology. **Trends in Biotechnology**, v. xx, p. 1–13, 2016.

SHI, Y.; XING, S.; WANG, X.; WANG, S. Changes of the reactor performance and the properties of granular sludge under tetracycline (TC) stress. **Bioresource Technology**, v. 139, p. 170–175, 2013.

SIMÕES, L. C.; SIMÕES, M.; VIEIRA, M. J. Biofilm interactions between distinct bacterial genera isolated from drinking water. **Applied and Environmental Microbiology**, v. 73, n. 19, p. 6192–6200, 2007.

SOBECK, D. C.; HIGGINS, M. J. Examination of three theories for mechanisms of cation-induced bioflocculation. **Water Research**, v. 36, n. 3, p. 527–538, 2002.

SONG, Z.; REN, N.; ZHANG, K.; TONG, L. Influence of temperature on the characteristics of aerobic granulation in sequencing batch airlift reactors. **Journal of Environmental Sciences**, v. 21, n. 3, p. 273–278, 2009.

STUBBÉ, S. M. L. **The Fate of Phosphate in Full-scale Aerobic Granular Sludge Systems**, 2016. Delft: Delft University of Technology.

TAY, J. H.; YANG, P.; ZHUANG, W. Q.; TAY, S. T. L.; PAN, Z. H. Reactor performance and membrane filtration in aerobic granular sludge membrane bioreactor. **Journal of Membrane Science**, v. 304, n. 1–2, p. 24–32, 2007.

TU, X.; ZHANG, S.; XU, L.; ZHANG, M.; ZHU, J. Performance and fouling characteristics in a membrane sequence batch reactor (MSBR) system coupled with aerobic granular sludge. **Desalination**, v. 261, n. 1–2, p. 191–196, 2010.

WAN, C.; LEE, D. J.; YANG, X.; et al. Calcium precipitate induced aerobic granulation. **Bioresource Technology**, v. 176, p. 32–37, 2015.

WAN, C.; YANG, X.; LEE, D.-J.; LIU, X.; SUN, S. Partial nitrification using aerobic granule continuous flow reactor. **Journal of the Taiwan Institute of Chemical Engineers**, v. 45, p. 2681–2687, 2014.

WANG, Y.; ZHONG, C.; HUANG, D.; WANG, Y.; ZHU, J. The membrane fouling

characteristics of MBRs with different aerobic granular sludges at high flux. **Bioresource Technology**, v. 136, p. 488–495, 2013.

WEI, D.; SHI, L.; YAN, T.; et al. Aerobic granules formation and simultaneous nitrogen and phosphorus removal treating high strength ammonia wastewater in sequencing batch reactor. **Bioresource Technology**, v. 171, n. 1, p. 211–216, 2014.

WIECZERZAK, M.; KUDŁAK, B.; YOTOVA, G.; et al. Modeling of pharmaceuticals mixtures toxicity with deviation ratio and best-fit functions models. **Science of the Total Environment**, v. 571, p. 259–268, 2016.

WINKLER, M.-K. H.; KLEEREBEZEM, R.; DE BRUIN, L. M. M.; et al. Microbial diversity differences within aerobic granular sludge and activated sludge flocs. **Applied Microbiology and Biotechnology**, v. 97, n. 16, p. 7447–7458, 2013.

WU, L.; PENG, C.; PENG, Y.; et al. Effect of wastewater COD/N ratio on aerobic nitrifying sludge granulation and microbial population shift. **Journal of Environmental Sciences**, v. 24, n. 2, p. 234–241, 2012.

YAN, L.; LIU, Y.; WEN, Y.; et al. Role and significance of extracellular polymeric substances from granular sludge for simultaneous removal of organic matter and ammonia nitrogen. **Bioresource Technology**, v. 179, p. 460–466, 2015.

YAN, Q.; FENG, G.; GAO, X.; et al. Removal of pharmaceutically active compounds (PhACs) and toxicological response of Cyperus alternifolius exposed to PhACs in microcosm constructed wetlands. **Journal of Hazardous Materials**, v. 301, p. 566–575, 2016.

YANG, X.; FLOWERS, R. C.; WEINBERG, H. S.; SINGER, P. C. Occurrence and removal of pharmaceuticals and personal care products (PPCPs) in an advanced wastewater reclamation plant. **Water Research**, v. 45, n. 16, p. 5218–5228, 2011.

YANG, X.; XU, X.; XU, X.; et al. Modeling and kinetics study of Bisphenol A (BPA) degradation over an FeOCl/SiO2 Fenton-like catalyst. **Catalysis Today**, v. 276, p. 85–96, 2016.

YU, X.; WAN, C.; LEI, Z.; et al. Use of aerobic granules for treating synthetic high-strength ammonium wastewaters. **Environmental Technology**, v. 35, n. 14, p. 1785–1790, 2014.

ZHANG, Q.; HU, J.; LEE, D.-J. Aerobic granular processes: Current research trends. **Bioresource Technology**, v. 210, p. 1–7, 2016.

ZHANG, X.; LIU, Y.-Q.; TAY, J.-H.; JIANG, W.-J. Fast granulation under extreme selection pressures and its formation mechanism. **Fresenius Environmental Bulletin**, v. 22, n. 5, p. 1330–1338, 2013.

ZHAO, X.; CHEN, Z.; WANG, X.; et al. Remediation of pharmaceuticals and personal care products using an aerobic granular sludge sequencing bioreactor and microbial community profiling using Solexa sequencing technology analysis. **Bioresource Technology**, v. 179, p. 104–112, 2015.

ZHENG, X.; WANG, X.; HUANG, X.; et al. Effects of Cu2+ on morphological structure, functional groups, and elemental composition of aerobic granular sludge. **Environmental Technology**, v. 34, n. 2, p. 219–224, 2013.

ZHOU, J.; YANG, F. L.; MENG, F. G.; AN, P.; WANG, D. Comparison of membrane fouling during short-term filtration of aerobic granular sludge and activated sludge. **Journal of Environmental Sciences**, v. 19, n. 11, p. 1281–1286, 2007.

ZITOMER, D. H.; DURAN, M.; ALBERT, R.; GUVEN, E. Thermophilic aerobic granular biomass for enhanced settleability. **Water Research**, v. 41, n. 4, p. 819–825, 2007.

ZOU, W.; XUE, B.; ZHI, W.; et al. Effects of antibiotic resistance genes on the performance and stability of different microbial aggregates in a granular sequencing batch reactor. **Journal of Hazardous Materials**, v. 304, p. 343–351, 2015.

CAPÍTULO 2

Stability and resistance of aerobic granular sludge in bioreactors after addition of calcium

Abstract

Granulation is a gradual process that makes flocculent sludge granular through the simultaneous densification and selection of aggregates via sedimentation. Aerobic granulation was established for the treatment of high organic load wastewater containing nitrogen, phosphorus, toxic substances, and xenobiotics. Damage to granule structure over time of bioreactor operation is one of the severest barriers to the practical application of aerobic granular sludge. The addition of calcium, magnesium, and other metal ions that contain coagulation properties may increase aggregation rates and granular structure stability. Four sequential batch reactors (SBR) fed with kraft pulp mill effluent were operated and monitored. Three reactors contained aerobic granular sludge and one operated with flocculent sludge. One granular sludge SBR received the addition of 100 $mg{\cdot}L^{-1}$ of Ca^{2+}; the second received 200 $mg.L^{-1}$ of Ca^{2+}, and the third received no intentional addition of calcium. Organic matter removal efficiency from the reactors and the effect of calcium on the morphological characteristics of the granules formed were evaluated. Chemical oxygen demand (COD) and biochemical oxygen demand (BOD) removal efficiencies were similar among all SBR, i.e., 60% and 90%, respectively. The addition of calcium did not affect granule size. The addition of 100 $mg.L^{-1}$ of Ca^{2+} increased the uniformity and the mechanical resistance of the granules and also increased granule settling velocity by approximately 36%.

Keywords: aerobic granular sludge, calcium, pulp mill effluent, sludge settling velocity

Introduction

Aerobic granulation is a promising technique for wastewater treatment and has significant potential for organic matter and nutrient removal, including recalcitrant and toxic effluents [1,2]. Granulation can be initiated by the adsorption and bacterial adhesion to inert materials and inorganic precipitates as well as by adhesion of microorganisms to other cells through physicochemical interactions. Filamentous bacteria increase the granulation process, forming structures that support the adhesion of new cells [3].

Microorganisms produced by cell lysis release biopolymers that are actively transported during the granulation process. These extracellular polymeric substances (EPS) are composed mainly of proteins, polysaccharides, humic acids, nucleic acids, and lipids [4,5] and form a matrix involving the microorganisms that facilitates their aggregation. EPS interactions and their characteristics are crucial for bioflocculation because they constitute a major part of the sludge mass [6].

Biofloc surfaces are often negatively charged due to the presence of functional groups in EPS. Carboxyl groups of uronic acids are deprotonated at pH values usually found in activated sludge systems and contribute to the negative charge of bioflocs. Proteins rich in amino acids containing carboxyl groups, such as glutamic and aspartic acids also contribute to the negative charge of the bioflocs [6].

In the presence of Ca^{2+}, Mg^{2+} and Fe^{2+} cations, the ionic bonds between carboxyl and phosphate groups are reduced and granulation is increased. Metallic cofactors improve the action of enzymes in the metabolism of viable cells [3]. Bivalent cations bind to negative functional groups in EPS thus stabilizing the biopolymer microorganism matrix, which favors aggregation. Addition of Ca^{2+} to aerobic granular sludge produced EPS with levels of polysaccharides higher than those of proteins, while the addition of Mg^{2+} increased the protein content in relation to polysaccharides. Granulation rate, particle size, and sludge settleability were rapidly increased by Ca^{2+} addition, whereas

sludge dewaterability was significantly enhanced by increasing Mg^{2+} [7]. These ions are probably constituents of polysaccharides or extracellular proteins. Although monovalent ions can also neutralize EPS functional groups, the extracellular polymers mainly bind to bivalent ions forming more stable complexes. The formation of calcium carbonate and the linkage of calcium to EPS and cells are non-specific processes controlled by the calcium ion gradient in the aqueous phase and by the granules. The addition of calcium or simply the replacement of sodium bicarbonate ($NaHCO_3$) by calcium hydroxide ($Ca(OH)_2$) for the neutralization of pH in the bioreactor can improve the granulation rate [8–11].

Calcium precipitates neutralize negatively charged cell surfaces and function as an inert support for bacteria. This phenomenon is important for the initial adsorption and initiation of the aggregation process. Thus, calcium deficiency or loss may decrease resistance or cause granule disruption [3].

In the presence of phosphates, the addition of calcium does not induce granulation or can even be detrimental to granule formation. Phosphates can form calcium precipitates inside the granules, damaging the environment required for maintenance of the granular structure and bacterial activity [3].

High concentrations of calcium may have a negative effect on the granulation process. Most studies with concentrations between 100 and 200 $mgCa^{+2}.L^{-1}$, presented a positive effect on granulation, whereas those above 300 $mgCa^{+2}.L^{-1}$ led to precipitate formation and had a negative influence on granulation [3].

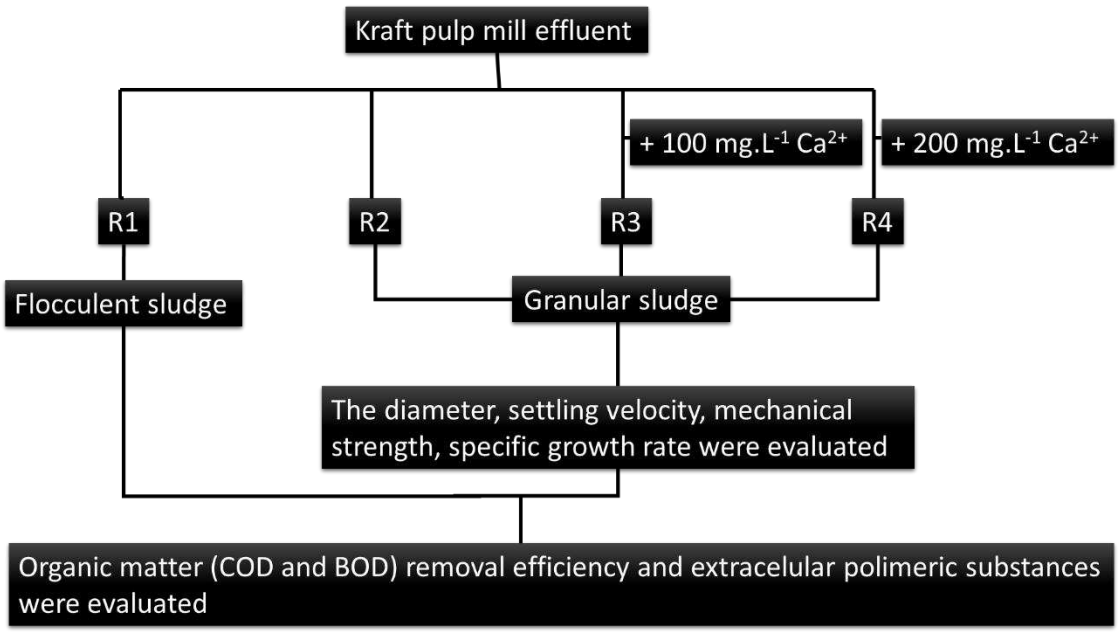
Added sodium and excess calcium can reduce floc properties by replacing bivalent cations. The ratio between the sum of the monovalent cations ($meq.L^{-1}$) divided by the sum of the bivalent cations with values greater than two indicates deterioration of the floc properties [6].

The objective of this study was to evaluate the characteristics of aerobic granular sludge in a system treating bleached kraft pulp mill effluent. Aerobic granular sludge characteristics such as size, settling velocity, strength, and organic matter removal efficiency and filtrability were evaluated with and without the addition of calcium chloride ($CaCl_2$).

Methodology

Biological treatment. Biological treatment was performed in four (R1, R2, R3 and R4) sequential batch reactors (SBR) operated in parallel (Figure 1). Each system had an aeration tank with a functional working volume of 1.6 L (height/diameter ratio of 4). Reactors temperature was maintained at 35°C by controllers connected to electric heaters. Fine bubble aerators were placed at the bottom of the reactors to supply oxygen to the biomass. Dissolved oxygen (DO) was maintained above 2 $mg.L^{-1}$. The duration of each cycle was 12 h and the volume exchange ratio was 50% resulting in a 24-hour hydraulic retention time (HRT). Influent pH was kept between 6.5 and 7.5 and nitrogen and phosphorus were added in the proportion COD: N: P equal to 200: 5: 1. The systems were fed with effluent from the pulp mill. The reactors were inoculated with biological sludge collected in the recirculation stream of an activated sludge treatment plant of a kraft pulp mill. Reactor R1 were operated with flocculent sludge and reactors R2, R3 and R4 were operated with granular sludge. Two SBR received $CaCl_2$ as a calcium source. The addition of calcium (100 $mg.L^{-1}$ and 200 $mg.L^{-1}$, respectively) in R3 and R4 aimed to evaluate the effect of this element on the aerobic granulation process.

Figure 1. Experimental plan



Physical, chemical, and biological analyses. Soluble COD, soluble BOD, pH and total suspended solids in the treated effluents were analyzed following the standard methods [12]. Calcium was determined by atomic absorption spectroscopy according to the Tappi standard T266 om-02 [13].

The size and the circularity of the granules were determined [14] using the ImageJ program [15] with measurements after SBR stabilization, i.e., after mature granules were observed. Average granule diameters were determined to evaluate whether addition of calcium affected their size. Diameters of the granules were compared among Reactors R2, R3, and R4. The distribution frequency of the granule diameters was evaluated throughout the test period after steady-state was achieved.

The specific growth rate of the granules (μ) was calculated based on the kinetic model [16].

The settling velocity and the mechanical strength (integrity coefficient) of the granules were determined [17].

Results and discussion

Characterization of the industrial effluent. The characteristics of the industrial effluent were determined (Table 1).

Table 1. Feeding effluent physical-chemical parameters, n = 12

Parameter	Unit	Results (mean ± standard deviation)		
pH	-	7.08 ± 0.25		
BOD_5	$mg.L^{-1}$	500 ± 13		
COD	$mg.L^{-1}$	1142 ± 140		
BOD/COD	-	0.44 ± 0.09		
Electrical conductivity	$mS.cm^{-1}$	2.31 ± 0.26		
TSS	$mg.L^{-1}$	44 ± 8		
Sodium	$mg.L^{-1}$	622 ± 102		
Calcium	$mg.L^{-1}$	R1 and R2	R3	R4
		170 ± 7	283 ± 2	385 ± 20

Effluent pH remained neutral, electrical conductivity and sodium concentration were typical of bleached kraft pulp mills [18,19]. Total suspended solid levels were low because they had been previously removed by primary sedimentation at the pulp mill treatment plant.

BOD and COD were similar to those reported for typical bleached kraft pulp mill effluents [18,19]. The organic matter in the effluent originates from wood (lignin, extractives, and carbohydrates) during the wood pulping process [20].

Calcium concentrations in R1 and R2 correspond to that of the industrial effluent used without added calcium from an external source (Table 1). The higher calcium concentration in R3 and R4 compared to R1 and R2 is due to the intentional addition of calcium chloride in these SBR.

Organic matter removal. The removal of organic matter was evaluated for soluble COD and soluble BOD_5 (Table 2).

Similar organic matter removal efficiency was achieved in the four SBR. A COD removal of 60% and a BOD_5 of 90% were obtained, which agrees with reported activated sludge systems treating effluents from pulp mills [20].

Table 2. Soluble COD and BOD_5 removal (mean ± standard deviation), n = 12 (BOD), n = 52 (COD).

Treatments	COD (%)	BOD_5 (%)
Reactor R1 (FS)	60 ± 7	91 ± 4
Reactor R2 (GS)	61 ± 6	90 ± 5
Reactor R3 (GS100)	64 ± 6	90 ± 5
Reactor R4 (GS200)	62 ± 6	91 ± 4

FS: flocculent sludge; GS: granular sludge; GS100: granular sludge with addiction of 100 $mg.L^{-1}$ Ca^{2+}; GS200: granular sludge with addiction of 200 $mg.L^{-1}$ Ca^{2+}.

The granular aerobic sludge in R2, R3, and R4 achieved similar effectiveness compared to the flocculent sludge in R1. This agrees with the organic matter removal obtained from SBR in the treatment of recycled paper mill effluent [2].

The addition of calcium to R3 and R4 did not reduce organic matter removal efficiency. No negative effect or reduced microbial activity was observed, demonstrating that the presence of calcium at the concentrations used was inert to biological degradation. Nevertheless, over the long term, the addition of calcium has been reported to increase removal efficiency of organic matter due to improved formation and stability of the granules [21,22].

Physical characteristics of the granules. The physical characteristics of the granules formed in R2, R3, and R4 were evaluated considering diameter size, settling velocity, and mechanical strength (Table 3).

Table 3. Diameter size (mm), settling velocity (Sett vel) and integrity coefficient (Int Coef) of the granules (mean ± standard deviation), n = 5 (settling velocity and integrity coefficient), n = 18 (diameter)

Treatments	Diameter (mm)	Sett vel ($m.h^{-1}$)	Int Coef ×100
Reactor R2 (GS)	11.31 ± 0.80	23.53 ± 6.90	16.97 ± 9.67
Reactor R3 (GS100)	11.16 ± 1.01	32.73 ± 6.25	0 ± 0
Reactor R4 (GS200)	11.33 ± 0.98	37.34 ± 8.53	1.56 ± 2.21

FS: flocculent sludge; GS: granular sludge; GS100: granular sludge with addiction of 100 $mg.L^{-1}$ Ca^{2+}; GS200: granular sludge with addiction of 200 $mg.L^{-1}$ Ca^{2+}.

The addition of calcium did not affect the size of the granules that had average diameters of 11.16 to 11.33 mm, similar in the three SBR R2, R3 and R4. The granules in the three SBR had larger diameters than those observed by other authors with and without the addition of bivalent Ca^{2+} and Mg^{2+} cations [3,7,23–27]. The formation of granules with diameters larger than 10 mm is greater than typical granules reported in the literature that are found to have a range from 0.8 to 4 mm [28–32]. The larger diameter of the granules produced in the present study could be the result of the characteristics of the industrial effluent that contains a significant portion of recalcitrant organic matter (low biodegradability) and by the SBR operational conditions. Compared to small granules, mass transfer and diffusion limitations of large granules are more severe, which can lead to EPS consumption by the cell core of the granules that would weaken the granular structure [33].

The diameter of the granules was greater than 9 mm in all SBR (Figure 2). Reactor R2, without calcium addition, presented 38.9% of the granule diameters between 10 and 11 mm, 27.8%, between 11 and 12 mm, and 27.8% greater than 12 mm. In SBR R3 and R4, with added calcium, granule diameters remained mostly (50% of the observations)

within the range of 11 to 12 mm, presenting a smaller size variation and indicating greater stability. Approximately, 86% of the granules formed in a bioreactor with the addition of Mn^{2+} had diameter size in a range of 2.5 to 3.5 mm, whereas less than 58% of those were within the same range without the addition of Mn^{2+}. Thus, the addition of Mn^{2+} promoted a narrower range and a greater similarity of the particle sizes of the granules [34]. The addition of low concentrations (0-1.05 mM) of a chelating agent, nitrilotriacetate (NTA), reduced the growth rate and homogeneity of the granule size. The results showed that 20% of the granules had diameter equal to 0.5 mm with the addition of 1.05 mM NTA, while 60% had a 0,5 mm diameter in the reactor without NTA [35].

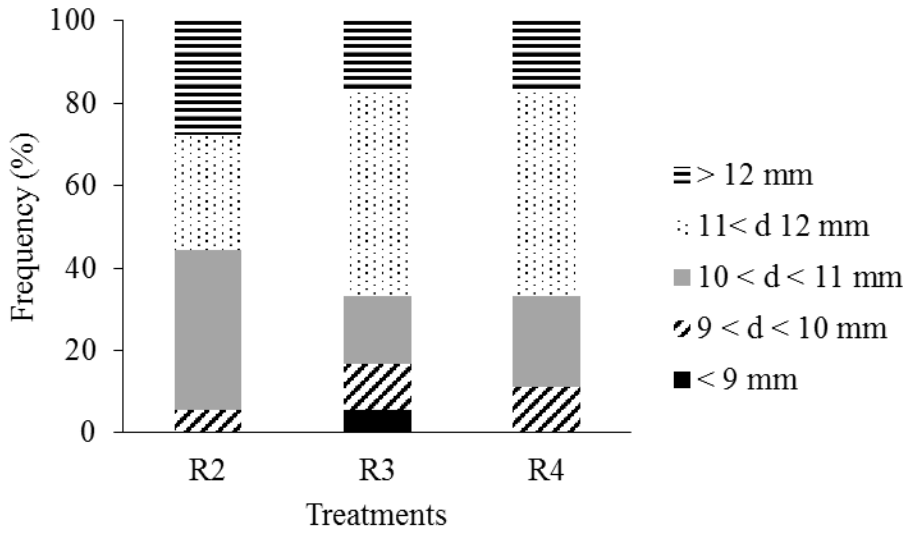


Figure 2. Frequency of the diameter of the granules produced in reactors R2, R3, and R4.

Granules produced in reactors R3 and R4 were more uniform and larger than those in reactor R2, with no added calcium. The granules in reactor R3 were denser and more compact than those from the other reactors (Figure 3). Visually, the granules of reactor R2 had a more fragile appearance than those from reactors R3 and R4.

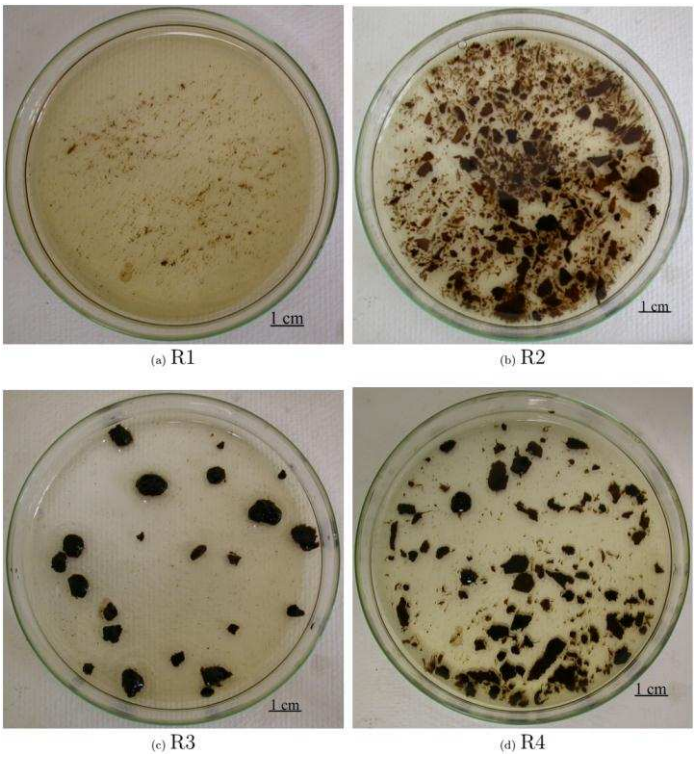


Figure 3. Uniformity and granule size of sludge produced in reactors R1, R2, R3, and R4.

The average coefficient of integrity of the granules was 16.97; 0.00 and 1.56 in reactors R2, R3, and R4, respectively (Table 3). The resistance of the granules in reactors R3 and R4, with added calcium, was higher than that in reactor R2, considering that the higher the integrity coefficient, the lower the physical strength of the granules [17]. The integrity coefficient of reactor R3 was zero in all the tests, indicating absence of rupture and proving the importance of increasing the resistance of the granules in the presence of calcium. These results is in agreement with the finding that resistance of the granules increased in the presence of 100 $mg.L^{-1}$ of Ca^{2+} due to calcium precipitation within the granules and the increase in polysaccharide content, forming a resistant core [36].

Granular aerobic sludge with high mechanical strength can improve the performance of membrane bioreactors (MBR). Aerobic granular sludge in MBR increases

membrane fouling due to the rupture of the granules undergoing transmembrane pressure [2]. Thus, the addition of calcium in MBR with aerobic granular sludge may reduce the fouling caused by the granule disruption.

Separation efficiency of the granules from the treated effluent depends on their sedimentation rate. The sedimentation rate in reactors R2, R3, and R4 was 23.53; 32.73, and 37.34 $m.h^{-1}$, respectively (Table 3) showing a 36% and 59% increase in the settling velocity of the granules in reactors R3 and R4 with the addition of calcium compared to that of reactor R2 without added calcium. Positive bivalent and trivalent ions can bind to negatively charged cells to form microbial nuclei, improving settling and strength properties [36].

Sedimentation rates between 24.2 and 36.4 $m.h^{-1}$, similar to those in reactors R2, R3 and R4 were reported for granules with diameters between 1.2 and 1.8 mm [37]. Velocities higher than 40 $m.h^{-1}$ were reported in granules with added calcium or magnesium [5], much greater than values reported for flocculent sludge, from 3 to 5 $m.h^{-1}$ [38].

Specific growth rate of aggregates. Growth of granules was initially slow (lag phase), followed by a more marked growth phase (log phase) until stabilizing at a steady state condition. The particle diameter was initially 0.6 to 0.8 mm, gradually increasing to values greater than 11 mm after equilibrium. These values allowed the calculation of the growth rate, which presented higher values in reactor R2 than in reactors R3 and R4 (Table 4).

Table 4. Specific growth rate (μ) of the granules produced in reactors R2, R3, and R4

Treatments	D_0 (mm)	D (mm)	D_{eq} (mm)	μ ($10^{-3}.d^{-1}$)
Reactor R2 (GS)	0.61	1.26	11.31	2.09
Reactor R3 (GS100)	0.78	1.38	11.16	1.99
Reactor R4 (GS200)	0.75	1.23	11.33	1.55

Size of microbial aggregates at lag phase (D_0), exponential (D) and at equilibrium (D_{eq}); GS: granular sludge; GS100: granular sludge with addiction of 100 $mg.L^{-1}$ Ca^{2+}; GS200: granular sludge with addiction of 200 $mg.L^{-1}$ Ca^{2+}.

The lower growth rates of the granules observed in reactors R3 and R4 are important and may be related to the presence of calcium. Because of the lower growth rate, the mechanical strength of the granules was higher. High growth rates are related to a rapid proliferation of microorganisms, increasing the granule size and deteriorating granule structure and density [36]. In other study, heterotrophic and autotrophic bacteria in aerobic granules had specific growth rates of 3.18 and 1.52 d^{-1}, respectively. The fast growing heterotrophs contribute to an increase of EPS, which is important for the maintenance of the integrity and stability of the structure of the matured granules [39]. The size of granules and their specific growth rates decreased from 2 to 0.45 mm and from 0.085 to 0.065 d -1, respectively, due to the increased ratio of nitrogen to COD (N/COD). The N/COD ratio contributes to the microbiological selection in the granules and increases the population of nitrifying bacteria that have a slower growth rate than that of heterotrophic bacteria [16].

Extracellular polymeric substances (EPS). The amount of EPS produced by the sludge in each reactor was determined and polysaccharide (PS) and protein (PN) content and the PS/PN ratio were quantified (Table 7).

Table 7. Polysaccharides (PS), proteins (PN) and PS/PN ratio in extracellular polymeric substances (mg g^{-1}VSS) of reactors R1, R2, R3 and R4

Treatments	PS	PN	PS/PN
Reactor R1	61	86	0.71
Reactor R2	105	179	0.59
Reactor R3	91	60	1.53
Reactor R4	80	70	1.14

The PS production in the systems with granular sludge in reactors R2, R3, and R4, was higher than in reactor R1 with flocculent sludge (Table 7). A large amount of PS facilitates adhesion between cells and strengthens the microbial structure by forming a strong, thick polymer matrix. The greater the microbial activity, the higher the PS, which increases initial granule resistance [40]. The higher PS content and the PS/PN>1 ratio of the granules formed in reactors R3 and R4 may have contributed to the higher sedimentation rate of these granules in relation to the granules formed in reactor R2, since amount of EPS, especially polysaccharides, showed a positive correlation with the sedimentability of the sludge [41,42].

The amount of protein was higher in the reactor with granular sludge without added calcium, in reactor R2. In addition, the specific growth rate of the granules was higher in reactor R2 (Table 4). It has been previously been shown that the increase in particle size is faster with high protein content [7].

The total amount of EPS (PS + PN) was higher in reactors R2, R3, and R4, with granular sludge, than in reactor R1, with flocculent sludge. EPS influences granulation by changing surface characteristics such as surface charge and hydrophobicity. A lower surface charge and greater hydrophobicity favors cell aggregation [43]. Reactor R2 produced about 90% more EPS (PS + PN) than the others and had a higher growth rate of the granules (Table 4). A large amount of EPS facilitates adhesion between cells due to the presence of functional groups such as hydroxyl, carboxyl and starch in the

molecules of the extracellular polymer substances, rapidly increasing the size of the granules [7].

Conclusions

The addition of calcium improved the physical characteristics of aerobic granular sludge, but did not affect the efficiency of removal of organic matter in the reactors. The addition of 100 and 200 $mg.L^{-1}$ of Ca^{2+} increased the settling velocity of the granules formed with calcium addition and its resistance, decreasing the integrity coefficient by 36% and 59%, respectively. The mean diameter of the granules was similar in all granular sludge reactors, but varied less in reactors with added calcium. The higher settling velocity and mechanical strength and the high organic matter removal efficiency achieved in the reactor with 100 $mg.L^{-1}$ of Ca^{2+} added guaranteed greater stability of the bleached kraft pulp mill effluent through the treatment process using granular aerobic sludge.

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References

1. Chen, Y.-Y., Pan, X., Li, J. & Lee, D.-J. Strengthening aerobic granule by salt precipitation. Bioresour. Technol. **218,** 1253–1256 (2016).
2. Morais, I. L. H., Silva, C. M. & Borges, C. P. Aerobic granular sludge to treat paper mill effluent: organic matter removal and sludge filterability. Desalin. Water Treat. **57,** 8119–8126 (2016).
3. Yu, H. Q., Tay, J. H. & Fang, H. H. P. The roles of calcium in sludge granulation during UASB reactor start-up. Water Res. **35,** 1052–1060 (2001).
4. Ren, N., Xie, T. & Xing, D. Composition of extracellular polymeric substances influences the autoaggregation capability of hydrogen-producing bacterium Ethanoligenens harbinense. Bioresour. Technol. **100,** 5109–5113 (2009).
5. Sajjad, M. & Kim, K. S. Influence of Mg2+ catalyzed granular sludge on flux sustainability in a sequencing batch membrane bioreactor system. Chem. Eng. J. **281,** 404–410 (2015).
6. Sobeck, D. C. & Higgins, M. J. Examination of three theories for mechanisms of cation-induced bioflocculation. Water Res. **36,** 527–538 (2002).
7. Sajjad, M. & Kim, K. S. Studies on the interactions of Ca2+ and Mg2+ with EPS and their role in determining the physicochemical characteristics of granular sludges in SBR system. Process Biochem. **50,** 966–972 (2015).
8. Cail, R. G. & Barford, J. P. The development of granulation in an upflow floc digester and an upflow anaerobic sludge blanket digest treating cane juice stillage. Biotechnol. Lett. **7,** 493–498 (1985).
9. Dolfing, J., Griffioen, A., van Neerven, A. R. W. & Zevenhuizen, L. P. T. M. Chemical and bacteriological composition of granular methanogenic sludge. Can. J. Microbiol. **31,** 744–750 (1985).
10. Mahoney, E. M., Varangu, L. K., Cairns, W. L., Kosaric, N. & Murray, R. G. E. The effect of calcium on microbial aggregation during UASB reactor start-up. Water Sci. Technol. **19,** 249–260 (1987).
11. Thiele, J. H., Wu, W. M., Jain, M. K. & Zeikus, J. G. Ecoengineering high rate biomethanation system: design of improved syntrophic biomethanation catalysts. Biotechnol. Bioeng. **35,** 990–999 (1990).
12. APHA. Standard methods for the examination of water and wastewater. Standard Methods (2012). doi:10.2105/AJPH.51.6.940-a
13. TAPPI. Determination of sodium, calcium, copper, iron and manganese in pulp and paper by atomic absorption spectroscopy. (2006).
14. Lochmatter, S., Gonzalez-Gil, G. & Holliger, C. Optimized aeration strategies for nitrogen and phosphorus removal with aerobic granular sludge. Water Res. **47,** 6187–6197 (2013).
15. Rasband, W. S. ImageJ. National Institutes of Health, Bethesda, Maryland, USA http://imagej.nih.gov/ij/ (1997). at <http://imagej.nih.gov/ij/>
16. Yang, S. F., Liu, Q. S., Tay, J. H. & Liu, Y. Growth kinetics of aerobic granules developed in sequencing batch reactors. Lett. Appl. Microbiol. **38,** 106–112 (2004).
17. Ghangrekar, M. M., Asolekar, S. R. & Joshi, S. G. Characteristics of sludge developed under different loading conditions during UASB reactor start-up and granulation. Water Res. **39,** 1123–1133 (2005).
18. Ketep, S. F., Fourest, E. & Bergel, A. Bioresource Technology Experimental and theoretical characterization of microbial bioanodes formed in pulp and paper mill

effluent in electrochemically controlled conditions. Bioresour. Technol. **149,** 117–125 (2013).
19. Patel, A., Arora, N., Pruthi, V. & Pruthi, P. A. Biological treatment of pulp and paper industry effluent by oleaginous yeast integrated with production of biodiesel as sustainable transportation fuel. J. Clean. Prod. **xxx,** 1–7 (2016).
20. Kamali, M. & Khodaparast, Z. Review on recent developments on pulp and paper mill wastewater treatment. Ecotoxicol. Environ. Saf. **114,** 326–342 (2015).
21. Ding, A. et al. Effect of calcium addition on sludge properties and membrane fouling potential of the membrane-coupled expanded granular sludge bed process. J. Memb. Sci. **489,** 55–63 (2015).
22. Ye, C., Yang, X., Zhao, F. J. & Ren, L. The shift of the microbial community in activated sludge with calcium treatment and its implication to sludge settleability. Bioresour. Technol. **207,** 11–18 (2016).
23. Adav, S. S., Lee, D. J. & Lai, J. Y. Effects of aeration intensity on formation of phenol-fed aerobic granules and extracellular polymeric substances. Appl. Microbiol. Biotechnol. **77,** 175–182 (2007).
24. Adav, S. S., Lee, D.-J. & Lai, J.-Y. Potential cause of aerobic granular sludge breakdown at high organic loading rates. Appl. Microbiol. Biotechnol. **85,** 1601–1610 (2010).
25. Tay, J. H., Yang, P., Zhuang, W. Q., Tay, S. T. L. & Pan, Z. H. Reactor performance and membrane filtration in aerobic granular sludge membrane bioreactor. J. Memb. Sci. **304,** 24–32 (2007).
26. Zitomer, D. H., Duran, M., Albert, R. & Guven, E. Thermophilic aerobic granular biomass for enhanced settleability. Water Res. **41,** 819–825 (2007).
27. Wan, C. et al. Calcium precipitate induced aerobic granulation. Bioresour. Technol. **176,** 32–37 (2015).
28. Liu, Y. Q. & Tay, J. H. Fast formation of aerobic granules by combining strong hydraulic selection pressure with overstressed organic loading rate. Water Res. **80,** 256–266 (2015).
29. Long, B. et al. Rapid cultivation of aerobic granular sludge in a pilot scale sequencing batch reactor. Bioresour. Technol. **166,** 57–63 (2014).
30. Long, B. et al. Rapid cultivation of aerobic granule for the treatment of solvent recovery raffinate in a bench scale sequencing batch reactor. Sep. Purif. Technol. **160,** 1–10 (2016).
31. Pronk, M. et al. Full scale performance of the aerobic granular sludge process for sewage treatment. Water Res. **84,** 207–217 (2015).
32. Yan, L. et al. Role and significance of extracellular polymeric substances from granular sludge for simultaneous removal of organic matter and ammonia nitrogen. Bioresour. Technol. **179,** 460–466 (2015).
33. Zhang, C., Zhang, H. & Yang, F. Diameter control and stability maintenance of aerobic granular sludge in an A/O/A SBR. Sep. Purif. Technol. **149,** 362–369 (2015).
34. Huang, L., Yang, T., Wang, W., Zhang, B. & Sun, Y. Effect of Mn2+ augmentation on reinforcing aerobic sludge granulation in a sequencing batch reactor. Appl. Microbiol. Biotechnol. **93,** 2615–2623 (2012).
35. Nancharaiah, Y. V., Joshi, H. M., Krishna Mohan, T. V., Venugopalan, V. P. & Narasimhan, S. V. Formation of aerobic granules in the presence of a synthetic chelating agent. Environ. Pollut. **153,** 37–43 (2008).

36. Lee, D. J., Chen, Y. Y., Show, K. Y., Whiteley, C. G. & Tay, J. H. Advances in aerobic granule formation and granule stability in the course of storage and reactor operation. Biotechnol. Adv. **28,** 919–934 (2010).
37. Liu, L., Wang, Z., Yao, J., Sun, X. & Cai, W. Investigation on the properties and kinetics of glucose-fed aerobic granular sludge. Enzyme Microb. Technol. **36,** 307–313 (2005).
38. Wang, Z. W., Liu, Y. & Tay, J. H. The role of SBR mixed liquor volume exchange ratio in aerobic granulation. Chemosphere **62,** 767–771 (2006).
39. Cui, F., Park, S., Kim, M. & CUI, F.; PARK, S.; KIM, M. Characteristics of aerobic granulation at mesophilic temperatures in wastewater treatment. Bioresour. Technol. **151,** 78–84 (2013).
40. Cao, X., Sheng, Y., Cao, H. & Zhang, Y. Comparison of Mg2+- and Ca2+-enhancing anaerobic granulation in an expanded granular sludge-bed reactor. Sci. China Chem. **57,** 1596–1601 (2014).
41. Chen, H., Zhou, S. & Li, T. Impact of extracellular polymeric substances on the settlement ability of aerobic granular sludge. Environ. Technol. **31,** 1601–1612 (2010).
42. Kim, K. S., Sajjad, M., Lee, J., Park, J. & Jun, T. Variation of extracellular polymeric substances (EPS) and specific resistance to filtration in sludge granulation process to the change of influent organic loading rate. Desalin. Water Treat. **52,** 4376–4387 (2014).
43. Zhu, L. et al. Specific component comparison of extracellular polymeric substances (EPS) in flocs and granular sludge using EEM and SDS-PAGE. Chemosphere **121,** 26–32 (2015).

Figure Legends

Figure 1. Experimental plan

Figure 2. Frequency of the diameter of the granules produced in reactors R2, R3, and R4.

Figure 3. Uniformity and granule size of sludge produced in reactors R1, R2, R3, and R4.

CAPÍTULO 3

Identificação dos microrganismos favoráveis à formação de grânulos aeróbios mesofílicos presentes no tratamento biológico de efluentes de fábrica de papel

RESUMO

Os grânulos aeróbios são grandes e compactos agregados microbianos que podem ser utilizados no tratamento de águas residuárias. A adoção de lodo aeróbio granular em biorreatores para o tratamento de efluentes é ainda considerada uma inovação e tem sido tema de diversas pesquisas recentes. No presente trabalho, dezenove isolados microbianos, obtidos em estudo realizado anteriormente, provenientes de grânulos aeróbios mesofílicos foram avaliados em testes de co-agregação e determinação das substâncias poliméricas extracelulares (SPE). Buscou-se avaliar a relação entre a quantidade de SPE produzida e a contribuição do isolado no processo de granulação. Os resultados indicaram que seis isolados dos gêneros Staphylococcus, Agrobacterium, Enterobacter e Rhodococcus foram considerados como efetivos para a produção e estabilidade dos grânulos aeróbios mesofílicos. A ausência destes isolados provocou um aumento da quantidade de carboidratos e uma redução da quantidade de ácidos húmicos e da relação proteína/polissacarídeo (PN/PS). A relação PN/PS está relacionada com a hidrofobicidade do lodo granular e a sua redução prejudicou a formação de grânulos.

1. INTRODUÇÃO

Novas tecnologias têm ampliado a eficiência e reduzido custos do tratamento de efluentes. O desenvolvimento do tratamento com lodo aeróbio granular tem demonstrado que o processo pode apresentar elevadas eficiências de remoção de matéria orgânica e nutrientes. Comparado ao lodo floculento do sistema de lodos ativados convencional, o maior tamanho, a estrutura compacta e a alta retenção de biomassa do lodo aeróbio granular implicam em uma maior velocidade de sedimentação e filtrabilidade do lodo granular, além de alta tolerância à cargas orgânicas e toxicidade (LI, YONGMEI et al., 2014; MORAIS; SILVA; BORGES, 2016; TAY et al., 2002; TOH et al., 2003).

A produção e a ação das substâncias poliméricas extracelulares (SPE) são importantes para determinar as características dos agregados microbianos nos processos

de tratamento de efluentes (TAY et al., 2002). São essenciais para a formação de agregados microbianos, uma vez que as bactérias ficam dispersas em uma matriz de SPE. Tais substâncias possuem função estrutural e protetora nos agregados e afetam a porosidade, a densidade, a carga elétrica, a hidrofobicidade, a estabilidade mecânica, além de outras propriedades dos biofilmes (LIU; LIU; TAY, 2004).

As SPE são macromoléculas orgânicas secretadas por bactérias sob certas condições ambientais, compostas principalmente por proteínas, polissacarídeos, ácidos húmicos, ácidos nucléicos e lipídios (GAO et al., 2011). A composição das SPE pode afetar a formação e as características dos grânulos aeróbios. A compactação do lodo granular é obtida tanto pelo aumento do teor de proteínas quanto de polissacarídeos dos SPE (CAUDAN et al., 2014). A resistência dos grânulos depende das condições de crescimento e do tipo de substrato. Grânulos mais densos são formados em consequência do crescimento de microrganismos com baixa taxa de crescimento e devido a interações específicas envolvendo as SPE da matriz do grânulo (CAUDAN et al., 2014).

Os grânulos aeróbios podem levar semanas para serem formados a partir de lodos ativados floculentos. Similar a outros tratamentos, a formação de grânulos pode ser obtida por meio da seleção de culturas microbianas. A inoculação de alguns tipos de microrganismos ao biorreator pode contribuir para o aumento da velocidade de formação e da resistência mecânica dos grânulos (ADAV, LEE E LAI, 2010; IVANOV et al., 2006; WAN et al., 2015). A mistura de uma cultura pura com elevada capacidade auto-agregação com o lodo de um sistema de lodos ativados, resultou na formação de grânulos aeróbios com diâmetro médio de 446 µm em apenas 8 dias (IVANOV et al., 2006). Bactérias do gênero Sphingomonas sp. estavam presentes em grande quantidade no início da granulação em reatores em batelada sequenciais (RBS) alimentados com efluente sintético e contribuíram para a manutenção da estrutura e função dos grânulos (WAN et al., 2016).

Interações físicas entre bactérias agregadas facilitam interações metabólicas, comunicação intercelular e trocas genéticas (MASZENAN, LIU E NG, 2011). Estudos sugerem que as características físicas dos grânulos são explicadas pela diversidade das comunidades microbianas e da composição das SPE associadas a estas comunidades (CAUDAN et al., 2014).

Em um trabalho prévio, determinou-se os tipos de microrganismos encontrados no lodo aeróbio granular que contribuiram para a agregação microbiana e aumento da resistência mecânica dos grânulos. Dezenove linhagens foram isoladas de um lodo granular aeróbio formado em um RBS que tratava efluente de fábrica de papel. A

formação dos granulos foi obtida a partir da seleção microbiana. Os testes de co-agregação indicaram que alguns isolados (2, 7, 9, 13, e 25) melhoraram a formação dos granulos, enquanto outros (10, 14, 18, e 26) inibiram a granulação (MORAIS et al., 2016).

O objetivo deste trabalho foi quantificar as substâncias poliméricas extracelulares produzidas pelos consórcios entre os microrganismos isolados no trabalho de Morais et al. (2016) envolvidos no processo de granulação aeróbia e verificar a relação de SPE com a resistência e estabilidade dos grânulos. Os microrganismos presentes nos grânulos foram identificados.

2. METODOLOGIA

2.1. OBTENÇÃO DOS GRÂNULOS AERÓBIOS E DOS ISOLADOS

Os grânulos aeróbios mesofílicos foram obtidos em um reator em batelada sequencial (RBS), alimentado com efluente de fábrica de papel reciclado (MORAIS et al., 2016).

Alíquotas de 5 mL de lodo granular foram submetidas a três etapas de centrifugação a 650 g por 2 minutos. Após cada centrifugação, o sobrenadante foi descartado e o sedimentado re-suspenso em solução salina 0,85%. Ao final da terceira centrifugação as amostras foram submetidas a pulsos de ultra-som (20 kHz, 4 s) para a desagregação do lodo, utilizando-se o Ultrasonic homogenizer (Cole Palmer Instrument Company 4710 series, Chicago).

As amostras desagregadas por ultra-som foram centrifugadas (650 g, 2 min) e retiradas alíquotas de 100 µL do sobrenadante para obtenção de diluições seriadas de 10^{-1} a 10^{-9}.

Amostras de 100 µL de cada diluição, foram inoculados em placas de Petri contendo meio R2A sólido (0,5 g de caseína hidrolisada; 0,5 g de extrato de levedura; 0,5 g de peptona; 0,5 g de dextrose; 0,3 g de fosfato dipotássico; 0,024 g de sulfato de magnésio; 0,3 g de piruvato de sódio, 15,0 g de ágar e 1000 mL de água destilada). As placas foram mantidas a temperatura ambiente e, após o surgimento das colônias, realizou-se a repicagem para placas de meio R2A sólido para obtenção de culturas puras. Cada cultura pura foi inoculada em meio R2A líquido e estocada em ultra-freezer a -80 °C.

2.2. INDICE DE CO-AGREGAÇÃO COM AUSÊNCIA DE UM ISOLADO

Foram realizados 20 ensaios de co-agregação a partir de consórcios dos isolados, um contendo todos os isolados e outras 19 combinações faltando um dos isolados em cada uma delas, para verificar a influência de cada isolado obtido na formação dos grânulos. O consórcio com todos os isolados foi denominado controle e os demais foram identificados por "-n" que se refere ao isolado ausente no consórcio. Por exemplo, o consórcio "-1" significa a combinação de 18 isolados na ausência do isolado 1, e assim por diante.

Cada isolado foi inoculado, inicialmente, em meio R2A líquido e incubado a 30 $\pm$ 1 ºC, sob agitação (110 $\pm$ 10 rpm), por 48 horas.

Foram inoculadas alíquotas das culturas líquidas de cada isolado com densidade ótica (DO) a 600 nm igual a 1,00 mL^{-1} em erlenmeyers de 250 mL contendo 75 mL de meio R2A líquido. Os erlenmeyers foram incubados a 30 $\pm$ 1 ºC, sob agitação (110 $\pm$ 10 rpm) e foram retiradas 6 amostras de 5 mL de cada erlenmeyer após 2, 4, 8, 24, 48 e 72 horas do início da incubação. As amostras foram submetidas à leitura de DO (600 nm) e armazenadas para extração e análise das substâncias poliméricas extracelulares (SPE).

2.3. EXTRAÇÃO E ANÁLISE DAS SPE

Foi transferido 1 mL de cada amostra para microtubos (2,0 mL), centrifugados (11200 g, 4 ºC, 15 min) e o sobrenadante foi armazenado em outro microtubo para quantificação das SPE livres. Os pellets foram re-suspensos em 20 mL de tampão fosfato, o pH foi corrigido para 11, por meio da adição de NaOH 1M, seguido de aquecimento em banho-maria (80 ºC, 30 minutos). Após essa etapa de extração, as amostras foram centrifugadas (11200 g, 4 ºC, 10 min) e o sobrenadante armazenado para posterior análise das SPE ligadas (MCSWAIN et al., 2005).

A caracterização química das substâncias poliméricas extracelulares produzidas foi realizada por meio das análises dos conteúdos de carboidratos, proteínas, ácidos húmicos e carbono orgânico total (COT).

2.3.1. **Quantificação de carboidratos**

Utilizou-se o método de dosagem fenol-ácido sulfúrico, no qual foram adicionados 0,25 mL de fenol (5%) e 1,25 mL de ácido sulfúrico em 0,5 mL de cada amostra. Após 10 minutos, os tubos foram agitados e colocados em banho de gelo por 20

minutos. Foi utilizada como padrão a curva de calibração utilizando sacarose (0 - 1,0 $mg.mL^{-1}$). As leituras das absorbâncias foram realizadas em espectrofotômetro a 490 nm (ALBALASMEH; BERHE; GHEZZEHEI, 2013).

2.3.2. **Quantificação de proteínas e ácidos húmicos**

Utilizou-se o método de Lowry modificado (FRØLUND et al., 1996), no qual é necessário o preparo de dois reagentes (A e B) para as reações. O reagente A foi produzido pela adição de 3 soluções, na proporção de 1:1:100 (10- 35 g de $CuSO_4$. L^{-1} de água; 20-70 g de $KNaC_4H_4O_6.4H_2O.L^{-1}$ de água e 3- 70 g de Na_2CO_3 .L^{-1} de NaOH 0,35N). O reagente B foi produzido com composição semelhante à do reagente A exceto pela substituição da solução de $CuSO_4$ por água deionizada. Nas reações foi utilizado também o reagente Folin Ciocalteau (2N) diluído 10 vezes.

Os reagentes foram adicionados na sequência Amostra:Reagente A:Reagente Folin, na proporção 1:1:1. Agitou-se a mistura e após 30 minutos realizou-se as leituras em espectrofotômetro a 750 nm. Realizou-se o mesmo procedimento com o Reagente B. Foi utilizada como padrão uma curva de calibração de 0-1,0 $mg.mL^{-1}$ de albumina.

Para a determinação das proteínas presentes utilizou-se a seguinte formula:

$$\mathbf{Abs_{proteínas} = 1{,}25 \cdot (Abs_A - Abs_B)}$$

A determinação de ácidos húmicos presentes nas amostras foi estimada utilizando a fórmula:

$$\mathbf{Abs_{ácidos\ húmicos} = Abs_B - (0{,}2 \cdot Abs_{proteínas})}$$

2.3.3. **Determinação de Carbono Orgânico Total (COT)**

O COT foi determinado pelo método da oxidação catalítica (XUAN et al., 2010) por meio de um analizador de carbono (TOC-V_{cph}, Shimadzu®, Quioto).

2.4. IDENTIFICAÇÃO DOS ISOLADOS OBTIDOS NA FORMAÇÃO DOS GRÂNULOS

2.4.1. Extração do DNA

Os isolados foram inoculados em microtubos (2,0 mL) contendo 1 mL de meio TY líquido (5 g de triptona, 3 g de extrato de levedura, 0,9 g de cloreto de cálcio dihidratado e 1000 mL de água destilada). Foi efetuada a extração do DNA, após 24 horas de incubação, conforme metodologia descrita por Kennedy et al., (2008), com modificações. Foi adicionado 1 mL do tampão de lise (EDTA 100 mM, Tris–HCL 100

mM, CTAB 2%, SDS 1% e NaCl 1,5 M) a cada cultura. A mistura reacional foi homogeneizada em vortex e aquecida em banho-maria a 65 ℃ por 20 min (com inversão dos tubos a cada 5 min). Em seguida, a mistura foi centrifugada por 20 min a 2800 g. A fase aquosa foi transferida para outro microtubo (2,0 mL) e adicionou-se a solução fenol:clorofórmio:álcool isoamílico (25:24:1) no mesmo volume do coletado e homogenizou-se em vortex, por 1 min, seguido de centrifugação por 10 min a 2800 g. O sobrenadante foi transferido para outro microtubo (2,0 mL) e adicionou-se 0,7 volume de isopropanol (100%) e 0,1 volume de acetato de sódio 3 M, inverteu-se os tubos suavemente (10 vezes) e incubou-se a -20 ºC durante aproximadamente 12 horas. Os tubos foram centrifugados por 10 min a 2800 g, descartando o sobrenadante, seguido da lavagem do pellet com álcool 70% e nova centrifugação, por duas vezes. O pellet foi deixado secar e então re-suspenso em 40 µL de água ultrapura.

2.4.2. **Amplificação de DNA**

O DNA de cada isolado foi amplificado utilizando os primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) e 1525R (5′-AAGGAGGTGATCCAGCC-3′). A mistura reacional do PCRcontinha 0,3 µL de Taq DNA polymerase (Invitrogen, Carlsbad, CA, USA), 0,5 µl de cada primer (10 µM), 2,5 µL do tampão de reação 10X, 0,5 µL de dNTP's (10 mM), 1,5 µL $MgCl_2$ (50 mM), 1 µL da amostra de DNA e 18,2 µL de água ultrapura, por amostra. A amplificação foi realizada em um termociclador (Mastercycler Personal; Eppendorf, Hamburg, Germany) sob as condições: 94°C por 5 min, seguido por 14 ciclos (94 °C por 30s, 65 °C por 40s e 72 °C por 1 min), mais 15 ciclos (94 °C por 30s, 50 °C por 40s e 72 °C por 1 min); e a etapa de extensão a 72°C por 7 min.

Após essa etapa, os amplicons foram sequenciados no ACTGene Laboratory (Centro de Biotecnologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brasil) em um ABI-Prism 3100 Genetic Analyzer com capilares de 50 cm e polímero POP6 (Applied Biosystems, Foster City, CA, USA).

Os dados obtidos após o sequenciamento foram tratados através dos programas MEGA (V. 6.0) e Bioedit, os contigues obtidos foram submetidos ao BLAST (NCBI, 2015) para o processo de identificação dos isolados.

3. RESULTADOS E DISCUSSÃO

3.1. ENSAIO DE CO-AGREGAÇÃO - DENSIDADE ÓTICA DOS CONSÓRCIOS

O crescimento das células nos consórcios foi monitorado pela densidade ótica durante 72 horas. Os consórcios apresentaram alterações mínimas na densidade ótica (Figura 1) após 24 horas, evidenciando a fase estacionária e indicando que, durante esse período, houve a produção/liberação das SPE que favorecem a formação de grânulos.

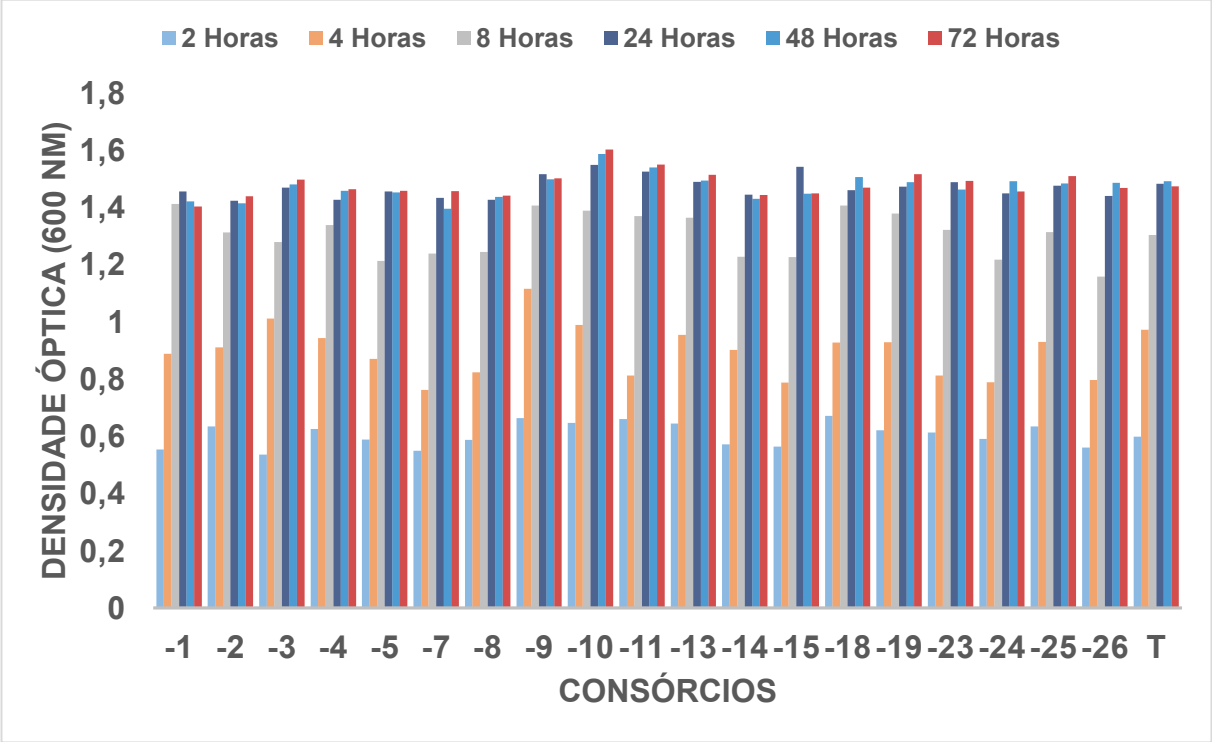

Figura 1. Densidade óptica (600 nm) das amostras coletadas de cada consórcio, em diferentes intervalos de tempo (horas), durante experimento de co-agregação (-n=consórcio com ausência do n-ésimo isolado, T = consórcio controle com todos os isolados).

Os valores de densidade ótica (DO) foram similares ao longo do tempo entre os consórcios. Houve um aumento gradual da DO ao longo do monitoramento até sua estabilização em 1,5, aproximadamente, após 24 horas (Figura 1). Dessa forma, a ausência de um dos isolados não afetou o crescimento microbiano dos consórcios.

A medição da densidade ótica é um método rápido e confiável, mas baseia-se na suposição de que as bactérias crescem como células únicas de igual tamanho e que as células são dispersas uniformemente na cultura líquida (HAABER et al., 2016). No caso de culturas formando aglomerados de células com uma estrutura em três dimensões, a

correlação entre os dados de densidade óptica e o número de células deve ser verificada, uma vez que parte da biomassa pode cobrir camadas internas de células (DE CARVALHO et al., 2005). Outros desafios que podem potencialmente afetar a medição da densidade óptica incluem células viáveis mas não cultiváveis ou células não viáveis mas intactas presentes na cultura, bem como células no processo de divisão (HAABER et al., 2016).

Em um biorreator, a densidade ótica pode se correlacionar com a concentração de sólidos suspensos voláteis no tanque de aeração (SSVTA). A DO acompanhou as variações dos SSVTA durante o processo de formação de grânulos aeróbios em um RBS para o tratamento de 2,4-diclorofenol (KHAN; KHAN; SABIR, 2011). Além disso, a presença de substâncias tóxicas inibe o crescimento e reduz a DO. A adição de 2 $mg.L^{-1}$ e $3mg.L^{-1}$ de Cr^{6+} provocou uma redução na DO de 1,0 para 0,8 e 0,3, respectivamente (YANG et al., 2016), enquanto que a adição de 3,5 mM de dodecil sulfato de sódio causou uma diminuição da DO de 1 para 0,2 em apenas 30 minutos de incubação (KLEBENSBERGER et al., 2006).

3.2. DETERMINAÇÃO DAS SPE

3.2.1. Quantificação de carboidratos

Os consórcios com ausência dos isolados que prejudicam a granulação (-4, -10, -14, -18 e -26) apresentaram, de maneira geral, uma redução da produção de carboidratos após 48 h, enquanto os consórcios com ausência dos isolados que contribuem para a formação de agregados (-2, -7, -9, -13, -19 e -25) mantiveram ou aumentaram a produção de carboidratos após 48 h levando a uma maior quantidade de carboidratos em relação ao controle (Figura 2). Além disso, o consórcio controle (T) apresentou baixa produção de carboidratos em comparação aos demais, 0,3 $mg.mL^{-1}$ e 0,16 $mg.mL^{-1}$ nas medições realizadas em 48 h e 72 h, respectivamente.

Os carboidratos são componentes hidrofílicos dos SPE e o excesso de carboidratos (principalmente se aliado à baixa relação proteína/carboidrato) resulta em um aumento do potencial zeta, prejudicando a formação de grânulos (TU et al., 2010). Dessa forma, os isolados indicados como prejudiciais ao processo de granulação podem ter reduzido a formação de agregados pela sua maior produção de carboidratos.

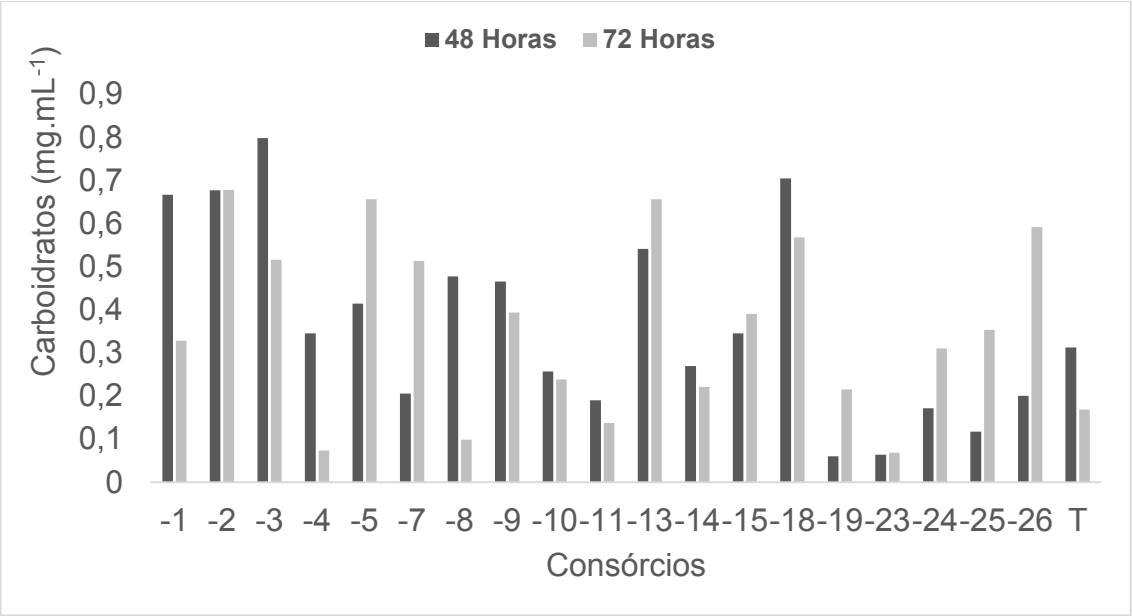

Figura 2. Quantificação de Carboidratos (mg.mL^{-1}) nas substâncias poliméricas extracelulares extraídas das amostras coletadas, em diferentes intervalos de tempo (horas), durante experimento de co-agregação (-n=consórcio com ausência do n-ésimo isolado).

De acordo com Seviour et al., (2012), os grânulos aeróbios contêm uma comunidade microbiana multiespecífica, que sintetizam uma grande diversidade de exopolissacarídeos desempenhando diferentes papéis estruturais e distinguem-se dos flocos do processo de lodos ativados convencional pela presença de exopolissacarídeos gelatinosos.

A sedimentabilidade do lodo granular está diretamente relacionada com a quantidade de SPE produzida (KIM et al., 2014). Os polissacarídeos são importantes na etapa de formação dos grânulos, mas a estabilidade dos mesmos depende do núcleo proteico (ZHU et al., 2015). A quantidade de SPE depende, entre outros fatores, da comunidade microbiana presente no grânulo e das condições de operação do reator. A diminuição do tempo de sedimentação do lodo provocou o arraste dos microrganismos de sedimentação lenta e o aumento a comunidade microbiana com boa floculação, o que resultou no aumento rápido dos teores de proteína (PN) e polissacarídeos (PS). No entanto, com a maturação do lodo granular, o teor de PS diminuiu devido ao consumo dos polissacarídeos pelos microrganismos durante a fase de inanição (ZHU et al., 2012).

3.2.2. Quantificação de Proteínas

A produção de proteínas dos consórcios variou entre 0,08 mg.mL^{-1} e 0,35 mg.mL^{-}

[1] e não houve um comportamento regular entre os consórcios na ausência dos isolados que beneficiam ou entre os que prejudicam a formação de agregados (Figura 3). A produção de proteínas nos consórcios -2, -4, -8, -23, -25, -26 e T aumentou entre 48 h e 72 h, indicando que a ausência destes isolados foi benéfica à formação de agregados.

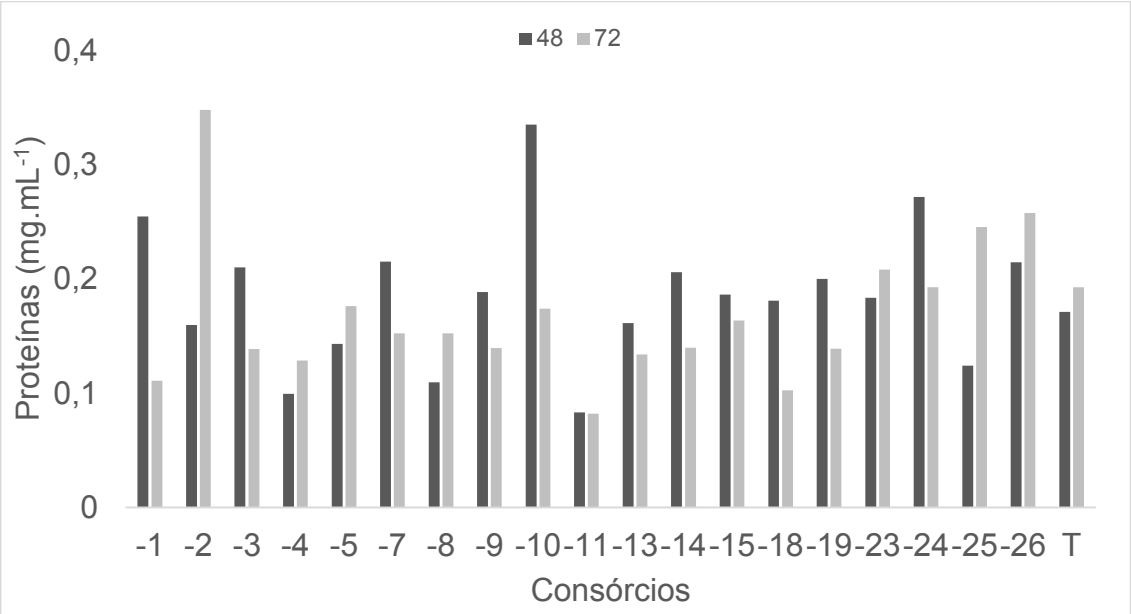

Figura 3. Quantificação de Proteínas ($mg.mL^{-1}$) nas substâncias poliméricas extracelulares extraídas das amostras coletadas, em diferentes intervalos de tempo (horas), durante experimento de co-agregação (-n=consórcio com ausência do n-ésimo isolado).

As proteínas são constituintes hidrofóbicos dos SPE e contribuem para a diminuição do potencial zeta, favorecendo a formação, além de aumentarem a estabilidade estrutural dos grânulos (DI LACONI; RAMADORI; LOPEZ, 2006; MCSWAIN et al., 2005; TU et al., 2010). Zhu et al. (2015) demonstrou que os teores de proteínas de lodos granulares aeróbios e anaeróbios foram maiores do que os de lodos floculentos, apresentando uma correlação linear negativa com a carga superficial do lodo e favorecendo a formação de grânulos.

A falta de uma tendência de aumento ou diminuição da produção de proteínas pelos consórcios dificulta a avaliação da influência deste parâmetro na formação dos agregados. Pode-se avaliar também a relação entre o conteúdo de substâncias nitrogenadas (PN) e polissacarídicas (PS). A relação PN/PS pode influenciar positivamente a hidrofobicidade e o potencial zeta, contribuindo para o aumento da habilidade de agregação (ZHANG et al., 2015). Além disso, grânulos formados com

relação PN/PS menor do que 1 são suscetíveis a desagregação, uma vez que as interações célula-célula são fracas (JIANG; TAY; TAY, 2002; LIU; LIU; TAY, 2004; ZHANG et al., 2015).

Os consórcios -4, -10, -14, -18 e -26, nos quais estavam ausentes os isolados prejudiciais à granulação, apresentaram, com exceção do -26, relação PN/PS ≥ 1 (Tabela 1). Uma maior relação PN/PS contribui para uma maior agregação e maior estabilidade dos agregados (ZHANG et al., 2015).

Tabela 1. Relação PN/PS dos consórcios de isolados

Consórcio	**PN/PS**	**Consórcio**	**PN/PS**
-1	1,79	-13	0,72
-2	0,88	-14	2,38
-3	0,92	-15	1,08
-4	7,14	-18	1,00
-5	0,79	-19	2,56
-7	1,25	-23	1,56
-8	5,56	-24	1,96
-9	1,15	-25	8,33
-10	1,79	-26	0,78
-11	3,45	T	3,25

A relação PN/PS dos grânulos é maior do que a dos flocos (SAJJAD; KIM, 2015; ZHU et al., 2015). Grânulos aeróbios apresentaram maior teor de proteínas (PN/PS entre 1,4 e 1,6) enquanto flocos apresentaram maior teor de polissacarídeos (PN/PS = 0,5) (BASUVARAJ; FEIN; LISS, 2015). As PN possuem ainda maior afinidade por cátions como o Ca^{2+} e o Mn^{2+} e facilitam a ligação das SPE com estes íons, reduzindo a carga superficial e promovendo a adesão celular (ZHU et al., 2015).

3.2.3. Quantificação de ácidos húmicos

A produção de ácidos húmicos (AH) variou entre 0,13 $mg.mL^{-1}$ e 0,53 $mg.mL^{-1}$ (Figura 4).

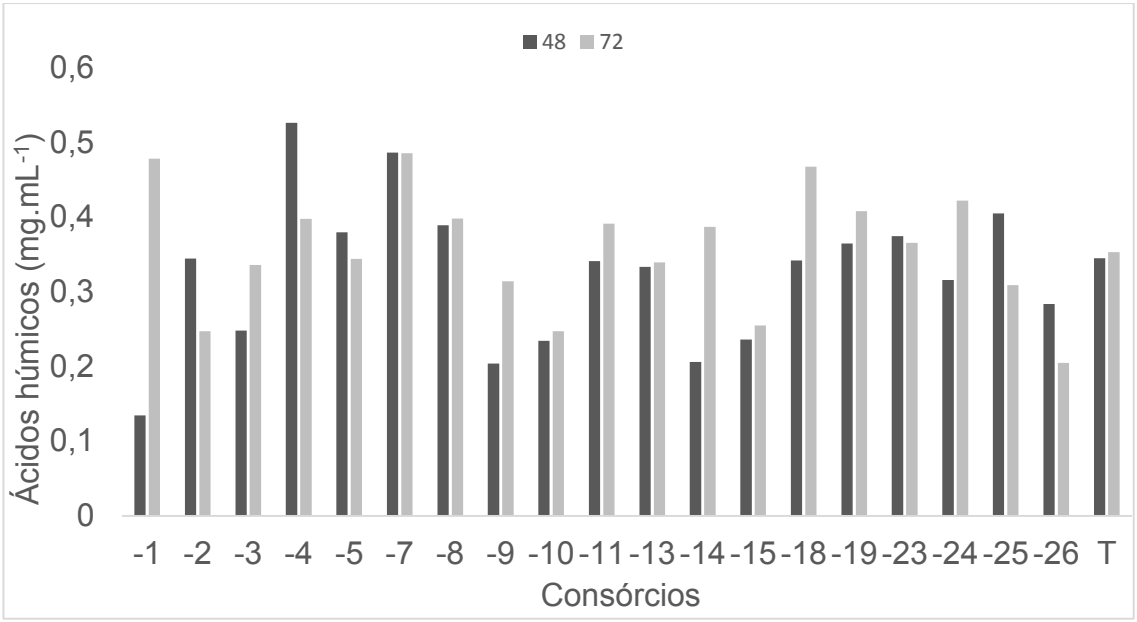

Figura 4. Quantificação de ácidos húmicos ($mg.mL^{-1}$) nas substâncias poliméricas extracelulares, em diferentes intervalos de tempo (horas), durante experimento de co-agregação (-n=consórcio com ausência do n-ésimo isolado).

Dentre os consórcios com ausência dos isolados favoráveis à granulação (-2, -7, -9, -13, -19 e -25), os consórcios -2, -9, -13 e -25 apresentaram menor concentração de ácidos húmicos do que o controle em 72 h, o que indica que a ausência destes isolados pode ser prejudicial à formação de grânulos pela redução da quantidade de AH produzidos. Por outro lado, entre os consórcios com ausência dos isolados prejudiciais à granulação (-4, -10, -14, -18 e -26), os consórcios -4, -14 e -18 produziram maior quantidade de ácidos húmicos do que o controle em 72 h indicando um favorecimento da produção de AH com a ausência destes isolados.

Os ácidos húmicos estão relacionados entre as substâncias poliméricas produzidas durante a formação dos grânulos aeróbios e atuam na estrutura dos grânulos (GAO et al., 2011). Os grânulos aeróbios podem ser considerados um biossorvente eficaz para o tratamento de metais pesados e os ácidos húmicos desempenham um papel importante na sorção destes íons no lodo aeróbio granular (WEI et al., 2016). Wang et al. (2014) observou que o aumento da concentração de Cr^{6+} até 30 $mg.L^{-1}$ resultou em um aumento do teor de substâncias proteicas e ácidos húmicos do lodo aeróbio granular. Além disso, os ácidos húmicos contribuem também para a remoção de compostos tóxicos. O teor de ácidos húmicos aumentou de 134,1 $mg.L^{-1}$ para 138,4 $mg.L^{-1}$ após a exposição do lodo a 20 $mg.L^{-1}$ de 2,6-diclorofenol (LI, KAI et al., 2016).

A concentração de ácidos húmicos pode sofrer pouca influência das condições

operação do reator. A variação da relação DQO/N (igual a 1, 2 e 4) não afetou a quantidade de proteína, ácido fúlvico e ácido húmico do lodo (LUO et al., 2014). O lodo alimentado com maior relação alimento / microrganismo (A/M) melhorou o processo de granulação e produziu mais polissacarídeos (PS) e proteínas (PN) do que o lodo com menor carga de DQO, enquanto as substâncias húmicas e os ácidos urônicos não apresentaram variação (KIM et al., 2014). O aumento da quantidade de ácido húmico e fúlvico pode ser resultante da decomposição de células mortas e de compostos orgânicos macromoleculares, como PN e PS (WANG et al., 2014).

3.3. IDENTIFICAÇÃO DOS ISOLADOS OBTIDOS

Os gêneros de todos os isolados obtidos a partir dos grânulos aeróbios foram identificados (Tabela 2**)**.

Tabela 2. Identificação dos isolados obtidos a partir dos grânulos aeróbios mesofílicos formados durante o tratamento de efluentes de indústria de papel reciclado.

Gênero	Isolados
Acinetobacter	1, 4, 5, 10, 15, 18 e 23
Agrobacterium	2, 13 e 19
----------	3
Enterobacter	7, 8, 11, 14, 24 e 26
Staphylococcus	9
Rhodococcus	25

Os isolados favoráveis à formação dos grânulos (2, 7, 9, 13, 19 e 25) pertencem a quatro dos cinco gêneros identificados (Tabela 3)**.** Bactérias desses quatro gêneros são descritas como produtoras de SPE com características que favorecem a formação de biofilmes e grânulos (LV et al., 2014).

Tabela 3. Identificação dos isolados favoráveis à formação dos grânulos aeróbios obtidos.

Isolado	Identificação
2	Agrobacterium sp.
7	Enterobacter sp.
9	Staphylococcus sp.
13	Agrobacterium sp.
19	Agrobacterium sp.
25	Rhodococcus sp.

Espécies de Enterobacter foram relatadas como produtoras de grande quantidade de polissacarídeos e SPE de adesão intracelular, os quais são substâncias primárias envolvidas diretamente na formação de biofilmes e grânulos (LIMOLI; JONES; WOZNIAK, 2015; TORRES et al., 2012). A curva de crescimento de Enterobacter aerogenes mostrou que a secreção dos SPE era paralela ao crescimento celular, com a máxima liberação de SPE observada na fase estacionária inicial (SALEHIZADEH; YAN, 2014).

Isolados de Agrobacterium sp. foram observados na superfície de grânulos.e geraram grande quantidade de proteína e floculantes. Além disso, foi avaliada a utilização de fósforo na produção de SPE de espécie de Agrobacterium e observou-se que limitação de fósforo aumenta a produção das substâncias que auxiliam na formação de biofilmes e grânulos por espécies desse gênero bacteriano (HUANG et al., 2012).

O Rhodobacter é um gênero bacteriano desnitrificante anaeróbio que também é capaz de secretar SPE para auxiliar a fixação. Estas espécies devem contribuir para a formação de grânulos aeróbios com a sua SPE segregada (LV et al., 2014). As SPE produzidas por espécies de Rhodococcus apresentam propriedades mecânicas, devido a formação de ligações não específicas. Além disso, algumas espécies têm sido amplamente utilizadas em processos de biorremediação para reduzir contaminantes na água e solo (PEN et al., 2015; RODRIGUES et al., 2006).

Exopolissacarídeos catiônicos como a adesina intercelular e poli-N-acetilglicosamina, encontradas em biofilmes de Staphylococcus epidermidis e Staphylococcus aureus, respectivamente, desempenham importante papel estrutural em biofilmes (SEVIOUR et al., 2012). O Staphylococcus aureus é um patógeno humano que

provoca uma grande quantidade de doenças que vão desde infecções de pele benignas até condições fatais, como bacteremia, endocardite infecciosa e infecções crônicas (HAABER et al., 2016). A patogenicidade do S. aureus está relacionada com a sua elevada capacidade de auto-agregação e de formar biofilmes. Além disso, S. aureus agrega-se eficientemente no plasma humano, uma condição que está ligada à virulência, e pode formar agregados planctônicos que protegem as células contra antibióticos (HAABER et al., 2016).

Os isolados prejudiciais à formação dos grânulos (4, 10, 14, 18 e 26) são espécies dos gêneros Acinetobacter e Enterobacter.

O gênero Acinetobacter possui membrana celular altamente hidrofóbica e pode produzir excesso de SPE, possuindo alta capacidade de auto-agregação e capacidade de aderência a superfície sólida (LV et al., 2014; PHUONG; KAKII; NIKATA, 2009). Interações hidrofóbicas são importantes para a co-agregação de isolados de Acinetobacter sp. e outras bactérias constituintes do lodo (PHUONG; KAKII; NIKATA, 2009).

Além disso, bactérias do gênero Acinetobacter possuem características que estão relacionadas com o aumento da resistência elevada degradação de substâncias tóxicas. Isolados de Acinetobacter calcoaceticus apresentaram autoagregação e elevada remoção de fenol, formando grânulos estáveis com diâmetros de 2,3mm. Os índices de autoagregação das Acinetobacter calcoaceticus tiveram correlação positiva com a quantidade de proteínas extraídas dos agregados (ADAV; LEE, 2008). Substâncias produzidas por espécies do gênero Acinetobacter apresentam polissacarídeos capsulares, os quais conferem vantagens de proteção para as células. Biofilmes de Acinetobacter baumannii protegeram as células contra exposição à tobramicina (DAVENPORT; CALL; BEYENAL, 2014).

4. CONCLUSÕES

Foram obtidos 19 isolados cultiváveis a partir dos grânulos aeróbios mesofílicos produzidos durante o tratamento de efluente de uma fábrica de papel. Com base nos resultados das SPE, constatou-se que há diferenças na efetividade da influência de cada isolado na formação dos grânulos. Os isolados 2, 7, 9, 13, 19 e 25 foram avaliados como favoráveis para a formação e estabilidade dos grânulos aeróbios mesofílicos e apresentaram, de maneira geral, maior produção de carboidratos, proteínas e relação PN/PS superior à 1, o que contribui para o processo de granulação aeróbia.

Esses isolados benéficos à grânulos aeróbios foram identificados como

pertencentes aos gêneros Agrobacterium, Enterobacter, Staphylococcus e Rhodococcus, que produzem grande quantidade de SPE auxiliando na formação e manutenção de biofilmes e grânulos.

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6. REFERENCIAS BIBLIOGRÁFICAS

ADAV, S. S.; LEE, D. J. Single-culture aerobic granules with Acinetobacter calcoaceticus. **Applied Microbiology and Biotechnology**, v. 78, n. 3, p. 551–557, 2008.

ADAV, S. S.; LEE, D.-J.; LAI, J.-Y. Potential cause of aerobic granular sludge breakdown at high organic loading rates. **Applied Microbiology and Biotechnology**, v. 85, n. 5, p. 1601–1610, 2010.

ALBALASMEH, A. A.; BERHE, A. A.; GHEZZEHEI, T. A. A new method for rapid determination of carbohydrate and total carbon concentrations using UV spectrophotometry. **Carbohydrate Polymers**, v. 97, n. 2, p. 253–261, 2013.

BASUVARAJ, M.; FEIN, J.; LISS, S. N. Protein and polysaccharide content of tightly and loosely bound extracellular polymeric substances and the development of a granular activated sludge floc. **Water research**, v. 82, p. 104–117, 2015.

DE CARVALHO, C. C. C. R.; MARQUES, M. P. C.; FERNANDES, P.; DA FONSECA, M. M. R. A simple imaging method for biomass determination. **Journal of Microbiological Methods**, v. 60, n. 1, p. 135–140, 2005.

CAUDAN, C.; FILALI, A.; SPÉRANDIO, M.; GIRBAL-NEUHAUSER, E. Multiple EPS interactions involved in the cohesion and structure of aerobic granules. **Chemosphere**, v. 117, n. 1, p. 262–270, 2014.

DAVENPORT, E. K.; CALL, D. R.; BEYENAL, H. Differential Protection from Tobramycin by Extracellular Polymeric Substances from Acinetobacter baumannii and Staphylococcus aureus Biofilms. **Antimicrobial Agents and Chemotherapy**, v. 58, n. 8, p. 4755–4761, 2014.

FRØLUND, B.; PALMGREN, R.; KEIDING, K.; NIELSEN, P. H. Extraction of extracellular polymers from activated sludge using a cation exchange resin. **Water Research**, v. 30, p. 1749–1758, 1996.

GAO, D.; FU, Y.; TAO, Y.; et al. Linking microbial community structure to membrane biofouling associated with varying dissolved oxygen concentrations. **Bioresource Technology**, v. 102, n. 10, p. 5626–5633, 2011.

HAABER, J.; COHN, M. T.; PETERSEN, A.; INGMER, H. Simple method for correct enumeration of Staphylococcus aureus. **Journal of Microbiological Methods**, v. 125, p.

58–63, 2016.

HUANG, L.; YANG, T.; WANG, W.; ZHANG, B.; SUN, Y. Effect of Mn2+ augmentation on reinforcing aerobic sludge granulation in a sequencing batch reactor. **Applied Microbiology and Biotechnology**, v. 93, n. 6, p. 2615–2623, 2012.

IVANOV, V.; WANG, X. H.; TAY, S. T. L.; TAY, J. H. Bioaugmentation and enhanced formation of microbial granules used in aerobic wastewater treatment. **Applied Microbiology and Biotechnology**, v. 70, n. 3, p. 374–381, 2006.

JIANG, H. L.; TAY, J. H.; TAY, S. T. L. Aggregation of immobilized activated sludge cells into aerobically grown microbial granules for the aerobic biodegradation of phenol. **Letters in Applied Microbiology**, v. 35, p. 439–445, 2002.

KENNEDY, J.; CODLING, C. E.; JONES, B. V; DOBSON, A. D. W.; MARCHESI, J. R. Diversity of microbes associated with the marine sponge, Haliclona simulans, isolated from Irish waters and identification of polyketide synthase genes from the sponge metagenome. **Environmental microbiology**, v. 10, n. 7, p. 1888–1902, 2008.

KHAN, M. Z.; KHAN, F.; SABIR, S. Aerobic granular treatment of 2,4-dichlorophenol. **Canadian Journal of Chemical Engineering**, v. 89, n. 4, p. 914–920, 2011.

KIM, K. S.; SAJJAD, M.; LEE, J.; PARK, J.; JUN, T. Variation of extracellular polymeric substances (EPS) and specific resistance to filtration in sludge granulation process to the change of influent organic loading rate. **Desalination and Water Treatment**, v. 52, n. 22–24, p. 4376–4387, 2014.

KLEBENSBERGER, J.; RUI, O.; FRITZ, E.; SCHINK, B.; PHILIPP, B. Cell aggregation of Pseudomonas aeruginosa strain PAO1 as an energy-dependent stress response during growth with sodium dodecyl sulfate. **Archives of Microbiology**, v. 185, n. 6, p. 417–427, 2006.

DI LACONI, C.; RAMADORI, R.; LOPEZ, A. Combined biological and chemical degradation for treating a mature municipal landfill leachate. **Biochemical Engineering Journal**, v. 31, p. 118–124, 2006.

LI, K.; WEI, D.; YAN, T.; DU, B.; WEI, Q. Responses of soluble microbial products and extracellular polymeric substances to the presence of toxic 2,6-dichlorophenol in aerobic granular sludge system. **Journal of Environmental Management**, v. 183, p. 594–600,

2016.

LI, Y.; ZOU, J.; ZHANG, L.; SUN, J. Aerobic granular sludge for simultaneous accumulation of mineral phosphorus and removal of nitrogen via nitrite in wastewater. **Bioresource Technology**, v. 154, n. 2, p. 178–184, 2014. Elsevier Ltd.

LIMOLI, D. H.; JONES, C. J.; WOZNIAK, D. J. Bacterial Extracellular Polysaccharides in Biofilm Formation and Function. **Microbiology spectrum**, v. 3, n. 3, p. 11–28, 2015.

LIU, Y. Q.; LIU, Y.; TAY, J. H. The effects of extracellular polymeric substances on the formation and stability of biogranules. **Applied Microbiology and Biotechnology**, v. 65, p. 143–148, 2004.

LUO, J.; HAO, T.; WEI, L.; et al. Impact of influent COD/N ratio on disintegration of aerobic granular sludge. **Water Research**, v. 62, p. 127–135, 2014.

LV, Y.; WAN, C.; LEE, D. J.; LIU, X.; TAY, J. H. Microbial communities of aerobic granules: Granulation mechanisms. **Bioresource Technology**, v. 169, p. 344–351, 2014.

MASZENAN, A. M.; LIU, Y.; NG, W. J. Bioremediation of wastewaters with recalcitrant organic compounds and metals by aerobic granules. **Biotechnology Advances**, v. 29, n. 1, p. 111–123, 2011.

MCSWAIN, B. S.; IRVINE, R. L.; HAUSNER, M.; WILDERER, P. A. Composition and Distribution of Extracellular Polymeric Substances in Aerobic Flocs and Granular Sludge. **Applied and environmental microbiology**, v. 71, n. 2, p. 1051–1057, 2005.

MORAIS, I. L. H.; SILVA, C. M.; BORGES, C. P. Aerobic granular sludge to treat paper mill effluent: organic matter removal and sludge filterability. **Desalination and Water Treatment**, v. 57, n. 18, p. 8119–8126, 2016.

MORAIS, I. L. H.; SILVA, C. M.; NASCIMENTO, A. G. DO; FILHO, N. P.; DIAS, J. C. T. Aerobic granular sludge to treat paper mill effluent : selection of ideal strains that contribute to the formation of strong aggregates. **Desalination and Water Treatment**, v. 57, n. 59, p. 28537–28550, 2016.

NCBI. Standard Nucleotide BLAST. Disponível em: <https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome>. Acesso em: 16/10/2015.

PEN, Y.; ZHANG, Z. J.; MORALES-GARCIA, A. L.; et al. Effect of extracellular polymeric substances on the mechanical properties of Rhodococcus. **Biochimica et biophysica acta**, v. 1848, n. 2, p. 518–526, 2015.

PHUONG, K.; KAKII, K.; NIKATA, T. Intergeneric coaggregation of non-flocculating Acinetobacter spp. isolates with other sludge-constituting bacteria. **Journal of Bioscience and Bioengineering**, v. 107, n. 4, p. 394–400, 2009.

RODRIGUES, J. L. M.; KACHEL, C. A.; AIELLO, M. R.; et al. Degradation of Aroclor 1242 Dechlorination Products in Sediments by Burkholderia xenovorans LB400(ohb) and Rhodococcus sp. Strain RHA1(fcb). **Applied and Environmental Microbiology**, v. 72, n. 4, p. 2476–2482, 2006.

SAJJAD, M.; KIM, K. S. Influence of Mg2+ catalyzed granular sludge on flux sustainability in a sequencing batch membrane bioreactor system. **Chemical Engineering Journal**, v. 281, p. 404–410, 2015.

SALEHIZADEH, H.; YAN, N. Recent advances in extracellular biopolymer flocculants. **Biotechnology Advances**, v. 32, n. 8, p. 1506–1522, 2014.

SEVIOUR, T.; YUAN, Z.; VAN LOOSDRECHT, M. C. M.; LIN, Y. Aerobic sludge granulation: A tale of two polysaccharides? **Water Research**, v. 46, n. 15, p. 4803–4813, 2012.

TAY, J.-H.; IVANOV, V.; PAN, S.; TAY, S. T.-L. Specific layers in aerobically grown microbial granules. **Letters in applied microbiology**, v. 34, n. 4, p. 254–257, 2002.

TOH, S. K.; TAY, J. H.; MOY, B. Y. P.; IVANOV, V.; TAY, S. T. L. Size-effect on the physical characteristics of the aerobic granule in a SBR. **Applied microbiology and biotechnology**, v. 60, n. 6, p. 687–695, 2003.

TORRES, C. A. V; ANTUNES, S.; RICARDO, A. R.; et al. Study of the interactive effect of temperature and pH on exopolysaccharide production by Enterobacter A47 using multivariate statistical analysis. **Bioresource technology**, v. 119, p. 148–156, 2012.

TU, X.; ZHANG, S.; XU, L.; ZHANG, M.; ZHU, J. Performance and fouling characteristics in a membrane sequence batch reactor (MSBR) system coupled with aerobic granular sludge. **Desalination**, v. 261, n. 1–2, p. 191–196, 2010.

WAN, C.; LEE, D. J.; YANG, X.; et al. Calcium precipitate induced aerobic granulation. **Bioresource Technology**, v. 176, p. 32–37, 2015.

WAN, C.; SHEN, Y.; CHEN, S.; et al. Microstructural strength deterioration of aerobic granule sludge under organic loading swap. **Bioresource technology**, v. 221, p. 671–676, 2016.

WANG, Z.; GAO, M.; WANG, S.; et al. Effect of hexavalent chromium on extracellular polymeric substances of granular sludge from an aerobic granular sequencing batch reactor. **Chemical Engineering Journal**, v. 251, p. 165–174, 2014.

WEI, D.; LI, M.; WANG, X.; et al. Extracellular polymeric substances for Zn (II) binding during its sorption process onto aerobic granular sludge. **Journal of Hazardous Materials**, v. 301, n. September 2015, p. 407–415, 2016.

XUAN, W.; BIN, Z.; ZHIQIANG, S.; et al. The EPS characteristics of sludge in an aerobic granule membrane bioreactor. **Bioresource technology**, v. 101, p. 8046–8050, 2010.

YANG, H.; MA, H.; SHI, B.; LI, L.; YAN, W. Experimental study of the effects of heavy metal ions on the hydrogen production performance of Rhodobacter sphaeroides HY01. **International Journal of Hydrogen Energy**, v. 41, n. 25, p. 10631–10638, 2016.

ZHANG, H.; YU, H.; ZHANG, L.; SONG, L. Stratification structure of polysaccharides and proteins in activated sludge with different aeration in membrane bioreactor. **Bioresource Technology**, v. 192, p. 361–366, 2015.

ZHU, L.; QI, H. YING; LV, M. LE; et al. Component analysis of extracellular polymeric substances (EPS) during aerobic sludge granulation using FTIR and 3D-EEM technologies. **Bioresource Technology**, v. 124, p. 455–459, 2012.

ZHU, L.; ZHOU, J.; LV, M.; et al. Specific component comparison of extracellular polymeric substances (EPS) in flocs and granular sludge using EEM and SDS-PAGE. **Chemosphere**, v. 121, p. 26–32, 2015.

II. Conclusões e recomendações gerais

O lodo aeróbio granular apresenta grandes vantagens em comparação a outros processos de tratamento biológico. As características físicas do lodo garantem uma melhor clarificação do efluente tratado, seja por sedimentação em um decantador secundário ou por filtração por membranas. Além disso, os grânulos aeróbios podem ser aplicados na remoção de nutrientes e substâncias tóxicas, apresentando elevadas eficiências. Tais vantagens contribuem para o aumento do número pesquisas visando uma maior compreensão do processo de granulação aeróbia. A revisão de literatura, apresentada no Capítulo 1, indicou os principais parâmetros que influenciam na formação do lodo aeróbio granular e mostrou que sua aplicação em plantas de larga escala ainda se restringe a um número reduzido de estações de tratamento de águas residuárias em todo o mundo, mas é previsto um aumento da implantação de ETE em larga escala com lodo granular.

A aplicação de 100 $mg.L^{-1}$ aumentou a velocidade de sedimentação e a resistência dos grânulos formados no RBS com efluente de fábrica de polpa celulósica. No entanto, o efeito de cátions divalentes pode variar em função da presença de agentes quelantes no reator. Assim, a avaliação da presença de agentes quelantes no efluente de polpa celulósica ou a adição destes no reator acrescentaria informações importantes para a melhoria da granulação aeróbia.

Dezenove isolados obtidos a partir de lodo aeróbio granular em RBS tratando efluente de fábrica de papel foram identificados. Isolados dos gêneros Agrobacterium, Enterobacter, Staphylococcus e Rhodococcus favoreceram a formação e estabilidade dos grânulos e produziram grande quantidade de SPE.

Os efluentes gerados no processo de polpação kraft apresentam temperaturas em torno de 55 °C e precisam ser resfriados antes da estação de tratamento de efluentes por lodos ativados convencional. A adaptação do lodo granular a temperaturas termofílicas reduziria o custo de resfriamento do efluente e favoreceria o reuso do efluente tratado no processo produtivo. No entanto, o aumento da temperatura dos sistemas com lodo granular leva à redução da resistência e desintegração dos grânulos. O conhecimento detalhado sobre os efeitos da temperatura sobre a granulação aeróbica ainda é limitado, embora alguns estudos sobre o lodo aeróbio granular tenham sido realizados a altas temperaturas.

O efeito da adição de cálcio na filtrabilidade do lodo aeróbio granular poderia ser tema de um estudo posterior. O diâmetro dos grânulos e o aumento da resistência mecânica provocado pela adição de 100 $mg.L^{-1}$ de Ca^{2+} pode reduzir a incrustação dos módulos de membranas e melhorar o funcionamento de um BRM.

ANEXOS

Determinação do tamanho médio dos grânulos

A determinação do tamanho e circularidade dos grânulos foi realizada por meio do uso do programa ImageJ (RASBAND, 1997). Os diâmetros foram calculados como diâmetros circulares equivalentes (LOCHMATTER; GONZALEZ-GIL; HOLLIGER, 2013).

Determinação da taxa específica de crescimento dos grânulos

A taxa específica de crescimento dos grânulos (μ) foi calculada com base no modelo cinético desenvolvido por Yang et al., (2004):

$$R - R_0 = (R_{eq} - R_0)[1 - e^{-\mu(t-t0)}]$$

Onde,
R = tamanho dos agregados no tempo t (mm)
R_{eq} = tamanho dos agregados no equilíbrio (mm)
μ = taxa específica de crescimento dos agregados (dia^{-1})
t_0 = tempo no final da fase lag
R_0 = tamanho dos agregados microbianos no tempo t_0.

Na determinação da taxa específica, o valor de R utilizado refere-se ao diâmetro dos grânulos 30 dias após o final da fase lag. Para este valor de tempo (t = 30) todos os sistemas estavam em fase de crescimento dos grânulos e não tinham atingido o estado estacionário.

Determinação da velocidade média de sedimentação

Para determinação da velocidade média de sedimentação, foi utilizado o procedimento proposto por Ghangrekar; Asolekar; Joshi, (2005). Tal procedimento se baseia na utilização de uma coluna de diâmetro igual a 7,5 cm e altura igual a 75 cm (coluna de sedimentação) preenchida com água de torneira (Figura 1).

Figura 1 - Colunas utilizadas para realização do teste de velocidade de sedimentação dos grânulos aeróbios.

Em cada coluna, foi acrescentado cerca de 25 mL de lodo diluído (5 – 10 vezes). Água de torneira foi utilizada para a determinação da velocidade de sedimentação, pois o pH e a força iônica da água de torneira é adequada para esta finalidade, e o tamanho dos grânulos é suficiente para permitir que estes não sejam afetados pelo estresse osmótico. A quantidade de lodo sedimentado ao fundo da coluna foi coletada em intervalos de tempo fixos (0,5; 1; 1,5; 3; 7,5; 15 e 60 minutos). Determinou-se o SST de cada amostra, para analisar a fração de lodo sedimentado em cada instante. A velocidade média de sedimentação foi calculada pela equação:

$$v_{méd.sedim.} = \frac{\sum(m\ x\ v)}{M}$$

Onde,

$v_{méd.sedim.}$ = velocidade média de sedimentação

m = massa da fração de lodo sedimentada;

v = velocidade de sedimentação da fração;

M = massa total da amostra de lodo.

Determinação da resistência dos grânulos

O procedimento utilizado por Ghangrekar; Asolekar; Joshi, (2005) foi utilizado para a determinação da resistência dos grânulos. O teste foi baseado na premissa de que

se os grânulos são submetidos à tensão de cisalhamento do fluido, a quantidade de lodo liberado no fluido (pelo desprendimento dos grânulos) será função da resistência dos grânulos. Uma amostra de lodo do reator foi diluída 10 vezes com água de torneira. Para separar os grânulos do lodo floculento, a amostra de lodo diluída (25 mL) foi deixada sedimentar na coluna de sedimentação (Figura 1). A fração de grânulos que sedimentaram em 1 minuto foi utilizada para a determinação da resistência. A força de cisalhamento foi introduzida, ainda que indiretamente, submetendo estes grânulos sedimentados em frascos cônicos contendo água de torneira em um volume total de 150 mL, os quais foram levados à um agitador ("shaker"). Cada amostra foi submetida ao mesmo grau de agitação (200 rpm por 5 minutos). Após a agitação a amostra foi mantida em repouso durante 1 minuto. Foi realizada a análise de sólidos suspensos totais do sobrenadante e do decantado. Os resultados das análises de SST foram utilizados para a determinação do coeficiente de integridade definido como a razão entre a massa de sólidos do sobrenadante dividida pela massa total de sólidos da amostra, ou seja:

$$coeficiente\ de\ integridade = \frac{massa\ de\ sólidos\ do\ sobrenadante}{massa\ total\ de\ sólidos\ da\ amostra}$$

Quanto maior a resistência dos grânulos, menor será a massa de sólidos no sobrenadante, visto que esta é resultado do rompimento dos grânulos pela agitação. Dessa forma, quanto menor o coeficiente de integridade de uma amostra, maior a resistência dos grânulos. Assim, um coeficiente de integridade igual a 1 indicaria a completa ruptura dos grânulos, indicando baixa resistência, e um coeficiente de integridade igual a 0 indicaria que não houve ruptura dos grânulos, indicando elevada resistência.

Observações microscópicas

As observações microscópicas foram realizadas, diariamente, com amostras do lodo fresco, utilizando um microscópio ótico com contraste de fase, da marca LEICA, modelo DMLS. Foi acompanhado o desenvolvimento dos grânulos e observado as características morfológicas do lodo biológico durante o processo de granulação. Foi utilizada uma máquina digital, da marca Nikon, modelo COOLPIX 4500 para obtenção de fotomicrografias dos grânulos e das características morfológicas do lodo biológico.

Purificação dos amplicons

Os amplicons foram purificados e submetidos a gel de agarose (1%) (Figura 2).

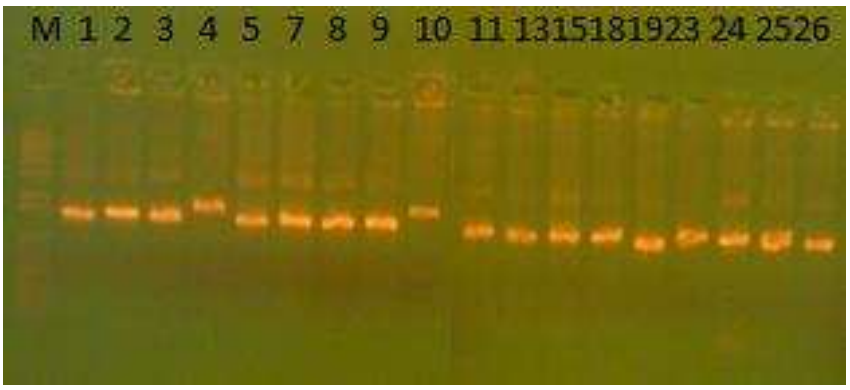

Figura 2. Gel de agarose (1%) das amostras de DNA, amplificadas com os primers 27F e 1525R (M= Marcador)