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Recuperação de polímeros semelhantes ao alginato a partir de lodo biológico de esgoto:
quantificação, caracterização e aplicação

Alginate-like polymers recovery from waste biological sludge: quantification,
characterization and application

Florianópolis
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O presente trabalho em nível de doutorado foi avaliado e aprovado por banca examinadora composta pelos seguintes membros:

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Florianópolis, 2020.

Este trabalho é dedicado aos meus pais Wilson e Vera.

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RESUMO

Dado o alto custo e o passivo ambiental do gerenciamento do lodo biológico excedente produzido em estações de tratamento de esgoto, a valorização desse resíduo deve ser priorizada. Dentro do contexto de economia circular, a extração de substâncias poliméricas extracelulares (EPS) a partir do lodo exibe um grande potencial de biorrefinaria uma vez que a presença de polímeros semelhantes a alginato (*alginate-like exopolymers* - ALE) formadores de hidrogéis possuem usos industriais promissores. Diante desse cenário, esse trabalho teve três objetivos que visam aprofundar conhecimentos em aspectos relacionados ao ALE: (1) avaliar a dinâmica da quantidade, fatores influenciadores e características químicas do ALE ao longo do tratamento de esgoto doméstico com grânulos aeróbios; (2) avaliar como a composição e propriedade de hidrogel do ALE são influenciadas pelo tipo de esgoto (sintético e real) e bioagregado (flocos e grânulos); e (3) testar o uso de hidrogéis de ALE como adsorventes de alguns ânions e cátions presentes em esgoto doméstico. Quanto ao objetivo (1), os resultados mostraram que a quantidade de ALE foi maior, mais estável e constantemente aumentou depois que a granulação foi obtida. Cerca de 236 ± 27 mg SV_{ALE}/g SV_{lodo} foram recuperados de grânulos e 187 ± 94 mg SV_{ALE}/g SV_{lodo} de flocos. A quantidade de ALE recuperada foi relacionada com a maior fração de grânulos, com a conversão da matéria orgânica e nutrientes, assim como com a estabilidade da comunidade microbológica. O ALE recuperado tanto de flocos como de grânulos apresentou propriedades de hidrogel sem diferenças na composição elementar e grupos funcionais. Quanto ao objetivo (2), os resultados de análises colorimétricas, FTIR e Raman mostraram que ALE é uma complexa mistura de proteínas, polissacarídeos e ácidos húmicos. De modo geral, o ALE recuperado de diferentes tipos de lodo teve uma quantidade e composição um tanto similar. Somente os grânulos aeróbios alimentados com esgoto sintético composto de acetato e propionato produziram de modo significativo mais ALE (261 ± 33 mg SV_{ALE}/g SV_{lodo} , +49%) e contiveram mais açúcares urônicos (254 ± 32 mg_{ácido glucurônico}/g SV_{ALE} , +62%). Ensaio reológico mostraram que a elasticidade do ALE recuperado de grânulos foi maior do que a de flocos quando o efluente era esgoto real. Testes enzimáticos indicaram que os ácidos manurônicos estão envolvidos na manutenção da estrutura de hidrogel do ALE. Quanto ao objetivo (3), os resultados mostraram que os hidrogéis de ALE removeram eficazmente somente fosfato do esgoto doméstico com remoções de até 90,8%. Contudo, os hidrogéis de ALE contribuíram para o aumento da concentração de DQO, nitrogênio e fósforo total no meio em até 117,6%, 118% e 42,5% respectivamente. Assim, modificações químicas na composição do ALE devem ser feitas a fim de aumentar sua estabilidade mecânica e evitar a liberação de outros compostos. O pH=6 prejudicou fortemente a eficiência de remoção de fosfato (diminuição para $28,9 \pm 0,8\%$), enquanto pH mais básicos na faixa de 8,5 foram os mais favoráveis. Portanto, os resultados obtidos nessa tese trazem novidades sobre aspectos quantitativos e qualitativos do ALE. Esses avanços podem ajudar na implementação de biorrefinarias para recuperação de recursos a partir de lodo biológico de esgoto contribuindo para uma economia circular.

Palavras-chave: Recuperação de recursos. Lodo biológico de esgoto. Polímeros que se comportam como alginato (ALE).

ABSTRACT

Due to the high cost and environmental liability of the management of waste biological sludge produced in wastewater treatment plants, the valorization of this waste must be prioritized. Within the context of circular economy, the extraction of extracellular polymeric substances (EPS) from the sludge exhibits a great potential for biorefinery since the presence of alginate-like polymers (ALE) which form hydrogels have promising industrial applications. Given this scenario, this work has three aims to deepen knowledge in aspects related to ALE: (1) to assess the dynamics of the quantity, environmental/operational influencing factors and chemical characteristics of ALE during the treatment of municipal wastewater by aerobic granular sludge; (2) to evaluate how the composition and the hydrogel property of ALE are influenced by the type of sludge (synthetic vs. real) and bioaggregate (flocs vs. granules); and (3) to test the use of ALE hydrogels as adsorbents for anionic and cationic compounds present in municipal wastewater. As for objective (1), the results showed that the content of ALE was higher, more stable and steadily increased after granulation was achieved. Approximately 236 ± 27 mg $VS_{ALE}/g VS_{sludge}$ were recovered from granules and 187 ± 94 mg $VS_{ALE}/g VS_{sludge}$ from flocs. The ALE content recovered was related to the large fraction of granules, with the conversion of organic matter and nutrients, as well as with the stability of the microbial community. ALE recovered from both flocs and granules were endowed with hydrogel properties, presenting no differences in elemental composition and functional groups. As for objective (2), the results of colorimetric assays, FTIR and Raman showed that ALE are a complex mixture of proteins, polysaccharides and humic acids. In general, ALE recovered from different types of sludge had a similar content and composition. Only aerobic granular sludge fed with synthetic wastewater composed of acetate and propionate produced significantly more ALE (261 ± 33 mg $VS_{ALE}/g VS_{sludge}$, + 49%) and contained more uronic sugars (254 ± 32 mg $glucuronic\ acid/g VS_{ALE}$, +62%). Rheological tests showed that the elasticity of ALE recovered from granules was greater than the elasticity from flocs when the effluent was municipal wastewater. Enzymatic tests indicated that mannuronic acids are involved in the maintenance of the structure of ALE hydrogels. As for objective (3), the results showed that ALE hydrogels were able to effectively remove only phosphate from municipal wastewater with removals of up to 90.8%. However, ALE hydrogels contributed to the increase in the concentration of COD, nitrogen and total phosphorus in the medium up to 117.6%, 118% and 42.5%, respectively. Thus, chemical changes in the composition of the ALE must be made to tailor its mechanical stability and avoid the release of other compounds. pH=6 strongly impaired the phosphate removal efficiency (decrease to $28.9 \pm 0.8\%$), while the basic pH in the 8.5 range was the most favorable. Therefore, the results obtained in this thesis provide advances on quantitative and qualitative aspects of ALE, which help consolidating the implementation of technologies for resource recovery from waste biological sludge.

Keywords: Resource recovery. Waste biological sludge. Alginate-like exopolymers (ALE).

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LISTA DE ABREVIATURAS E SIGLAS

AGS	<i>Aerobic granular sludge</i> (lodo granular aeróbio)
ALE	<i>Alginate-like exopolymers</i> (polímeros que se comportam como alginato)
BSA	<i>Bovine serum albumin</i> (albumina sérica bovina)
COD	<i>Chemical oxygen demand</i> (demanda química de oxigênio)
DBO	Demanda bioquímica de oxigênio
DO	<i>Dissolved oxygen</i> (oxigênio dissolvido)
DQO	Demanda química de oxigênio
EAWAG	<i>Swiss Federal Institute of Aquatic Science and Technology</i> (Instituto Federal Suíço de Ciências Aquáticas e Tecnologia)
EDS	Espectroscopia de raios-x por dispersão de energia
EDX	<i>Energy disperse X-ray Spectroscopy</i> (espectroscopia de raios-x por dispersão de energia)
EPS	<i>Extracellular polymeric substances</i> (substâncias poliméricas extracelulares)
ETE	Estação de tratamento de esgoto
ETH	<i>Eidgenössische Technische Hochschule</i> (Instituto Federal de Tecnologia)
FTIR	Espectroscopia de infravermelho por transformada de Fourier
GG	Gulurônico-gulurônico
GM	Gulurônico-manurônico
ICP-OES	<i>Inductively Coupled Plasma Optical Emission Spectrometry</i> (Espectrometria de emissão atômica por plasma acoplado indutivamente)
INSA	<i>Institut National des Sciences Appliquées</i> (Instituto Nacional de Ciências Aplicadas)
IVL	Índice volumétrico de lodo
LABEFLU	Laboratório de efluentes líquidos e gasosos
LGA	Lodo granular aeróbio
MEV	Microscopia eletrônica de varredura
MM	Manurônico-manurônico
OTU	<i>Operational taxonomic unit</i> (unidade taxonômica operacional)
PAO	<i>Polyphosphate-accumulating organisms</i> (organismos acumuladores de fósforo)
PC	<i>Principal Component</i> (componentes principais)
PCA	<i>Principal component analysis</i> (análise dos componentes principais)

pH	Potencial hidrogeniônico
PHA	Polihidroxiclcanoato
PPGEA	Programa de pós-graduação em engenharia ambiental
RBS	Reator em bateladas sequenciais
sCOD	<i>Soluble chemical oxygen demand</i> (demanda química de oxigênio solúvel)
SEM	<i>Scanning electron microscopy</i> (microscopia eletrônica de varredura)
SSV	Sólidos suspensos voláteis
SV	Sólidos voláteis
SVI	<i>Sludge volumetric index</i> (índice volumétrico de lodo)
tCOD	<i>Total chemical oxygen demand</i> (demanda química de oxigênio total)
TP	<i>Total phosphorus</i> (fósforo total)
TS	<i>Total solids</i> (sólidos totais)
TSS	<i>Total suspended solids</i> (sólidos suspensos totais)
UASB	<i>Upflow anaerobic sludge blanket</i> (reator anaeróbico de fluxo ascendente e manta de lodo)
UFSC	Universidade Federal de Santa Catarina
VFA	<i>Volatile fatty acid</i> (ácido graxo volátil)
VS	<i>Volatile solids</i> (sólidos voláteis)
VSS	<i>Volatile suspended solids</i> (sólidos suspensos voláteis)
WRRF	<i>Wastewater resource recovery facility</i> (unidade de recuperação de recursos de efluentes)
WWTP	<i>Wastewater treatment plant</i> (estação de tratamento de esgoto)

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1 CONTEXTUALIZAÇÃO

Nas áreas urbanas, um dos principais agentes poluidores de águas são os esgotos que na maioria das vezes são lançados diretamente nos corpos de água. Frente a essa degradação intensa dos recursos hídricos, os efluentes devem ser tratados em estações de tratamento de esgoto (ETE), onde a água retorna à natureza com melhor grau de qualidade diminuindo os impactos no meio ambiente. Contudo, diversos subprodutos sólidos são gerados ao longo do processo de tratamento, como sólidos grosseiros, areia e lodo. Esse último é formado também a partir de processos biológicos, onde parte da matéria orgânica é absorvida e convertida em biomassa microbiana, caracterizando-se como um resíduo semissólido, pastoso (cerca de 95% de água) e de natureza predominantemente orgânica (ANDREOLI *et al.*, 2014; HEALY *et al.*, 2015).

Uma das definições para lodo de esgoto é resíduo sólido gerado nos processos de tratamento de esgoto sanitário (CONAMA, 2020). Em termos globais, a produção de lodo seco na União Europeia foi de 8.909.000 toneladas em 2010, nos Estados Unidos de 6.514.000 toneladas em 2004 e na China de 2.966.000 em 2006 (MATEO-SAGASTA; SALLY-RASCGID; THEBO, 2015). Em um levantamento feito em 275 ETE localizadas em 17 estados brasileiros, a produção anual de lodo úmido foi de 308.633 toneladas sendo que cerca de 50% do lodo produzido é disposto em aterro sanitário (MACHADO; FIGUEIREDO, FILHO, 2004). Em Santa Catarina, a Companhia Catarinense de Água e Saneamento (CASAN) produz cerca de 18 mil toneladas ao ano hoje enviadas para aterros sanitários, procedimento considerado ecologicamente correto, porém visto como um desperdício pela própria concessionária (CASAN, 2016). Embora o lodo represente apenas 1% a 2% do volume de esgoto tratado (ANDREOLI; VON SPERLING; FERNANDES, 2014), seu gerenciamento é bastante complexo e tem um custo que pode chegar a até 57% do total gasto com a operação de uma ETE (LEBLANC; MATTHEWS; RICHARD, 2008). Observa-se que esses quantitativos são bastante elevados, ressaltando a grande problemática do gerenciamento do lodo, que se for mal executado, pode comprometer os benefícios ambientais e sanitários esperados das estações de tratamento (ANDREOLI, 2006).

Dentre as alternativas de disposição final do lodo, encontra-se: descarga oceânica, incineração, aterro sanitário, *landfarming*, recuperação de áreas degradadas e reciclagem agrícola (VON SPERLING, 2002). Na maior parte dos países em desenvolvimento, mais de 90% dos resíduos coletados são dispostos em lixões ou em aterros sanitários (NIZAMI *et al.*,

2017), inclusive o Brasil (ANDREOLI; VON SPERLING; FERNANDES, 2014). Na Europa, embora as principais vias de disposição final de lodo de esgoto sejam reuso na agricultura, aterro sanitário e incineração, diversas legislações têm dado ênfase à produção de energia e subprodutos (HEALY *et al.*, 2015). Alguns países inclusive já possuem incentivos financeiros implementados a fim de diminuir a disposição de lodo em aterros e estimular a sua reciclagem (JENSEN; JEPSEN, 2005). Com os avanços da tecnologia ambiental e a busca de soluções compatíveis com a complexidade exigida pelo tratamento do lodo, é possível promover o reaproveitamento e a valorização do resíduo, o qual passa a ser visto como subproduto útil e economicamente viável. Embora outras opções possam ser mais econômicas, como a incineração, o gerenciamento de resíduos é baseado também em outros critérios como os ambientais (GÓMEZ *et al.*, 2010), convergindo para a tendência de diminuição de envio de resíduos para aterro.

A valorização do lodo é amparada também por uma base legal, conforme previsto na Lei Federal nº 12.305/2010, a qual institui a Política Nacional de Resíduos Sólidos (BRASIL, 2010). Segundo a lei, na gestão e gerenciamento de resíduos sólidos, deve ser observada a seguinte ordem de prioridade: não geração, redução, reutilização, reciclagem, tratamento dos resíduos sólidos e disposição final ambientalmente adequada dos rejeitos. Desse modo, somente os rejeitos devem encaminhados para os aterros. Ademais, o fomento ao desenvolvimento científico e tecnológico, a adoção de tecnologias apropriadas e a difusão dos conhecimentos gerados de interesse para o saneamento básico são alguns dos objetivos da Política Nacional de Saneamento Básico instituída pela Lei Federal nº 11.445/2007 (BRASIL, 2007). Assim, estudos que subsidiem o desenvolvimento de tecnologias que possibilitem extrair o máximo possível de novos usos para os resíduos gerados, como os lodos, são estimulados pela legislação ambiental brasileira.

A tendência atual de expansão da cobertura dos serviços de saneamento com a implantação de sistemas de coleta e tratamento de esgoto conseqüentemente aumenta a geração de lodo (HEALY *et al.*, 2015). Dentre as tecnologias disponíveis para tratamento, aquelas que fazem uso de biomassa na forma de grânulos aeróbios - tipicamente chamados de lodo granular aeróbio (LGA) - têm provado ser uma biotecnologia ambiental efetiva e altamente promissora para o tratamento de efluentes, estando em foco atualmente (BOLTZ *et al.*, 2017; ZHANG; HU; LEE, 2016). Essa técnica de origem holandesa é baseada em características específicas da biomassa granular aeróbia onde é possível obter elevadas remoções de matéria orgânica e de nutrientes produzindo um efluente final de alta qualidade (COMA *et al.*, 2012; DERLON *et al.*, 2016; SARMA; TAY, 2018a). Essa tecnologia tem se mostrado capaz de competir com outras

já consolidadas na área de tratamento de efluentes como os lodos ativados, espalhando-se aos poucos pelo mundo com a progressiva instalação e operação de plantas em escala real (SARMA; TAY, 2018a). A formação dos grânulos é mediada pela produção de substâncias poliméricas extracelulares (EPS, do inglês *extracellular polymeric substances*) pelos microrganismos as quais fornecem uma matriz para a agregação da biomassa (DING *et al.*, 2015). Estudos da composição de EPS de lodo granular aeróbio de esgoto real mostraram que a maior parte dos exopolímeros é formada por polímeros que se comportam como alginato (*Alginate-like exopolymers* – ALE), apresentando propriedade de hidrogel¹ (FELZ *et al.*, 2016; LIN *et al.*, 2010). Não somente de grânulos, mas o ALE também já foi recuperado a partir de biomassa predominantemente flocular (LIN; SHARMA; VAN LOOSDRECHT, 2013; SAM; DULEKGURGEN, 2015). O ALE vem se mostrando um polímero versátil com diversas aplicações industriais, como material impermeabilizante à água e retardante de chamas de superfícies, assim como potencial bioissorvente de corantes e fosfato (DALL'AGNOL *et al.*, 2020; KIM *et al.*, 2020; LADNORG *et al.*, 2019; LIN *et al.*, 2015). Logo, dada a grande variedade de composição do EPS e as possíveis aplicações comerciais dos polímeros que vierem a ser extraídos, questões sobre o porquê, como e sob quais circunstâncias os polímeros são sintetizados estão para serem respondidas. Isso poderá ajudar a entender melhor o processo de desenvolvimento dos grânulos e como usá-los como fonte de matéria-prima. Assim, há necessidade de estudos que avaliem técnicas de recuperação de biomateriais de lodo granular, metodologias de caracterização e aplicações dos biomateriais recuperados (LIN *et al.*, 2015).

Visto que há uma tendência de aumento do número de ETE usando lodo granular aeróbio nos próximos anos, conseqüentemente uma grande quantidade de lodo excedente será produzida. A fim de alcançar os padrões cada vez mais restritivos das políticas ambientais, a operação da ETE deve ser direcionada à recuperação de produtos assim como à economia de energia (MILFERSTEDT *et al.*, 2017), entrando dentro do conceito de biorrefinarias. Muitas das ETE, especialmente as grandes, se transformarão de unidades tradicionais de tratamento em locais de recuperação de recursos, podendo contribuir para alcançar a economia circular (VAN LEEUWEN *et al.*, 2018). O foco atual dessa abordagem está em aliar uma alta eficiência de tratamento com o reaproveitamento dos resíduos. Assim, pesquisas que foquem na formação de EPS devem ser realizadas para avaliar o potencial de reaproveitamento dos polímeros presentes na biomassa descartada no lodo (ZHANG; HU; LEE, 2016). Se biomateriais puderem

¹ Material polimérico que exibe a capacidade de inchar e reter uma fração significativa de água dentro de sua estrutura, mas sem se dissolver nela.

ser recuperados do lodo e aplicados em um novo uso, a sustentabilidade e o ciclo econômico do tratamento de efluentes podem ser fortemente aumentados.

Perante o exposto, fica claro que estudos relacionados à temática de EPS objetivando sua posterior recuperação e uso é de considerável importância na área das ciências ambientais. Assim, esse trabalho foi motivado basicamente pela problemática atual e recorrente da grande quantidade de lodo com potencial poluidor diariamente produzido em ETE que necessita de gerenciamento adequado, podendo ser valorizado para outros usos. Nesse contexto, o estudo da dinâmica de produção, caracterização e teste de possíveis aplicações do ALE extraído de lodo biológico pode ajudar a trazer soluções a esse problema.

1.1 O GRUPO DE PESQUISA

O LABEFLU (Laboratório de Efluentes Líquidos e Gasosos) vinculado ao Departamento de Engenharia Sanitária e Ambiental da Universidade Federal de Santa Catarina (UFSC) desenvolve há quase 20 anos atividades de pesquisa, ensino e extensão na área de tratamento de efluentes líquidos e gasosos. Nos últimos anos, o departamento vem desenvolvendo pesquisas quanto ao tratamento e gerenciamento de lodos de esgotos sanitários (PINTO, 2006; MANZOCHI, 2008; SUNTTI, 2010; FOGOLARI, 2011; LEITE, 2011; FEDRIZZI, 2012; FURTADO, 2012; KÄFER, 2015; LEITE, 2015; VELHO, 2015), o que estimula a continuidade de estudos dentro deste tema em novos projetos científicos. No LABEFLU, Velho (2015) avaliou processos físico-químicos e biológicos de minimização da produção de lodo em sistemas de lodos ativados, enquanto Leite (2011; 2015), trabalhou com digestores anaeróbios para, entre outros, reduzir o volume de sólidos de lodo adensado de ETE. Além disso, recentemente, alguns trabalhos têm sido feitos pelo LABEFLU focando na recuperação de EPS de lodo granular aeróbio. Dentre esses, citam-se estudos sobre a avaliação da comunidade microbiana presente nos processos de granulação, remoção de nutrientes e produção de EPS e ALE (GUIMARÃES, 2017) e a aplicação de ALE como biossorvente para remoção de fósforo (DALL'AGNOL, 2020). Contudo, um estudo mais aprofundado que quantifique, caracterize e teste uma aplicação dos polímeros recuperáveis de lodo biológico de esgoto ainda não foi realizado. É então sobre o estudo dessa lacuna em aberto que se baseia uma das novas linhas de pesquisa do laboratório e onde se insere o presente trabalho de doutorado, o qual foi realizado em duas etapas. A primeira etapa ocorreu no LABEFLU com apoio financeiro do projeto PRONEX/FAPESC (Núcleo de Excelência - Projeto PRONEX: Tecnologias Inovativas Para a Sustentabilidade do Saneamento Básico em Santa Catarina) e do

projeto Universal do Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). A segunda etapa foi feita durante período de doutorado sanduíche na Suíça no *Swiss Federal Institute of Aquatic Science and Technology* (Eawag) com auxílio financeiro também do CNPq e do Programa de Internacionalização da Pós-Graduação - PRINT-CAPES.

1.2 HIPÓTESES E PERGUNTAS DA PESQUISA

Essa pesquisa foi conduzida pelas seguintes hipóteses:

1. O desenvolvimento de grânulos durante o tratamento de esgoto doméstico é relacionado com a produção de EPS e ALE pela biomassa.
2. As características químicas do ALE variam ao longo do processo de granulação com esgoto doméstico.
3. A composição química e a propriedade de hidrogel do ALE variam conforme a natureza do efluente a ser tratado, assim como o tipo de bioagregado (grânulo ou floco).
4. O ALE pode ser valorizado e utilizado como bioissorvente de diferentes compostos presentes em esgoto doméstico.

Frente a essas hipóteses, foram elaboradas as seguintes perguntas que motivaram essa pesquisa:

1. Como se dá a dinâmica de produção e características de EPS e ALE ao longo da granulação no tratamento de esgoto doméstico?
2. Quais parâmetros físicos, químicos e biológicos do esgoto e da biomassa que mais se relacionam com a dinâmica de produção de ALE ao longo da granulação no tratamento de esgoto doméstico?
3. Diferentes tipos de efluentes e bioagregados influenciam na composição química do ALE e nas suas propriedades de hidrogel?
4. Quais compostos presentes em esgoto doméstico hidrogéis de ALE podem adsorver?

1.3 OBJETIVOS

1.3.1 Objetivo geral

O objetivo geral dessa pesquisa foi quantificar, caracterizar e testar uma aplicação do ALE recuperado a partir de lodo biológico de esgoto a fim de dar suporte à valorização desse resíduo dentro do conceito de economia circular.

1.3.2 Objetivos específicos

Frente ao exposto, os objetivos específicos desse trabalho de doutorado foram:

1. Quantificar o EPS/ALE produzidos, avaliar sua correlação com fatores operacionais/ambientais e avaliar mudanças nas propriedades de hidrogel, composição elementar e grupos funcionais do ALE ao longo do processo de granulação no tratamento de esgoto doméstico.
2. Avaliar a influência do tipo de efluente (sintético e real) e de bioagregado (grânulos e flocos) na quantidade recuperada de ALE assim como na sua composição química e propriedade de hidrogel.
3. Avaliar o potencial de hidrogéis de ALE remover diferentes compostos presentes em esgoto doméstico.

1.4 ESTRUTURA DA TESE

Após essa contextualização inicial, a tese segue com uma revisão bibliográfica (Seção 2) onde se busca expor os principais fundamentos teóricos que embasaram essa pesquisa. É apresentado o conceito de biorrefinaria e economia circular, detalhando alguns biomateriais e biopolímeros já que a tese é sobre o estudo de um recurso (ALE) recuperado a partir de um resíduo (lodo biológico). Foco especial é dado ao alginato dada sua semelhança com o ALE. Em seguida, é exposto um pouco de conceitos relacionados ao processo de granulação e EPS, culminando com as últimas pesquisas e informações que se têm atualmente sobre o ALE.

Posteriormente, inicia-se a seção de Resultados e Discussões (Seção 4). Os resultados obtidos são apresentados na forma de três artigos científicos redigidos em inglês. O artigo 1 (Seção 4.1) buscou quantificar o ALE e principalmente responder às perguntas “*Há mudança qualitativa e quantitativa ao longo do tempo do ALE produzido em um sistema que trata esgoto*”

real?” e “*Quais parâmetros físicos, químicos e biológicos do esgoto e da biomassa se relacionam com a dinâmica de produção de ALE?*”. Para isso, foi explorada a variação da quantidade e de algumas características químicas do EPS e ALE ao longo da granulação em um reator em bateladas sequenciais piloto que tratou esgoto doméstico. Além disso, também se elucidou quais fatores ambientais estão mais relacionados à dinâmica de produção de EPS e ALE. Esse trabalho foi desenvolvido no Brasil nas instalações do LABEFLU (UFSC) no período de setembro de 2016 a abril de 2018. Os resultados dessa parte da pesquisa possibilitaram sobretudo quantificar o ALE que pode ser extraído de um sistema com flocos ou grânulos: mais ALE pode ser recuperado de grânulos que de flocos. Esses dados são importantes para futuras avaliações econômicas de implantação de biorrefinarias em escala real. Também foi possível demonstrar a relação da quantidade de ALE com o processo de granulação, com a conversão da matéria orgânica e nutrientes e com a estabilidade da comunidade microbiana.

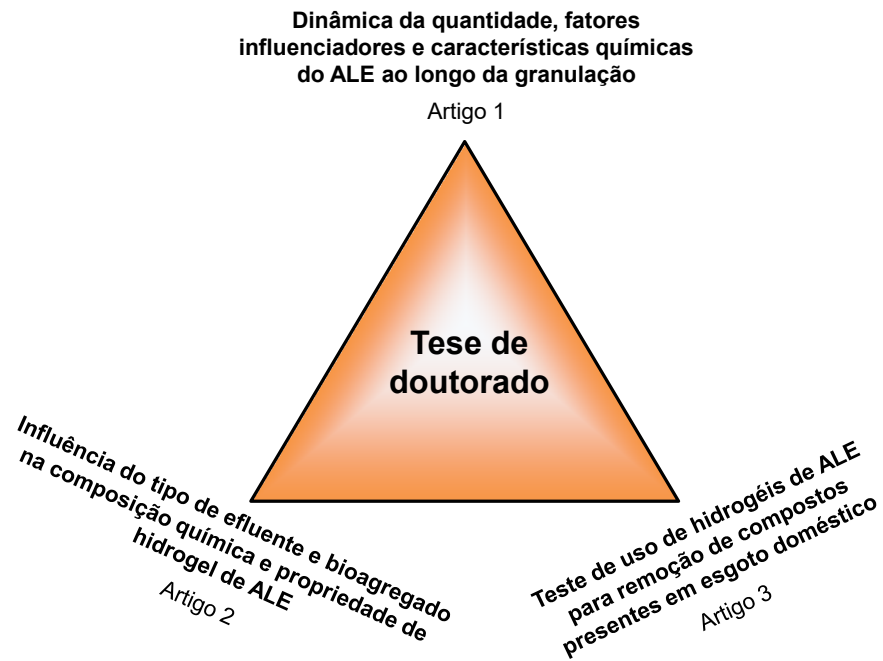
O artigo 2 (Seção 4.2) buscou fazer uma análise mais qualitativa do ALE e responder à pergunta “*Como diferentes tipos de esgoto e bioagregados podem influenciar na composição química do ALE e nas suas propriedades de hidrogel?*” Para isso, ALE extraído de esgoto doméstico e sintético assim como de flocos e grânulos foi analisado quimicamente por diferentes análises complementares. Também se focou em analisar mais profundamente a propriedade de hidrogel do ALE em função do lodo do qual ele é extraído. Esse trabalho foi desenvolvido na Suíça nas instalações do *Swiss Federal Institute of Aquatic Science and Technology* (Eawag) no período de junho de 2018 a janeiro de 2019 em parceria com o *Institute of Food, Nutrition and Health (ETH Zürich)*, *Laboratoire de Biotechnologies Agroalimentaire et Environnementale (Université Paul Sabatier – Toulouse, França)* e o *Toulouse Biotechnology Institute (INSA – Toulouse, França)*. Os resultados dessa parte da pesquisa possibilitaram sobretudo elucidar a composição química do ALE por proteínas, ácidos húmicos e polissacarídeos e como ela varia em função do lodo de origem, ou seja, do tipo de esgoto e de bioagregados. Também foi possível demonstrar os polímeros envolvidos no estabelecimento da propriedade de hidrogel do ALE: os ácidos manurônicos. Por fim, mostrou-se a influência do lodo de origem nas propriedades reológicas dos hidrogéis de ALE. Hidrogéis de ALE extraídos de grânulos são mais rígidos que os extraídos de flocos. De modo geral, os resultados foram relevantes para a implantação de biorrefinarias já que se demonstrou o impacto do lodo biológico de esgoto na composição química e propriedades de hidrogel do ALE.

O artigo 3 (Seção 4.3) buscou testar uma aplicação do ALE e responder à pergunta “*Hidrogéis de ALE podem ser usados como adsorventes de compostos presentes em esgoto doméstico?*” Para isso, hidrogéis de ALE extraído de lodo granular aeróbio foram usados como material adsorvente em reatores de bancada para remover diferentes compostos comumente presentes em esgoto doméstico. Testou-se a influência de diferentes concentrações de ALE, altas concentrações de outros íons como amônia, nitrato e fosfato assim como diferentes valores de pH. Esse trabalho foi desenvolvido na Suíça nas instalações do *Swiss Federal Institute of Aquatic Science and Technology* (Eawag) no período de janeiro de 2019 a maio de 2019. Os resultados dessa parte da pesquisa possibilitaram mostrar de uma maneira preliminar o potencial do ALE para remoção de fosfato de esgoto doméstico. Com isso, um bioproduto recuperado de lodo biológico de esgoto (ALE) tem potencial de ser utilizado para remoção e recuperação de um nutriente (fósforo).

Na seção Discussão Integrada (Seção 5) é apresentada uma discussão integrada com base nos resultados obtidos nos três artigos assim como são apresentadas perspectivas futuras em relação ao tema pesquisado dentro do contexto brasileiro. Na seção Conclusões (Seção 6) são apresentadas as conclusões gerais seguidas de recomendações para trabalhos futuros (Seção 7). Para ajudar o leitor, ao final da tese, há um Glossário com uma lista alfabética de termos técnicos que aparecem nessa tese seguidos da sua definição.

Sucintamente, essa pesquisa buscou elucidar três aspectos em relação ao ALE extraído de lodo biológico de esgoto: (1) fatores influenciadores e a dinâmica da quantidade e das características químicas do ALE ao longo do tratamento de esgoto doméstico com grânulos aeróbios; (2) como a composição e propriedade de hidrogel do ALE são influenciadas pelo tipo de efluente e bioagregado; (3) o potencial de uso de hidrogéis de ALE como adsorvente de compostos presentes em esgoto doméstico. Uma ilustração desses três aspectos em torno do ALE que juntos compõem essa tese está exposta na Figura 1.

Figura 1 - Estrutura da pesquisa desta tese de doutorado formada por três artigos científicos.



Fonte: Elaborada pelo autor (2020).

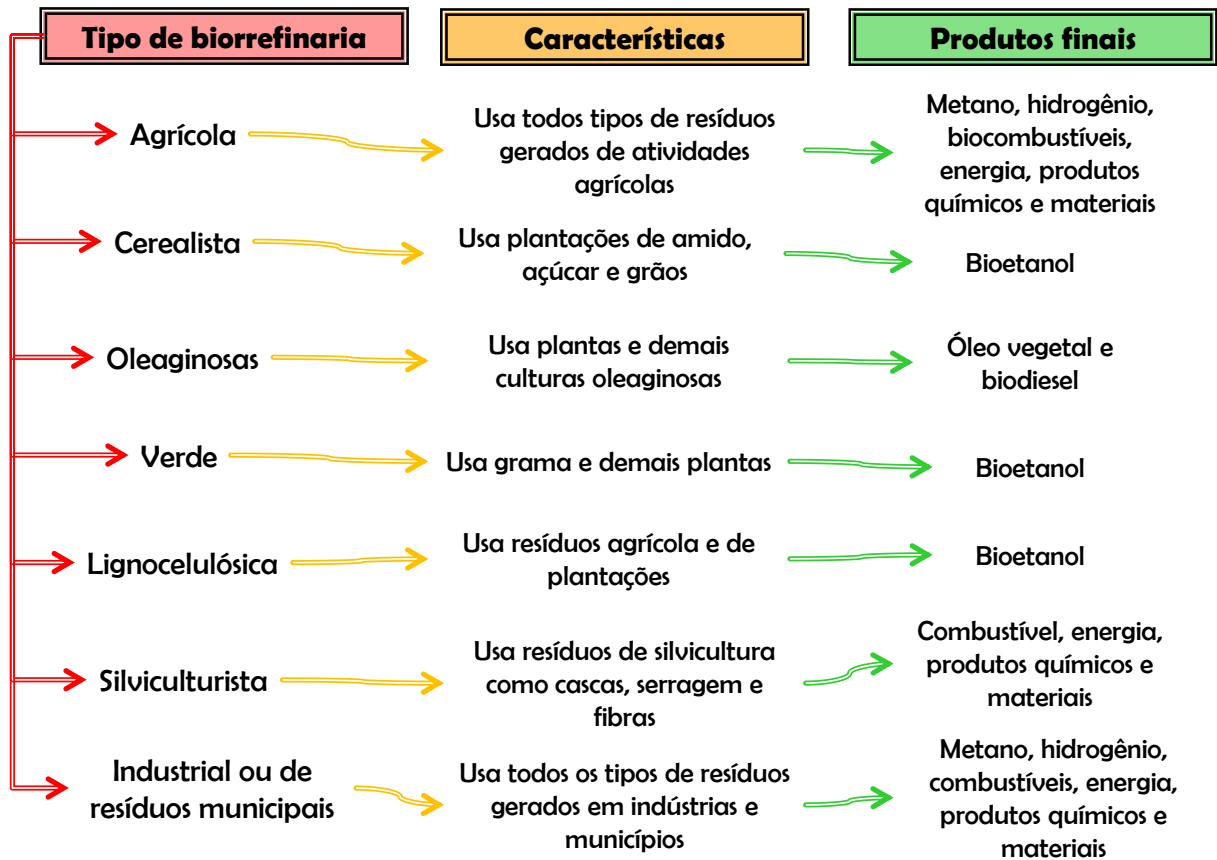
2 REVISÃO BIBLIOGRÁFICA

2.1 BIORREFINARIAS E A ECONOMIA CIRCULAR

Em função das preocupações ambientais atuais, há uma busca mundial crescente por fontes renováveis de matérias-primas. Atualmente, está ocorrendo uma mudança de paradigma onde os resíduos gerados não são mais vistos somente como um custo de um processo, mas também como um meio de extrair recursos (MOHAN *et al.*, 2016). Eles representam uma fonte potencial de biomassa, materiais recicláveis, produtos químicos, energia e renda se sabiamente gerenciados e usados como fonte de matéria-prima (NIZAMI *et al.*, 2017). Com isso, um modo de mitigar os efeitos negativos dos processos produtivos atuais, valorizando a biomassa e minimizando a geração de resíduo, é a realização da sua conversão em produtos com valor agregado. Dentro desse contexto, surge o conceito de biorrefinarias no qual resíduos com baixo valor agregado se tornam uma potencial fonte de matéria-prima renovável, oferecendo uma opção sustentável para utilizá-los e produzir uma gama de bioprodutos comercializáveis (MOHAN *et al.*, 2016). Similarmente a refinarias de petróleo, onde muita energia e produtos químicos são produzidos a partir do petróleo cru, as biorrefinarias podem produzir energia, calor, produtos químicos e outros materiais com valor agregado a partir de resíduos (CLARK; DESWARTE, 2008).

Dentre os diversos benefícios advindos das biorrefinarias, sobretudo em países em desenvolvimento, pode-se citar a geração de energia, criação de novos negócios e consequente criação de empregos, diminuição dos custos e áreas de aterros sanitários, redução na emissão de gases de efeito estufa e economia no uso de recursos naturais de solo e água subterrânea. Assim, a implantação de biorrefinarias de resíduos ajudaria não somente a fazer a transição de economias lineares para circulares, onde os resíduos são reintroduzidos no ciclo produtivo ao invés de ser descartados, mas também a contribuir para melhorar a saúde pública e ambiental (NIZAMI *et al.*, 2017). Exemplos de biorrefinarias baseadas nas suas características e produtos finais gerados estão apresentados na Figura 2.

Figura 2 - Tipos de biorrefinarias baseadas nas suas características e produtos finais.

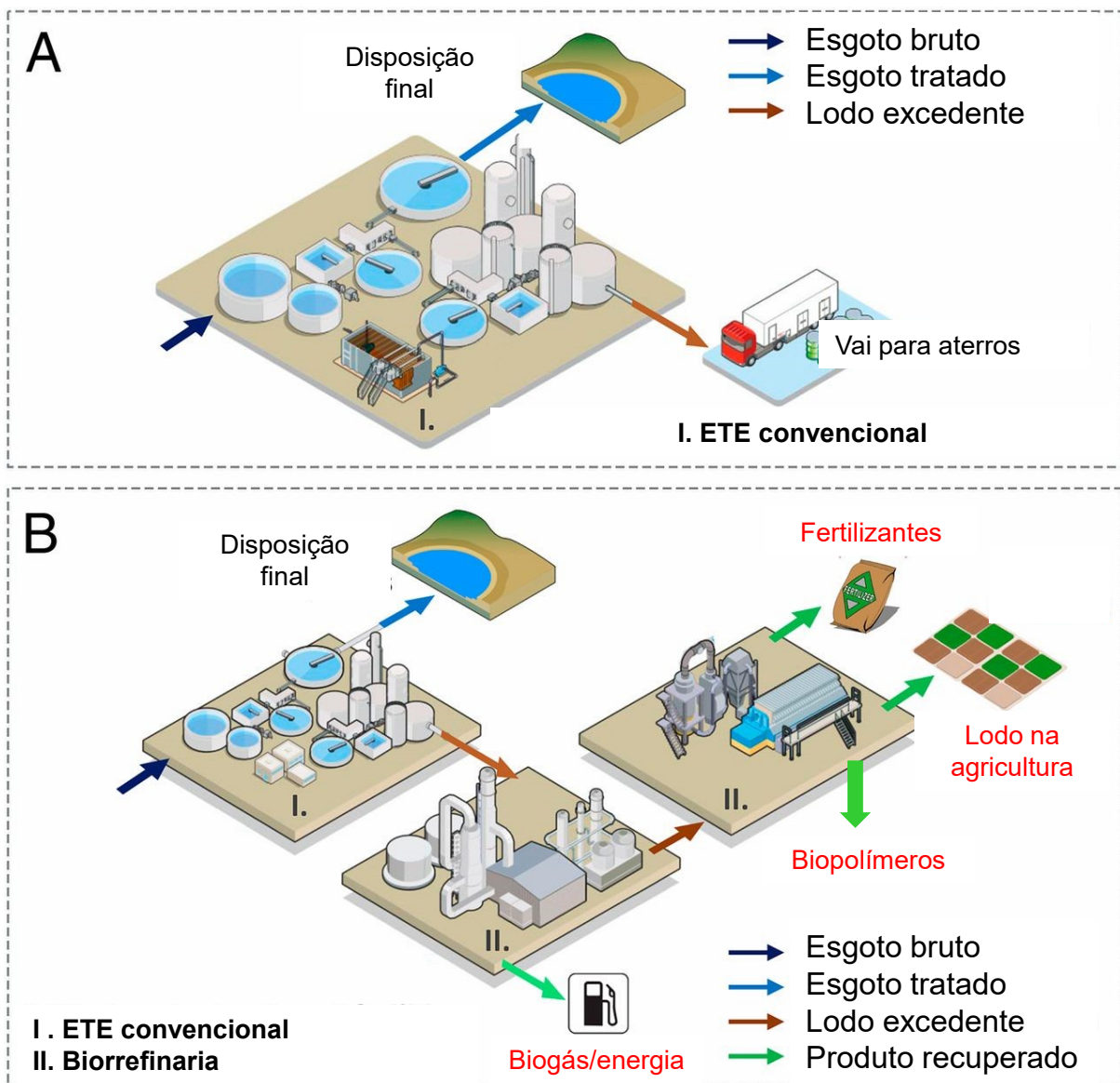


Fonte: adaptado de Nizami et al. (2017).

Um bom exemplo de biorrefinaria em potencial são as estações de tratamento de esgotos (ETE), cuja abordagem como local de obtenção de produtos com valor agregado vem tendo sucesso em aplicações como recuperação de nutrientes, biogás e biopolímeros (SAM; DULEKGURGEN, 2015). Efluentes podem ser usados como fonte de matérias-primas já que contém diversos recursos como matéria orgânica, fósforo, nitrogênio, metais pesados, biogás, biopolímeros, energia térmica, entre outros (SOLON *et al.*, 2019; VAN DER HOEK; DE FOOIJ; STRUKER, 2016). A recuperação de materiais com alto valor agregado a partir de lodo de esgoto em quantidades e custos compatíveis com a demanda e preços do mercado atual está em crescente expansão (VAN LOOSDRECHT; BRDJANOVIC, 2014). Análises buscando determinar quais recursos estão presentes no esgoto, sua quantidade e onde se localizam, assim como identificar e caracterizar meios de sua recuperação, têm sido realizadas em sistemas de coleta e tratamento de esgoto já operantes em Amsterdam (Holanda) com foco em biogás, celulose, bioplástico, fósforo e polímeros que se comportam como alginato (ALE) (VAN DER

HOEK; FOOIJ; STRUKER, 2016). Estima-se que de cerca 230 milhões de Euros por ano a partir de 2030 podem ser gerados a partir da recuperação de recursos em ETE da Holanda (VAN LEEUWEN *et al.*, 2018). Uma ETE instalada na cidade de Alperdoorn (Holanda) contribui atualmente para o conceito de economia circular através da recuperação de biogás para a produção de eletricidade, estruvita para uso como fertilizante agrícola e agora foca também na obtenção de alginato (ROYAL HASKONINGDHV, 2017). Essa nova abordagem ajuda a realizar a transição do conceito e imagem de ETE para fonte promissora de recuperação de produtos com valor agregado (Figura 3).

Figura 3 – (A) modelo de estação de tratamento de esgoto (ETE) convencional e (B) acoplada com a recuperação de recursos dentro do conceito de biorrefinaria.



Fonte: adaptado de Wang et al. (2015).

Apesar dos benefícios e do grande apelo das biorrefinarias, algumas ressalvas merecem ser feitas. Do ponto de vista econômico e de mercado, o uso de resíduos como matéria-prima comumente impacta a qualidade do produto final. Assim, a escolha das matérias-primas deve ser feita considerando aspectos técnico-econômicos como o uso final requerido e os métodos necessários para alcançar um produto com o necessário grau de pureza (FREITAS; TORRES; REIS, 2017; SOLON *et al.*, 2019). Do ponto de vista ambiental, é essencial que seja avaliado o impacto dos produtos gerados nas biorrefinarias através de análise de ciclo de vida (ACV). Isso assegura que eles sejam verdadeiramente sustentáveis e apresentem reais vantagens ambientais e sociais quando comparados com outros produtos análogos (NIZAMI *et al.*, 2017). Além disso, os resultados da ACV podem ser utilizados para elaboração e aplicação de políticas reguladoras, padronizando e aumentando a confiabilidade dos processos empregados (MOHAN *et al.*, 2016).

Perante o exposto, nota-se que as biorrefinarias de resíduos ou de geração de energia fornecem uma solução economicamente viável e promissora tanto para o aumento das demandas energéticas quanto para o gerenciamento ambientalmente adequado de resíduos (NIZAMI *et al.*, 2017). Entretanto, o uso em escala real é desafiador visto que muitos fatores devem ser levados em consideração, como o desenvolvimento de unidades pilotos que requerem colaborações intersetoriais e atração de investidores para construção de biorrefinarias, assim como um aprofundamento dos métodos de produção (CLARK; DESWARTE, 2008). Além disso, estudos mostram que para que a recuperação de recursos em ETE de países em desenvolvimento (como o Brasil) seja promissora, deve-se avaliar e adaptar as possibilidades de recuperação com base na realidade local ao invés de simplesmente replicar os modelos de países desenvolvidos (WANG *et al.*, 2015). Logo, uma maior exploração dos biorrecursos com base em cenários específicos pode ajudar a transformá-los em modelos tecnológicos factíveis, estabelecer mercados econômicos e, conseqüentemente, caminhar em direção a um planeta com máxima da valorização de resíduos (NIZAMI *et al.*, 2017).

2.1.1 Biomateriais e biopolímeros

A maior parte dos polímeros sintéticos produzidos hoje provém de petroquímicos e não são biodegradáveis (AVÉROUS; POLLET, 2012). Entretanto, a produção de materiais biodegradáveis tem um potencial significativo de contribuir para o atendimento da crescente demanda de matérias-primas (MOHAN *et al.*, 2016) e superar a limitação dos recursos naturais

no futuro (AVÉROUS; POLLET, 2012). Com isso, produtos naturais derivados de biomassa vêm atraindo considerável atenção como substitutos ambientalmente amigáveis de produtos poliméricos sintéticos (ANDERSON; ISLAM; PRATHER, 2018; SALEHIZADEH; YAN; FARNOOD, 2018). Um dos principais incentivos para o uso de bioprodutos são os seus potenciais benefícios ambientais, como redução de emissão de dióxido de carbono (CO₂) e serem biodegradáveis (CLARK; DESWARTE, 2008).

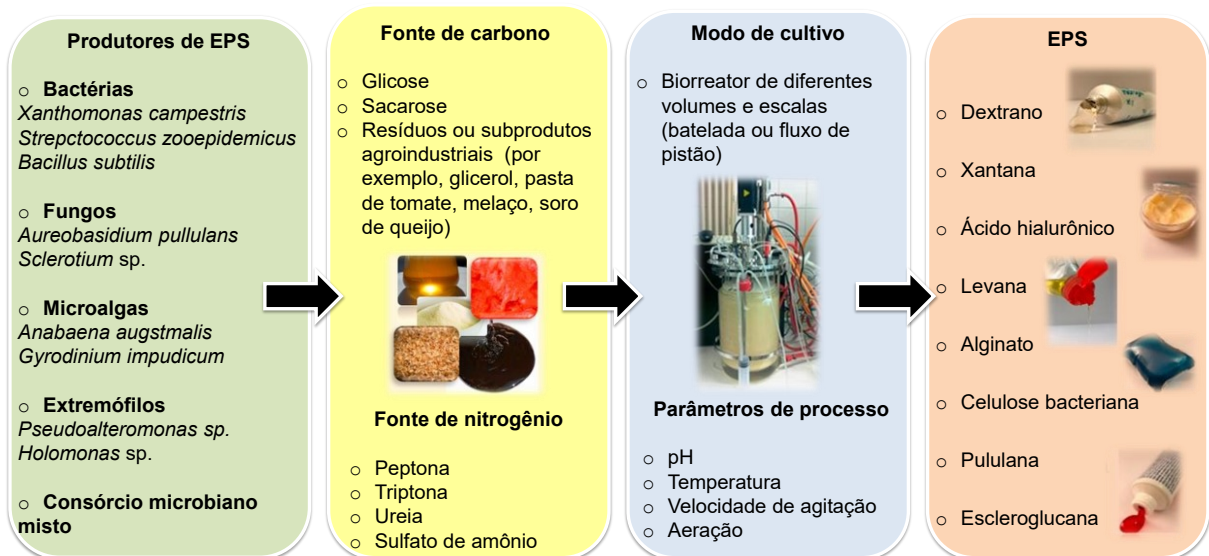
A recuperação de biomateriais a partir lodo de esgoto em processos biológicos de tratamento proporciona uma nova fonte de materiais sustentáveis (LIN *et al.*, 2015; ZHANG; HU; LEE, 2016). O lodo pode ser usado para produção de energia, bioplásticos, polímeros, materias de construção e outros compostos potencialmente úteis, assim como para recuperação de nutrientes (SHI *et al.*, 2018). Os principais produtos recuperáveis de lodo de esgoto são (HAELEY *et al.*, 2015):

- Nitrogênio (2,4-5% dos sólidos totais);
- Fósforo (0,5-0,7% dos sólidos totais);
- Metais pesados (principalmente níquel, zinco e cobre com recuperação de 99, 100 e 93% respectivamente);
- Lodo seco ou suas cinzas depois de incineração (para uso como materiais de construção);
- Bioplásticos (polihidroxialcanoatos (PHA) acumulados na faixa de 0,3 a 22,7 mg_{polímero}/g_{lodo});
- Enzimas;
- Ácidos graxos voláteis;
- Proteínas dos vermes e fungos;
- Demais biopolímeros.

A matriz de substâncias poliméricas extracelulares (EPS, do inglês *extracellular polymeric substances*) da biomassa tem se mostrado uma interessante fonte de biopolímeros para usos diversos. Durante os últimos anos, produtores microbianos de EPS como bactérias, fungos e algas têm sido objeto de extensivas pesquisas, demonstrando o grande potencial de esses sistemas microbianos gerarem biopolímeros com novas estruturas químicas e diferentes propriedades funcionais (FREITAS; TORRES; REIS, 2017). A absorção de compostos orgânicos pelo EPS é relatada como um dos fatores responsáveis pelo seu decaimento no meio líquido em reatores (PRONK *et al.*, 2015a). Assim, a bioadsorção por lodo granular aeróbio tem um grande potencial de remover metais pesados, corantes e compostos hidrofóbicos tanto de

esgoto doméstico quando industrial (SARMA; TAY, 2018a; WANG *et al.*, 2018). Lodo granular aeróbio já foi utilizado para avaliar o papel do EPS na biossorção de efluentes com cor, comprovando o seu grande potencial como adsorvente para remover corantes de soluções aquosas (WEI *et al.*, 2015). O EPS dos grânulos é rico em diversos grupos funcionais químicos que geralmente têm uma capacidade de captura de íons maior que o próprio grânulo (WANG *et al.*, 2018). Ademais, EPS já foram recuperados de lodo granular e caracterizados como material de revestimento e impermeabilização de superfícies, fornecendo funcionalidades interessantes enquanto as propriedades ambientais do material foram mantidas, como biodegradabilidade (LIN *et al.*, 2015; LOTTI *et al.*, 2019). Esse biomaterial facilmente formou um filme flexível e homogêneo funcionando como uma barreira resistente à água, assim como se mostrou anfifílico. No caso do lodo granular aeróbio, além de ser fonte de produtos orgânicos, o seu EPS também pode fornecer minerais que podem ser utilizados como fertilizantes, como é o caso da estruvita ($MgNH_4PO_4 \cdot 6H_2O$) a qual corresponde à maior parte da fração inorgânica dos grânulos (≈ 286 mg/g SST de grânulos) (LIN; BASSIN; VAN LOOSDRECHT, 2012). Na Figura 4, apresentam-se alguns aspectos envolvidos no processo de recuperação industrial de EPS para obtenção de diversos biopolímeros.

Figura 4 - Aspectos do processo da recuperação industrial de EPS para obtenção de biopolímeros.



Fonte: adaptado de Freitas et al. (2017).

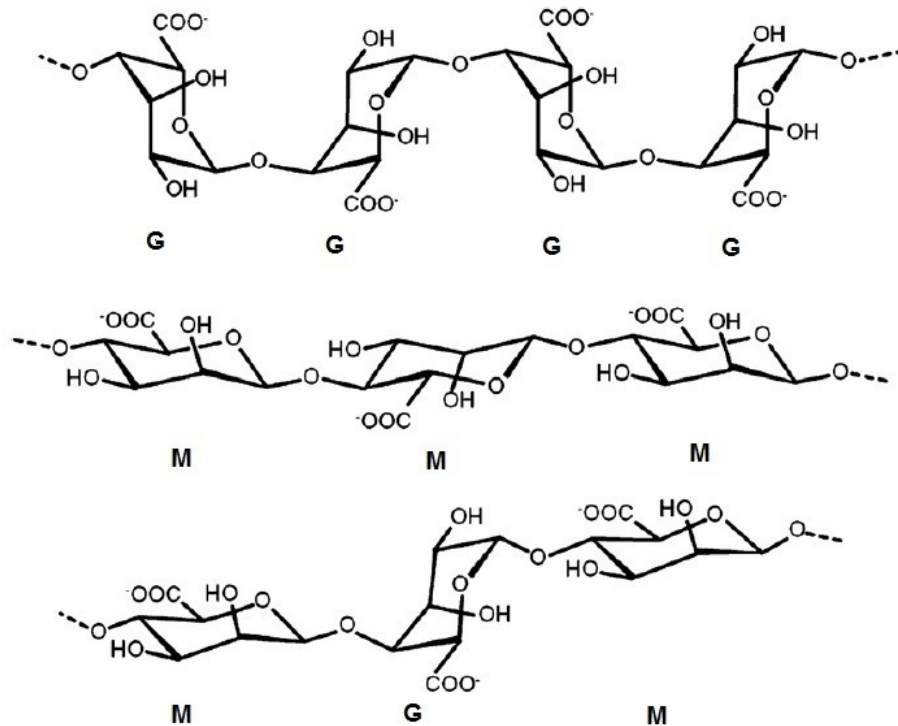
Como se pode perceber, polímeros derivados de fontes naturais vêm ganhando cada vez mais destaque quanto ao seu uso como biomateriais. No entanto, até o momento, os biopolímeros não tem encontrado grande aplicação nas indústrias devido ao seu alto custo de produção e por apresentarem algumas propriedades com desempenho inferior ao material convencional (AVÉROUS; POLLET, 2012). Para eliminar os tradicionais gargalos no custo e desempenho relacionados ao seu emprego, o uso de biomassa de baixo custo e desenvolvimento de novas estratégias de recuperação para produções em larga escala são recomendados como diretrizes-chaves de estudos futuros (SALEHIZADEH; YAN; FARNOOD, 2018).

2.1.2 Alginato

O alginato é um polissacarídeo e copolímero linear constituído dos ácidos urônicos α -L-gulurônicos e β -D-manurônicos com ligações 1-4 glicosídicas (HAY *et al.*, 2013; MÜLLER; DOS SANTOS; BRIGIDO, 2011; PAWAR; EDGAR, 2012). Dependendo da natureza da matéria-prima, sua composição varia em termos da proporção, comprimento e sequência de blocos dos resíduos de ácidos manurônicos (M) e gulurônicos (G) e grau de polimerização (LEE; MOONEY, 2012), como apresentado na Figura 5. Os blocos são compostos por resíduo consecutivos G (GGGGGG), M (MMMMMM) e alternando resíduos G e M (GMGMGM) (LEE; MOONEY, 2012). A composição (ou seja, proporção M/G), sequência, comprimento do bloco G e o peso molecular são fatores críticos que afetam as propriedades físicas do alginato e seus hidrogéis resultantes (LEE; MOONEY, 2012; MÜLLER; DOS SANTOS; BRIGIDO, 2011) e variam conforme a fonte de produção (PAWAR; EDGAR, 2012). Maiores concentrações de blocos G resultam em géis mais rígidos (HAY *et al.*, 2013)

O alginato possui propriedades espessantes, estabilizantes e gelificantes que fazem com que este biopolímero seja aplicado na indústria de alimentos, papel, agricultura, impressão, cosméticos, têxtil, além da área farmacêutica e médica (BORTOLIN *et al.*, 2012; HAY *et al.*, 2013; MÜLLER; DOS SANTOS; BRIGIDO, 2011). Ele pode ser usado como agente de encapsulação, de gelificação, de formação de filmes e de fibras sintéticas, entre outras possibilidades (MÜLLER; DOS SANTOS; BRIGIDO, 2011). Dada sua biocompatibilidade e capacidade de formação de gel, destaque se dá na área biomédica, particularmente nas áreas de cicatrização de ferimentos, transporte de fármacos, cultura celular *in vitro* e na engenharia de tecidos (LEE; MOONEY, 2012).

Figura 5 - Estrutura química de polímeros de alginato formados pelos monômeros do ácido gulurônico (G) e manurônico (M) como blocos GGGG, MMM e MGM.



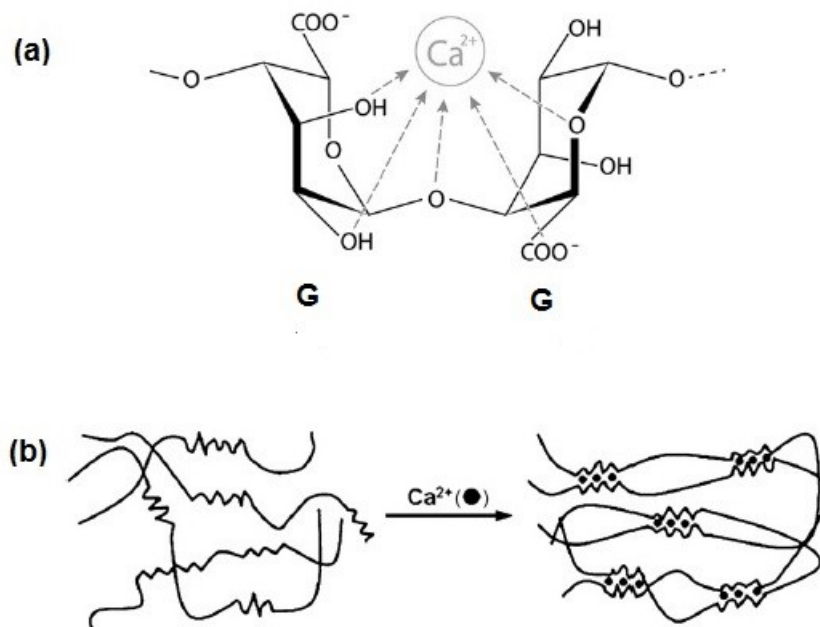
Fonte: Lee e Mooney (2012).

O alginato bacteriano pode ser obtido a partir de biossíntese se utilizando microrganismos do gênero *Pseudomonas aeruginosa*, *Azotobacter indicus* e *Azotobacter Vinelandii* (HAY *et al.*, 2013; LEE; MOONEY, 2012; MÜLLER; DOS SANTOS; BRIGIDO, 2011; REMMINGHORST; REHM, 2006). Ambos os gêneros produzem alginato como um polissacarídeo exopolimérico durante sua fase de crescimento (REMMINGHORST; REHM, 2006). Atualmente, a principal fonte de alginato em escala comercial vem de diversos gêneros de algas marinhas marrons (HAY *et al.*, 2013; LEE; MOONEY, 2012; MÜLLER; DOS SANTOS; BRIGIDO, 2011; PAWAR; EDGAR, 2012). A diferença chave entre os alginatos de algas e os bacterianos é que os últimos são acetilados no oxigênio dos carbonos 2 e 3 dos blocos M (HAY *et al.*, 2013; PAWAR; EDGAR, 2012).

O alginato é um polissacarídeo negativamente carregado, o que confere propriedades de formação de soluções viscosas e estruturas em forma de gel na presença de cátions divalentes (REMMINGHORST; REHM, 2006). Hidrogéis são compostos de polímeros hidrofílicos que possuem estruturas tridimensionais porosas com formas bem definidas, estreita distribuição de tamanho de poros e alta capacidade de absorção de água (BORTOLIN *et al.*, 2012; LEE;

MOONEY, 2012). As propriedades físico-químicas dos hidrogéis são altamente dependentes do tipo e densidade de reticulação, além do peso molecular e composição química dos polímeros (LEE; MOONEY, 2012). O método mais comum de preparo de hidrogéis a partir de uma solução aquosa de alginato é sua combinação com agentes iônicos reticulantes, como os cátions divalentes Ca^{2+} , Sr^{2+} e Ba^{2+} . Os blocos G do polímero formam, então, junções com os blocos G de cadeias poliméricas adjacentes no chamado modelo de reticulação *egg-box* (caixa de ovo), resultando na estrutura em gel (HAY *et al.*, 2013; LEE; MOONEY, 2012), como visualizado na Figura 6. Embora existam outros métodos, o cátion Ca^{2+} liberado em solução de cloreto de cálcio (CaCl_2) é um dos agentes mais frequentemente usados para reticular alginato via reticulação iônica (PAWAR; EDGAR, 2012). Acredita-se que somente os blocos G de alginato participem da reticulação intermolecular com cátions divalentes (como Ca^{2+}) para formar hidrogéis (LEE; MOONEY, 2012).

Figura 6 - (a) Reticulação de blocos de ácido gulurônico através da ligação com cálcio. (b) Modelo caixa de ovo (*egg-box model*) representando a reticulação iônica do alginato com íons de cálcio para formação de hidrogel.



Fonte: Adaptado de Lee e Mooney (2012) e Hay *et al.* (2013).

2.2 REATORES EM BATELADAS SEQUENCIAIS COM LODO GRANULAR AERÓBIO

O reator em bateladas sequenciais com lodo granular aeróbio é um tipo de reator com uso de biofilme na forma de grânulos aeróbios (BOLTZ *et al.*, 2017) utilizado para tratamento tanto de esgoto doméstico quanto industrial (SARMA; TAY, 2018a). Esses reatores possuem certas características atraentes como uma ótima sedimentabilidade da biomassa, menor geração de volume de lodo, menor perda e maior retenção de biomassa no reator durante o tratamento, o que explica a crescente popularidade da tecnologia de tratamento com grânulos (SARMA; TAY; CHU, 2017).

Os grânulos aeróbios são produzidos principalmente em reatores em bateladas sequenciais (RBS) (SARMA; TAY, 2018a; SARMA; TAY; CHU, 2017). Esse sistema biológico de tratamento de águas residuárias é constituído por tanque único onde se realiza, sequencialmente em uma mesma unidade, a oxidação da matéria carbonácea, a remoção de nutrientes e a separação sólido/líquido através da sedimentação, compreendendo basicamente as seguintes fases: enchimento, reação, sedimentação, retirada e repouso (JORDÃO; PESSÔA, 2005). Essas fases do reator podem acontecer em volume constante ou variável. Em volume constante, os reatores operaram com alimentação de esgoto bruto pelo fundo e descarte do efluente tratado pelo topo, onde ambos processos ocorrem simultaneamente. Com isso, a entrada inferior do esgoto bruto no reator “empurra” o efluente tratado na parte superior, mantendo o volume do reator sempre constante em um regime de fluxo de pistão (DERLON *et al.*, 2016; PRONK *et al.*, 2015b; WAGNER *et al.*, 2015). Já no modo de volume variável, cada fase ocorre separadamente com aumento do volume de líquido no reator durante o enchimento e diminuição no descarte, após ocorrer a mistura completa (DE KREUK *et al.*, 2010; NI *et al.*, 2009; PRONK *et al.*, 2015b; QIN; LIU; TAY, 2004; ROCKTÄSCHEL *et al.*, 2015; WAGNER *et al.*, 2015; WAN *et al.*, 2011; WAN; BESSIÈRE; SPÉRANDIO, 2009; YANG *et al.*, 2014).

ETE usando a tecnologia de grânulos aeróbios têm sido implantadas com sucesso em escala real, alcançando ótimas eficiências de tratamento como, por exemplo, a em operação na cidade de Gamerwolde na Holanda (Figura 7) (PRONK *et al.*, 2015b). Nessa ETE, há dois reatores com capacidade de tratamento de 2.500 m³/h tratando cerca de 41% do esgoto afluente, sendo o excedente direcionado para um reator de lodos ativados convencional. Em média, o efluente tratado sai com 20 mg/L de sólidos suspensos, 64 mg/L de DQO, 9,7 mg/L de DBO₅, 0,9 mg/L de fósforo total e 1,1 mg/L de nitrogênio na forma de amônia (NH₄-N), o quais são valores que podem ser considerados excelentes quando comparados com os padrões de

lançamento de esgoto tratado previstos na legislação federal brasileira (CONAMA, 2011).

Além disso, conforme tendências atuais de pesquisas, uma promissora característica do lodo granular aeróbio é a possibilidade de remoção de contaminantes emergentes como poluentes orgânicos persistentes e nanomateriais, os quais podem ser simultaneamente tratados via biodegradação, bioacumulação e biosorção (SARMA; TAY; CHU, 2017). Contudo, ainda há limitações para serem estudadas, como o longo tempo de granulação, pouco entendimento dos mecanismos moleculares, desintegração e morfologia imprevisível dos grânulos e casos com ineficiente remoção de nutrientes (MILFERSTEDT *et al.*, 2017).

Figura 7 - reatores em bateladas sequenciais com lodo granular (em destaque) operantes na ETE de Gamerwolde na Holanda.



Fonte: NEREDA®, 2018.

2.2.1 Granulação

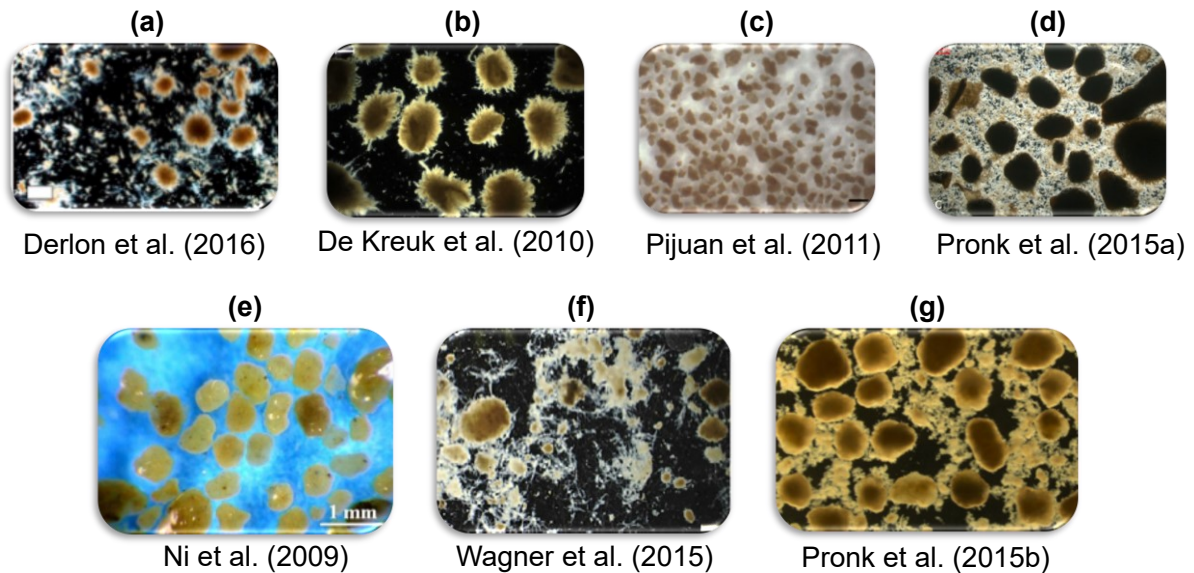
A granulação aeróbia é um processo gradual onde o lodo disperso flocular evolui para grânulos maduros com tamanho estável (LIU; LIU; TAY, 2004). O processo de formação de grânulos é a base da tecnologia via lodo granular aeróbio, contudo, ainda é um dos aspectos menos elucidados (SARMA; TAY; CHU, 2017). A granulação pode ser afetada por inúmeros fatores operacionais como inóculo do lodo utilizado, composição do substrato a ser tratado, carga orgânica, estratégia de alimentação e desenho do reator, taxa de troca volumétrica, intensidade da aeração e conseqüente força hidrodinâmica de cisalhamento (ADAV *et al.*, 2008).

Os grânulos são agregados esféricos de culturas microbianas mistas autoimobilizadas via *quorum sensing* (SARMA; TAY, 2018a; SARMA; TAY; CHU, 2017) cuja superfície exterior é dominada por microrganismos aeróbios, enquanto o interior pode conter microrganismos facultativos e estritamente anaeróbios, assim como biomassa morta (SARMA; TAY; CHU, 2017). Diferentes populações microbianas existem ao longo de seu volume como metazoários, protozoários, bactérias, fungos e archaea (DE KREUK *et al.*, 2010; WINKLER *et al.*, 2012) sendo que geralmente as bactérias são os organismos dominantes (SARMA; TAY, 2018a). Os grânulos exibem forma regular, lisa e arredondada, estrutura microbiana densa e forte, resistência a altas cargas orgânicas e tolerância à toxicidade devido à proteção pelo EPS (ADAV; LEE; TAY, 2008). Eles têm diferentes formas, cores e tamanhos que resultam das diferentes espécies microbianas envolvidas nos seus mecanismos de formação (MILFERSTEDT *et al.*, 2017). Com uma menor proporção de compostos hidrofílicos que os flocos (TU *et al.*, 2012), os grânulos possuem em média as seguintes características (MILFERSTEDT *et al.*, 2017):

- Diâmetro de 0,2-16 mm;
- Densidade de 1,004 a 1,065 g/cm³;
- Velocidade de sedimentação de 18-130 m/h;
- Índice volumétrico de lodo (IVL) menor que 80 mL/g;
- Porosidade 0,68-0,93;
- 94-97% de água.

Na Figura 8, podem ser visualizadas imagens microscópicas de grânulos capturadas em diversos estudos, utilizando diferentes tipos de efluentes.

Figura 8 - Imagens de microscopia de grânulos aeróbios obtidos em diferentes condições de cultivo: (a, d, e) esgoto doméstico, (b) amido particulado, (c) efluente de abatedouro, (f) efluente particulado e solúvel composto por amido, acetato e peptona, e (g) acetato, metanol, propanol e butanol. Para a escala o leitor deve consultar o trabalho original de cada imagem.



Fonte: indicada em cada imagem.

O desenvolvimento de grânulos aeróbios pode levar de diversas semanas a alguns meses e a sua desintegração ainda é um desafio para aplicação dessa tecnologia (SARMA; TAY, 2018a). O processo de granulação pode ser categorizado em três fases: aclimatação, granulação e maturação indicada pelo tamanho estável dos grânulos (LIU; LIU; TAY, 2004). A densificação das partículas ocorre primeiramente antes do crescimento dos grânulos (WAN; BESSIÈRE; SPÉRANDIO, 2009). Segundo Wan *et al.* (2011), o passo inicial para granulação corresponderia a um aumento da coesão do agregado com grande diminuição do índice volumétrico de lodo (IVL). Ao mesmo tempo, o agregado se torna cada vez mais compacto que os flocos iniciais e, provavelmente, mais denso. A segunda fase acontece quando os grânulos crescem rapidamente, mas pequenos agregados ainda são observados. Isso pode ser devido tanto ao crescimento disperso dos flocos quanto ao desprendimento de partículas da superfície dos grânulos devido a sua erosão pelo cisalhamento. Essas partículas provavelmente contêm microcolônias que funcionam como "sementes" iniciadoras para desenvolvimento de novos grânulos.

A composição do efluente tem sido reportada como fator de peso no processo de granulação. A carga orgânica aplicada no sistema altera a estrutura da comunidade microbiana dos grânulos aeróbios (ADAV; LEE; LAI, 2009), onde uma alta carga de DQO é favorável para

a estabilidade do grânulo, aumentando a concentração do lodo granular e o tamanho dos bioagregados (WANG *et al.*, 2009). Entretanto, grânulos cultivados com esgoto real, onde os substratos são complexos e a carga orgânica é relativamente baixa, são menores que aqueles formados a partir de esgoto sintético (DERLON *et al.*, 2016; ROCKTÄSCHEL *et al.*, 2015), embora haja trabalhos que reportaram o aumento do tamanho dos grânulos com esgoto doméstico (WANG *et al.*, 2009). O tipo de substrato prontamente biodegradável (solúvel) ou lentamente biodegradável (particulado) pode impactar a morfologia dos grânulos, assim como todo o processo de granulação, o que explica o porquê da formação de grânulos com esgoto real geralmente levar mais tempo que quando usado esgoto sintético (WAGNER *et al.*, 2015). Isso possivelmente também explica o fato dos reatores que operam em condições reais terem sempre uma fração de lodo flocular (PRONK *et al.*, 2015b). Embora o processo de granulação seja lento, sobretudo com esgoto real, uma baixa taxa de crescimento dos grânulos pode levar a uma estrutura mais forte, principalmente pela seleção de bactérias nitrificantes que possuem crescimento lento (LIU; YANG; TAY, 2004), onde a boa estrutura granular compensaria o tempo de sua formação. De modo geral, evidências apontam que a granulação com esgoto real requer a redução da pressão de seleção da biomassa e aumento do período anaeróbio para favorecer a hidrólise de particulados e utilização de substrato orgânico (WAGNER *et al.*, 2015).

A síntese de EPS, *quorum sensing*, hidrofobicidade da superfície celular e ligações iônicas têm sido reportados como fatores primários essenciais para granulação (SARMA; TAY; CHU, 2017; ZHANG *et al.*, 2019a). Contudo, os mecanismos de bioagregação ainda não são claros e as conclusões do papel do EPS são na maior parte feitas com base em correlações e hipóteses (DING *et al.*, 2015). Portanto, o foco atual de pesquisas com lodo granular aeróbio tem sido em investigações sobre os mecanismos envolvidos na dinâmica de produção de EPS e seus constituintes, a fim de preencher lacunas existentes na elucidação do processo de granulação.

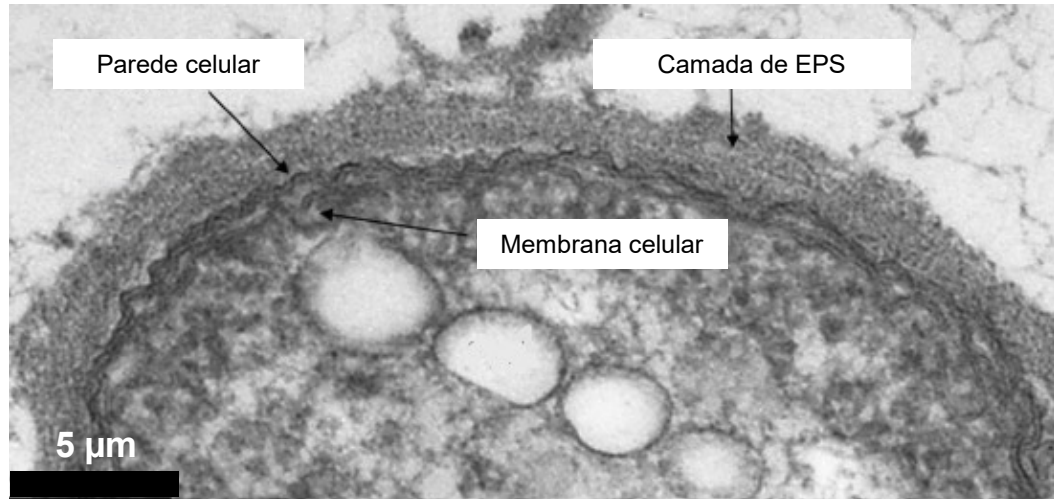
2.2.2 Substâncias poliméricas extracelulares (EPS - *Extracellular polymeric substances*)

Microrganismos aglomerados na forma de biofilmes vivem em uma matriz gelatinosa de substâncias poliméricas extracelulares chamada de EPS (sigla em inglês para *extracellular polymeric substances*) que eles mesmos produzem (BOLTZ *et al.*, 2017). O EPS é o principal componente da matriz de grânulos tanto aeróbios quanto anaeróbios (LIU; LIU; TAY, 2004) cuja composição é complexa, compreendendo proteínas, polissacarídeos, glicoproteínas, ácidos

nucleicos, ácidos húmicos, lipídeos, entre outros (ADAV; LEE, 2008; BOLEIJ *et al.*, 2018; BOLTZ *et al.*, 2017; LIU; LIU; TAY, 2004). O EPS fornece aos biofilmes estabilidade mecânica além de servir como rede polimérica tridimensional que interconecta e imobiliza as células bacterianas a superfícies e dentro do biofilme (BOLTZ *et al.*, 2017), conforme pode ser visualizado na Figura 9.

A formação de biofilme pode efetivamente mitigar efeitos adversos de condições ambientais externas (MIAO *et al.*, 2016), onde o EPS tem um papel essencial em manter a integridade e estabilidade da estrutura dos grânulos (LIU; LIU; TAY, 2004). Embora ainda não completamente compreendidas, diversas funções fisiológicas importantes têm sido propostas para o EPS microbiano, como proteção contra pressões ambientais (stress osmótico, temperatura, pH, danos pela luz ultravioleta, metais pesados e produtos oxidantes), adesão celular a superfícies e reserva de armazenamento de carbono ou água (FREITAS; TORRES; REIS, 2017).

Figura 9 - Localização da camada de EPS ao redor da bactéria *Defluviicoccus* sp. presente em lodo granular aeróbio que trata efluente sintético composto por acetato em reator operado a 35 °C.



Fonte: adaptado de Pronk *et al.* (2017).

A formação de grânulos aeróbios não depende da produção de EPS exclusivamente por uma única população microbiana (SEVIOUR *et al.*, 2012) visto que a capacidade de secreção é amplamente distribuída entre bactérias, fungos e microalgas (FREITAS; TORRES; REIS, 2017). Contudo, as bactérias são geralmente os organismos dominantes em grânulos (SARMA; TAY, 2018a) como, por exemplo, as produtoras de polissacarídeos *Acinetobacter* sp., *Thauera* sp., *Bdellovibrio* sp. e *Paracoccus* sp. (WAN *et al.*, 2013; GUIMARÃES *et al.*,

2017; 2018). Como os grânulos contêm várias comunidades de espécies, uma grande diversidade de polímeros possivelmente com diferentes papéis estruturais é sintetizada (SEVIOUR *et al.*, 2012). Além da produção diretamente pela biomassa, o EPS pode ser constituído por substâncias intracelulares expelidas durante a morte celular (NI *et al.*, 2009).

A quantidade e a composição de EPS produzida dependem de inúmeros fatores como: comunidade microbiana, composição do substrato, tipo do substrato limitante (carbono, nitrogênio ou fósforo), limitação de oxigênio, força iônica, temperatura, tensão de cisalhamento, entre outros (LIU; LIU; TAY, 2004). Seviour *et al.* (2009) observaram que as características dos grânulos e seus biopolímeros foram afetadas pelo pH, temperatura e concentração de sais. Agitação e/ou aeração levando ao cisalhamento (TAY; LIU; LIU, 2004), aumento na carga orgânica (ADAV; LEE; LAI, 2009) e a limitação de nitrogênio (FREITAS; TORRES; REIS, 2017) têm sido reportados como situações favoráveis para produção de EPS pelos microrganismos. Além disso, outros elementos como aminoácidos, açúcares, fósforo, potássio e cátions metálicos são necessários para a síntese de EPS (FREITAS; TORRES; REIS, 2017). Por outro lado, a salinidade do efluente diminui a quantidade de EPS em grânulos (LI *et al.*, 2017a), influenciando nas diferentes frações e composição do EPS (ZHAO *et al.*, 2016). De modo geral, a produção de EPS é aumentada quando a comunidade microbiana está sujeita a condições de cultivo estressantes, o que favorece a formação dos grânulos (QIN; LIU; TAY, 2004).

Independentemente se o foco é a extração do EPS total da biomassa ou de componente(s) específico(s), a sua solubilização é o primeiro passo para, posteriormente, coletá-lo e analisá-lo (BOLEIJ *et al.*, 2018; FELZ *et al.*, 2016; LIN *et al.*, 2018; PRONK *et al.*, 2017; SEVIOUR *et al.*, 2019). Com isso, a quantidade e a composição de EPS recuperado a partir da biomassa variam também significativamente de acordo com a metodologia de extração empregada, a qual influencia fortemente as características do extraído (ADAV; LEE, 2008; HONG *et al.*, 2017). McSwain *et al.* (2005) observaram que a extração de EPS de lodo granular e flocular via dois métodos distintos - um com resina de troca iônica e outro com alta temperatura mais hidróxido de sódio - afetaram sobremaneira o resultado em termos de composição de carbono orgânico total, proteínas e polissacarídeos. Assim, a seleção do método de extração é crucial para alcançar uma efetiva recuperação do componente alvo do EPS requerido (HONG *et al.*, 2017).

Sucessivas extrações do EPS a partir da mesma amostra de lodo granular aeróbio podem aumentar em até 50% a recuperação do biomaterial através da sua depleção da biomassa,

garantindo uma representativa amostragem do EPS (CAUDAN *et al.*, 2012; FELZ *et al.*, 2016). A deterioração da estrutura compacta do grânulo é aparentemente um pré-requisito para uma extração efetiva do EPS (ADAV; LEE, 2008) podendo haver materiais intracelulares extraídos junto com o EPS (FELZ *et al.*, 2016). Embora haja métodos que possibilitem uma apropriada extração sem significativa lise celular (CAUDAN *et al.*, 2012), boa parte deles fazem uso de condições mais amenas de extração (pH em torno de 7 e temperatura em 30 °C) onde poucos polímeros estruturais são solubilizados e, assim, podem escapar da extração (LIN *et al.*, 2018; PRONK *et al.*, 2017). Frente a isso, para uma adequada análise da estrutura do EPS, a destruição das células microbianas é inevitável (PRONK *et al.*, 2017; SEVIOUR *et al.*, 2019).

A quantidade e a composição do EPS extraído variam grandemente em função da metodologia empregada, do tipo de lodo, do tipo de tratamento e da operação do reator conforme disposto na Tabela 1. Assim, embora haja vários métodos físicos e químicos de extração de EPS, não há um procedimento padrão, o que faz a comparação quantitativa e qualitativa do EPS muito difícil (LIU; LIU; TAY, 2004; SEVIOUR *et al.*, 2019). Isso também dificulta a postulação de teorias genéricas sobre a quantidade e composição do EPS nos grânulos e flocos.

Conforme as pesquisas avançam, novos tipos de EPS vão sendo descobertos e recuperados por métodos de extração diferentes dos habitualmente empregados. Pronk *et al.* (2017) extraíram uma nova substância polimérica extracelular ácida (chamada de EPS solúvel ácido) de lodo granular aeróbio dominado pelas bactérias *Deffluviococcus* sp. em reator alimentado com acetato operado a 35 °C. Ácido acético, ao invés das frequentes condições de extração alcalinas, foi necessário para dissolver os grânulos e solubilizar a matriz polimérica. O trabalho mostrou que não há um método único que possa ser usado para extrair todos os EPS da biomassa, devendo ser desenvolvido com foco no tipo de grânulo e EPS de interesse. Logo, avaliações criteriosas a fim de adequar o método de extração com as características da biomassa de onde serão extraídos os biopolímeros são necessárias para obter produtos recuperados representativos.

Tabela 1 - Quantificação e caracterização de EPS extraído por métodos variados a partir de diferentes lodos (biomassa) cultivados em diferentes condições operacionais.

Origem do EPS	Reator	Método de extração	Composição	Referência
Flocos e biofilme anammox tratando chorume de aterro sanitário	RBS com biomassa imobilizada. Ciclo de 24 h com 5 h de enchimento. Aeração, sedimentação e descarte variável conforme desempenho	Solução buffer com Na ₃ PO ₄ , NaH ₂ PO ₄ , NaCl e KCl e ultrassonificação	Carboidratos (71-73%), proteínas (≈20%) e ácidos nucleicos (<10%)	Miao et al. (2016)
Grânulos anammox tratando esgoto sintético	UASB	Resina de troca iônica	113-127.2 mg _{EPS} /g _{SSV} , proteínas (56,6-75,3 mg/g _{SSV}), carboidratos (70,8-36 mg/g _{SSV})	Ni et al. (2015)
Grânulos aeróbios tratando fenol	RBS com 5 min de enchimento, 320 minutos de aeração, 30 min de sedimentação e 5 min de descarte	Formamida e NaOH	Proteínas (240 ± 13 mg/g _{SSV}), carboidratos (61 ± 9,4 mg/g _{SSV}) e lipídios (51,1 ± 7,8 mg/g _{SSV})	Adav et al. (2008b)
Grânulos aeróbios tratando efluente salino sintético	RBS com 6 min de enchimento, 120 min de aeração, 5 min de sedimentação, 5 min de decantação e 8 min de inatividade	Formaldeído e NaOH	250-75 mg _{EPS} /g _{SSV} conforme o tempo de operação e porcentagem de salinidade no efluente	Li et al. (2017)
Grânulos aeróbios tratando esgoto doméstico	RBS com 5 min de enchimento, 125-200 min de aeração, 15-30 min de sedimentação e 20 min de descarte	EDTA	Carboidratos (62,2 ± 5,8 mg/g _{SSV}), proteínas (26,5 ± 4,5 mg/g _{SSV}),	Ni et al. (2009)
Grânulos aeróbios e flocos tratando esgoto sintético com acetato	RBS com 5 min de enchimento, 65 min de anoxia, 270 min de aeração, 10 min de sedimentação e 10 min de descarte	Centrifugação, lavagem com água deionizada e alta temperatura	Flocos: proteínas (20,75 mg/g _{SSV}) carboidratos (15,22 mg/g _{SSV}) Grânulos: proteínas (15,05 mg/g _{SSV}) carboidratos (9,94 mg/g _{SSV})	Lv et al. (2014)

Origem do EPS	Reator	Método de extração	Composição	Referência
Grânulos aeróbios tratando esgoto sintético	RBS com 5 min de enchimento, 3h 45 min de aeração e 10 min de descarte	Ultrassonificação e formaldeído	140 a 300 mg EPS/g _{SSV}	Adav et al. (2009)
Grânulos aeróbios e flocos de lodo ativado tratando fenol	RBS com ciclo de 12 horas	Variado, usando formaldeído, formamida, calor, ultrassonificação e NaOH	Flocos: proteínas (35,9-62,2 mg/g _{SSV}), carboidratos (44,1-59,1 mg/g _{SSV}), substâncias húmicas (45,8-77,5 mg/g _{SSV}) e lipídeos (10,7-18,3 mg/g _{SSV}) Grânulos: proteínas (455,2-238,2 mg/g _{SSV}), carboidratos (57,2-109,3 mg/g _{SSV}), substâncias húmicas (49,3-85,2 mg/g _{SSV}) e lipídeos (38,2-68,4 mg/g _{SSV})	Adav e Lee (2008)
Grânulos aeróbios tratando efluente sintético	RBS com 15 min de enchimento, 20 min mistura anóxica, 145 min aeração, 30 min sedimentação e 30 min de descarte	Ultrassonificação, detergente não-iônico e EDTA	Proteínas (211,5 ± 24,6 mg/g _{SSV}), polissacarídeos (58,3 ± 11,9 mg/g _{SSV})	Caudan et al. (2012)
Grânulos aeróbios tratando esgoto doméstico	Sem descrição	Na ₂ CO ₃ e alta temperatura	Aproximadamente 325 mg EPS/g _{SSV}	Felz et al. (2016)
Grânulos aeróbios sob condições termofílicas tratando acetato	RBS com 1 hora de enchimento, 112 min de aeração, 3 min de sedimentação e 5 min de descarte	Na ₂ CO ₃ , alta temperatura e ácido acético	166 ± 6 mg _{EPS} /g _{SSV}	Pronk et al. (2017)
Flocos e grânulos de RBS tratando acetato e efluente de cervejaria	Sem descrição	Resina de troca iônica	34.6 mg _{EPS} /g _{SSV}	Sam e Dulekgurgen (2015)
Flocos tratando esgoto doméstico	Tanque de aeração de lodos ativados	NaOH e alta temperatura	421 mg _{EPS} /g _{SSV} , proteínas (186 mg/g _{SSV}), polissacarídeos (46 mg/g _{SSV}), substâncias húmicas (56 mg/g _{SSV})	Hong et al., (2017)

Conceitualmente, algumas funções básicas, propriedades e constituintes da matriz de EPS são conhecidas, mas sua cinética de produção e consumo, sua contribuição para conversão de materiais e para as taxas de transformações bioquímicas em biofilmes são pouco definidas (BOLTZ *et al.*, 2017). Além disso, há diferentes aspectos do EPS inadequadamente explicados que requerem investigação detalhada como, por exemplo, se algumas espécies particulares ou quase todos os microrganismos são responsáveis pela sua produção (SARMA; TAY; CHU, 2017). Frente a essas lacunas e à grande diversidade de polímeros passíveis de serem recuperados com métodos consolidados ou novos, a realização de pesquisas com EPS se torna relevante na área do tratamento de efluentes atualmente. Um melhor entendimento sobre a matriz de EPS levará ao desenvolvimento de melhores estratégias tanto para recuperação de bioprodutos quanto para o gerenciamento de biofilmes em sistemas biológicos de tratamento (SEVIOUR *et al.*, 2019).

2.2.3 O papel dos polímeros que formam géis: polímeros que se comportam como alginato (ALE)

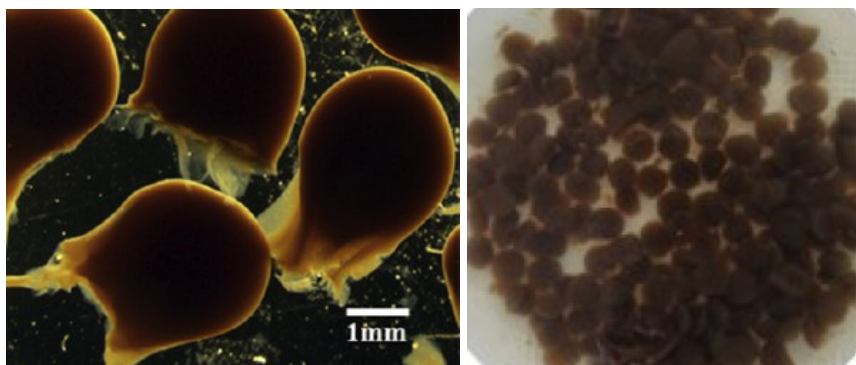
Os atributos apresentados pelos grânulos fazem com que sejam caracterizados como hidrogéis, assim há a formação de uma rede polimérica reticulada que possibilita a gelificação (SEVIOUR *et al.*, 2009), a qual é dita como o principal mecanismo para desenvolvimento da granulação aeróbia (LI *et al.*, 2014). Em vista disso, os hidrogéis são componentes chaves para granulação (YANG *et al.*, 2014) e o conhecimento da sua dinâmica de produção e consumo é relevante para o controle de processos de tratamento. Nesse contexto, o estudo da presença de polímeros no EPS que possibilitem essa formação de gel tem despertado grande interesse, impulsionando pesquisas a fim de elucidar mecanismos de granulação assim como a recuperação desses materiais.

O emprego da terminologia de hidrogel como característica essencial de polímeros de lodo granular aeróbio surgiu na última década. Seviour *et al.* (2012) extraíram um exopolissacarídeo com propriedades de hidrogel a partir de grânulos alimentados com acetato o qual foi chamado de “Granulan”. Com efluente real, o termo ALE (do inglês *alginate-like exopolymers*) foi descrito primeiramente na literatura por Lin *et al.* (2010) em estudo onde exopolissacarídeos semelhantes ao alginato foram extraídos de lodo granular aeróbio e caracterizados. Mais recentemente, em um trabalho onde a metodologia de extração de ALE foi refinada, Felz *et al.* (2016) o denominaram de polímeros extracelulares semelhantes ao

alginato (do inglês *alginate-like extracellular polymers*), possivelmente pelo fato de não somente os polissacarídeos, mas outros compostos orgânicos comporem o biomaterial. No processo de recuperação de ALE, primeiramente se extrai o EPS da biomassa em condições de alta temperatura (80 °C), alto pH (10-11) e agitação. Em seguida, ocorre a precipitação do ALE na forma ácida (pH \approx 2.2) (FELZ *et al.*, 2016). Alguns trabalhos que caracterizaram o ALE confirmaram que ele é um biopolímero complexo composto por proteínas, polissacarídeos (neutros e urônicos), glucosaminas, glicoconjugados sulfatados, ácidos húmicos e lipídeos (FELZ *et al.*, 2019, 2020a; LIN *et al.*, 2015).

ALE possui semelhança com alginato comercial em termos de propriedades de formação de hidrogel (KARAKAS *et al.*, 2020; SAM; DULEKGURGEN, 2015). Em testes para formação de hidrogel, ALE de cálcio é produzido na reação entre ALE de sódio e íons de cálcio em solução (FELZ *et al.*, 2016), formando estruturas em forma de pequenas esferas chamadas comumente nos trabalhos da área como *beads* (Figura 10). Além do cálcio, ALE pode formar hidrogéis com diversos íons metálicos, contudo, a química de formação de hidrogéis de ALE é diferente da do alginato devido as suas diferentes composições (FELZ *et al.*, 2020b). Além disso, hidrogéis de ALE podem conter até 98% de água (SAM; DULEKGURGEN, 2015). Essa alta capacidade de absorção de água também estimula o reaproveitamento de ALE a partir da sua recuperação da biomassa, visto que a desidratação e disposição do lodo contribuem para grande parte dos custos de operação de uma ETE (SEVIOUR *et al.*, 2009).

Figura 10 - Morfologia das esferas de ALE reticuladas em solução de CaCl₂ extraídas de lodo granular aeróbio.



Fonte: Lin *et al.* (2013) (esquerda) e Dall' Agnol (2020) (direita).

ALE faz parte do EPS e tem sido extraído com sucesso não só de grânulos, mas também de flocos de lodo proveniente de tratamento de esgoto real, apresentando propriedade

de hidrogel e elasticidade (LIN *et al.*, 2010; LIN; SHARMA; VAN LOOSDRECHT, 2013), embora possa haver diferenças em suas características químicas e mecânicas (LIN; SHARMA; VAN LOOSDRECHT, 2013). O ALE é um dos polímeros dominantes em grânulos, fornecendo alta hidrofobicidade além de uma estrutura compacta, forte e elástica que dá proteção aos microrganismos assim como aumenta a sedimentabilidade (LIN *et al.*, 2010; SAM; DULEKGURGEN, 2015). Ele é um importante polímero estrutural com relevante importância na formação e estabilidade de grânulos (FELZ *et al.*, 2016; LIN *et al.*, 2010) onde o alginato é um componente (LIN *et al.*, 2010). A sua alta fração de blocos de ácido gulurônico (GG)² (aproximadamente 69%) contribui para a alta hidrofobicidade, força e flexibilidade do lodo granular aeróbio (LIN; SHARMA; VAN LOOSDRECHT, 2013). ALE tem se mostrado o principal componente do EPS (cerca de 63%) de grânulos de sistemas que tratam esgoto doméstico (FELZ *et al.*, 2016), podendo contribuir para o desenvolvimento da biomassa e assim melhorar a performance do tratamento (SUN; ZHAN; WANG, 2017). O aumento da concentração de ALE tem sido reportado como um pré-requisito para formação de grânulos maduros, entretanto somente o seu aumento não garante a granulação (YANG *et al.*, 2014).

Na Tabela 2 estão resumidos alguns trabalhos que extraíram ALE de grânulos e flocos tratando diferentes tipos de efluentes, mostrando detalhes da metodologia de extração empregada, rendimento e caracterização do ALE. A metodologia mais consolidada para extração de ALE até o momento foi estabelecida por Felz et al. (2016). A faixa de rendimento de recuperação de ALE a partir do lodo biológico varia de 23 mg_{ALE}/g SV_{floco} a 253 ± 14 mg_{ALE}/g SV_{grânulo} (expresso em termos de sólidos voláteis). Como se pode observar na Tabela 2, há diferentes tempos de extração assim como presença ou não de etapa de agitação e/ou purificação (lavagem com água, diálise e/ou e precipitação com etanol). As variações no rendimento e composição do ALE podem ser devido às diferentes origens do lodo assim como a diferenças em etapas do método de extração. Lembra-se que a possível influência do tipo de lodo é um dos objetivos da presente tese de doutorado e será elucidada na seção 4.2, assim como será feita uma discussão sobre a influência do protocolo de extração.

² A caracterização química detalhada do alginato é apresentada na seção 2.1.2.

Tabela 2 - Quantificação e caracterização do ALE extraído em condições alcalinas e de alta temperatura a partir de diferentes lodos (biomassa) cultivados em diferentes condições operacionais. O rendimento é expresso em termos de sólidos voláteis (SV).

Origem do ALE	Método de extração	Rendimento	Composição	Referência
Lodo granular aeróbio peneirado proveniente de esgoto doméstico (Nereda®)	80 °C, alto pH, agitação (35 min) e posterior diálise	Não informado	Variou conforme o método colorimétrico e padrão empregado: <ul style="list-style-type: none"> • Proteínas (43-38%) • Açúcares neutros (14-11%) • Açúcares urônicos (13-7%) • Compostos fenólicos (29-5%) • Açúcares alcoólicos • Aminoaçúcares 	Felz et al. (2019)
Lodo granular aeróbio peneirado proveniente de esgoto doméstico (Nereda®)	80 °C, alto pH, agitação (35 min) e posterior diálise	$253 \pm 14 \text{ mg}_{\text{ALE}}/\text{g SV}_{\text{grânulo}}$	Compostos semelhantes à ácido hialurônico e glicosaminoglicanos sulfatados: $87 \pm 6 \text{ mg}_{\text{glicosaminoglicanos}} / \text{g SSV}_{\text{ALE}}$	Felz et al. (2020)
Lodo granular aeróbio peneirado proveniente de esgoto doméstico (Nereda®)	80 °C, alto pH e agitação (35 min)	$\approx 205 \text{ mg}_{\text{ALE}}/\text{g SV}_{\text{grânulo}}$	Não caracterizado	Felz et al. (2016)
Lodo granular aeróbio (Nereda®) e flocos provenientes de esgoto sanitário composto por 25% de efluente de abatedouro	80 °C e alto pH (60 min) com lavagem do ALE com água deionizada e precipitação com etanol	$72 \pm 6 \text{ mg}_{\text{ALE}}/\text{g SV}_{\text{floco}}$ $160 \pm 4 \text{ mg}_{\text{ALE}}/\text{g SV}_{\text{grânulo}}$	Grânulo com $69.1 \pm 8.9\%$ de blocos GG Flocos com $35.3 \pm 8.4\%$ de blocos GG	Lin et al. (2013)

Origem do ALE	Método de extração	Rendimento	Composição	Referência
Lodo granular aeróbio de reator operado a 35 °C tratando esgoto sintético com acetato	80 °C e alto pH (30 min)	75 mg _{ALE} /g SV _{grânulo}	Não caracterizado	Pronk et al. (2017)
Lodo granular aeróbio proveniente de esgoto salino sintético com propionato	80 °C e alto pH (210 min) com lavagem do ALE com água deionizada e precipitação com etanol	≈ 100 mg _{ALE} /g SV _{grânulo}	Os principais blocos do ALE foram o MG ao longo da granulação	Yang et al. (2014)
Lodo granular aeróbio proveniente de esgoto salino sintético com acetato, glicose e leveduras	80 °C e alto pH (60 min) com lavagem do ALE com água deionizada e precipitação com etanol	≈ 25 - 55 mg _{ALE} /g SV _{grânulo}	Maior proporção de blocos GG (≈ 75%) com o aumento da salinidade	Li et al. (2017)
Lodo granular aeróbio proveniente de esgoto salino sintético com acetato e glicose	80 °C, alto pH e agitação (60 min) com lavagem do ALE com água deionizada e precipitação com etanol	Até 49,8 mg _{ALE} /g SV _{grânulo}	Mais blocos GG (23 mg/g SV _{ALE}) com 1% de salinidade, >80% de moléculas com peso acima de 100 kDa, Galactosidade, glicosamina, lipídeos e octadecanamida	Meng et al. (2019)
Lodo granular aeróbio (Nereda®) e flocos de lodo ativado provenientes de esgoto doméstico	80 °C, alto pH e agitação (40 min) com lavagem do ALE com água deionizada e precipitação com etanol	150 ± 20 mg _{ALE} /g SV _{floco} 253 ± 14 mg _{ALE} /g SV _{grânulo}	Significante presença de compostos fosfatados	Kim et al. (2020)
Lodo granular aeróbio e flocos de esgoto sintético com acetato e efluente de cervejaria	80 °C e alto pH (60 min) com lavagem do ALE com água deionizada e precipitação com etanol	≈ 23 mg _{ALE} /g SV _{floco} ≈ 43 ± 14 mg _{ALE} /g SV _{grânulo}	37.5 mg _{glicose} /g SV _{floco} 78.0 mg _{glicose} /g SV _{grânulo}	Sam & Dulekgurgen (2015)

Origem do ALE	Método de extração	Rendimento	Composição	Referência
Lodo granular aeróbio peneirado proveniente de esgoto sanitário composto por 25% de efluente de abatedouro (Nereda®)	80 °C e alto pH (60 min) com lavagem do ALE com água deionizada e precipitação com etanol	$160 \pm 4 \text{ mg}_{\text{ALE}}/\text{g SV}_{\text{grânulo}}$	$486 \pm 22 \text{ mg}_{\text{alginato}}/\text{g SV}_{\text{ALE}}$ 69 ± 9 % blocos GG 15 ± 2% blocos MG 2 ± 1% blocos MM	Lin et al. (2010)
Lodo granular aeróbio proveniente de reator operado a 20 °C tratando de esgoto sintético com acetato	80 °C e alto pH (60 min) com lavagem do ALE com água deionizada e precipitação com etanol	$125 \pm 14 \text{ mg}_{\text{ALE}}/\text{g SV}_{\text{grânulo}}$	Não caracterizado	Lin et al. (2012)
Lodo granular aeróbio proveniente de esgoto sintético com acetato em reator com recirculação de ar	80 °C, agitação e alto pH (60 min) com lavagem do ALE com água deionizada e precipitação com etanol	Não caracterizado	Cerca de 50% de blocos GG, seguidos pelos blocos MG e MM	Zhang et al. (2019)
Lodo granular aeróbio proveniente de esgoto doméstico	80 °C, alto pH e agitação (35 min)	$213 \pm 17 \text{ mg}_{\text{ALE}}/\text{g SV}_{\text{grânulo}}$	Não caracterizado	Dall' Agnol et al. (2020)
Lodo granular aeróbio proveniente de esgoto doméstico	80 °C, alto pH e agitação (35 min)	$175 \pm 24 \text{ mg}_{\text{ALE}}/\text{g SV}_{\text{grânulo}}$	Não caracterizado	Ladnorg et al. (2019)

Conforme características operacionais, do efluente e das comunidades microbianas, o ALE pode apresentar diferentes características embora mantenha suas propriedades de hidrogel (SAM; DULEKGURGEN, 2015). Acredita-se que microrganismos de crescimento lento como os do grupo PAO (organismos acumuladores de poli-fosfato) são importantes na produção de EPS e ALE (GUIMARÃES, 2017). Em estudo que explorou os efeitos da alta salinidade na agregação de grânulos para o tratamento de efluente salino sintético, observou-se que o ALE pode ser um componente ativo para estimular a granulação através da reticulação com íons metálicos (LI *et al.*, 2017a). Efluentes salinos aumentam a quantidade de ALE extraída de lodo granular aeróbio assim como a proporção de blocos GG (LI *et al.*, 2017a; MENG *et al.*, 2019), o que faz com que o hidrogel resultante seja mais rígido (HAY *et al.*, 2013). Ademais, baixas concentrações de oxigênio dissolvido favorecem a produção de alginato (FREITAS; TORRES; REIS, 2017), o que pode ser uma condição que também influencie no rendimento de ALE extraído de lodo biológico. A aceleração da granulação pode ser alcançada pelo estímulo da produção de hidrogel alternando diferentes cargas orgânicas (WHITELEY; LEE, 2015; YANG *et al.*, 2014). Assim, cargas de choque comumente encontradas em ETEs em escala real podem favorecer a produção de ALE, tornando-se uma interessante fonte de recuperação desse biopolímero.

Apesar de o “granulan” e o ALE serem estruturas que explicam a formação de gel nos grânulos, a contribuição de outros polímeros não pode ser descartada (SEVIOUR *et al.*, 2012). Há certamente possibilidade que outros tipos de EPS formem diferentes tipos de hidrogéis existindo em diferentes tipos de grânulos (FELZ *et al.*, 2016). Em efluente não caracterizado como esgoto doméstico, o ALE representou menos de 1% da fração orgânica dos grânulos, o que confirma que o EPS difere em cada tipo de lodo (PRONK *et al.*, 2017). Logo, novas pesquisas que esclareçam a composição, propriedades e fatores que interferem na produção desses diferentes biopolímeros contribuem para a ampliação do controle e conhecimento de processos de granulação, assim como para a futura recuperação de recursos e aplicação industrial.

2.3 POLÍMEROS QUE SE COMPORTAM COMO ALGINATO (ALE) COMO FONTE DE MATÉRIA-PRIMA

A produção microbiana de biopolímeros é uma alternativa sustentável que não depende somente do uso de ecossistemas oceânicos como no caso do alginato (ANDERSON;

ISLAM; PRATHER, 2018). Já que o alginato é um produto proveniente de limitadas fontes naturais, a possibilidade de desenvolver novas classes de alginato com características químicas e físicas específicas pode revolucionar o uso desse material (LEE; MOONEY, 2012). Novas fontes de biopolímeros que apresentem características semelhantes ao alginato, como o ALE, têm grande potencial de abrir um novo nicho de mercado dentro do contexto das biorrefinarias (GUIMARÃES, 2017).

As condições de mercado atuais favorecem a produção de ALE a partir de esgoto doméstico. Em estudos de viabilidade de recuperação de diversos recursos de ETE, o ALE tem sido classificado como o mais valioso, compreendendo cerca de 50% do valor monetário que pode ser gerado considerando também a recuperação de bioplástico, fósforo, biogás e celulose (VAN DER HOEK; FOOIJ; STRUKER, 2016). Na Holanda, espera-se que cerca de 85.000 toneladas de ALE sejam recuperadas de 10 diferentes ETE até 2030, o que gerará até 170 milhões de euros (VAN LEEUWEN *et al.*, 2018). Em termos da realidade nacional, com esgoto doméstico brasileiro tratado em um reator piloto, a produção de ALE foi cerca de 29 g SV_{ALE}/m^3 de esgoto tratado (DALL'AGNOL *et al.*, 2020), o que é um dado inédito obtido nas condições locais que pode dar suporte a futuras análises de custo-benefício.

A extração comercial de ALE em larga escala já é uma realidade em que a Holanda é o país que está vanguarda. O nome comercial do ALE é Kaumera® (KIM *et al.*, 2020). A primeira unidade em escala real de recuperação de ALE foi oficialmente aberta em outubro de 2019 na cidade de Zutphen (Holanda) com estimativa de produção de 400 toneladas por ano, e a segunda unidade espera ser aberta até o fim de 2020 (KAUMERA, 2020). A implantação dessas biorrefinarias faz parte de um programa nacional holandês para recuperação de ALE com participação de organismos públicos, empresas privadas e universidades (ROYAL HASKONINGDHV, 2017).

Recentemente, a literatura tem reportado com mais frequência usos mais detalhados do ALE, o qual tem se mostrado um bioproduto bastante versátil. De modo geral, ALE pode ser usado para melhorar a qualidade de papel, como espessante de tintas, massas e géis, para melhorar a cura do concreto, para produção de fertilizantes, pode ser aplicado em superfícies para resistência ao fogo e para produção de tecidos adsorventes de fluidos (VAN LEEUWEN *et al.*, 2018; KAUMERA, 2020). ALE extraído tanto de flocos quanto de grânulos apresenta comportamento de resistência ao fogo, o que o faz adequado para uso como material de revestimento retardante de chamas, alcançando requerimentos da Agência de Regulação de Aviação Americana (KIM *et al.*, 2020). O ALE tem propriedade anfifílica e forma filme flexível e homogêneo em superfícies, o que dá a ele o potencial de ser usado como agente de

revestimento impermeabilizante à água em superfícies (LIN *et al.*, 2015). Além disso, hidrogéis de ALE também tem se mostrado promissores biossorventes com capacidade de remover corantes, como o azul de metileno (LADNORG *et al.*, 2019), e fósforo de soluções sintéticas (DALL'AGNOL *et al.*, 2020). Além do aproveitamento da fração orgânica, a parte inorgânica do ALE pode ser uma fonte do mineral fertilizante estruvita (LIN; BASSIN; VAN LOOSDRECHT, 2012), o que faz com que esse biomaterial possa ser aproveitado de diferentes maneiras.

O desenvolvimento industrial de processos microbianos com recuperação de EPS e comercialização dos biopolímeros (como o ALE) requer um melhor entendimento das suas configurações estruturais e propriedades físico-químicas. Além disso, ainda é necessária pesquisa em biossíntese, produção e recuperação (FREITAS; TORRES; REIS, 2017). Logo, mais estudos que esclareçam essas lacunas existentes podem contribuir para a futura consolidação de ETE como biorrefinarias para recuperação de ALE.

3 MATERIAIS E MÉTODOS

Conforme já exposto anteriormente, essa tese é composta por três diferentes artigos científicos que exploraram os objetivos inicialmente propostos. Essa seção busca mostrar de forma resumida o delineamento experimental utilizado em cada um dos três artigos. Maiores detalhes metodológicos podem ser consultados diretamente nas seções referentes a cada artigo. Parte do presente trabalho foi conduzido no Brasil na UFSC (Artigo 1) e parte na Suíça na Eawag (Artigos 2 e 3). A Figura 11 expõe de modo esquemático o fluxograma metodológico utilizado na tese.

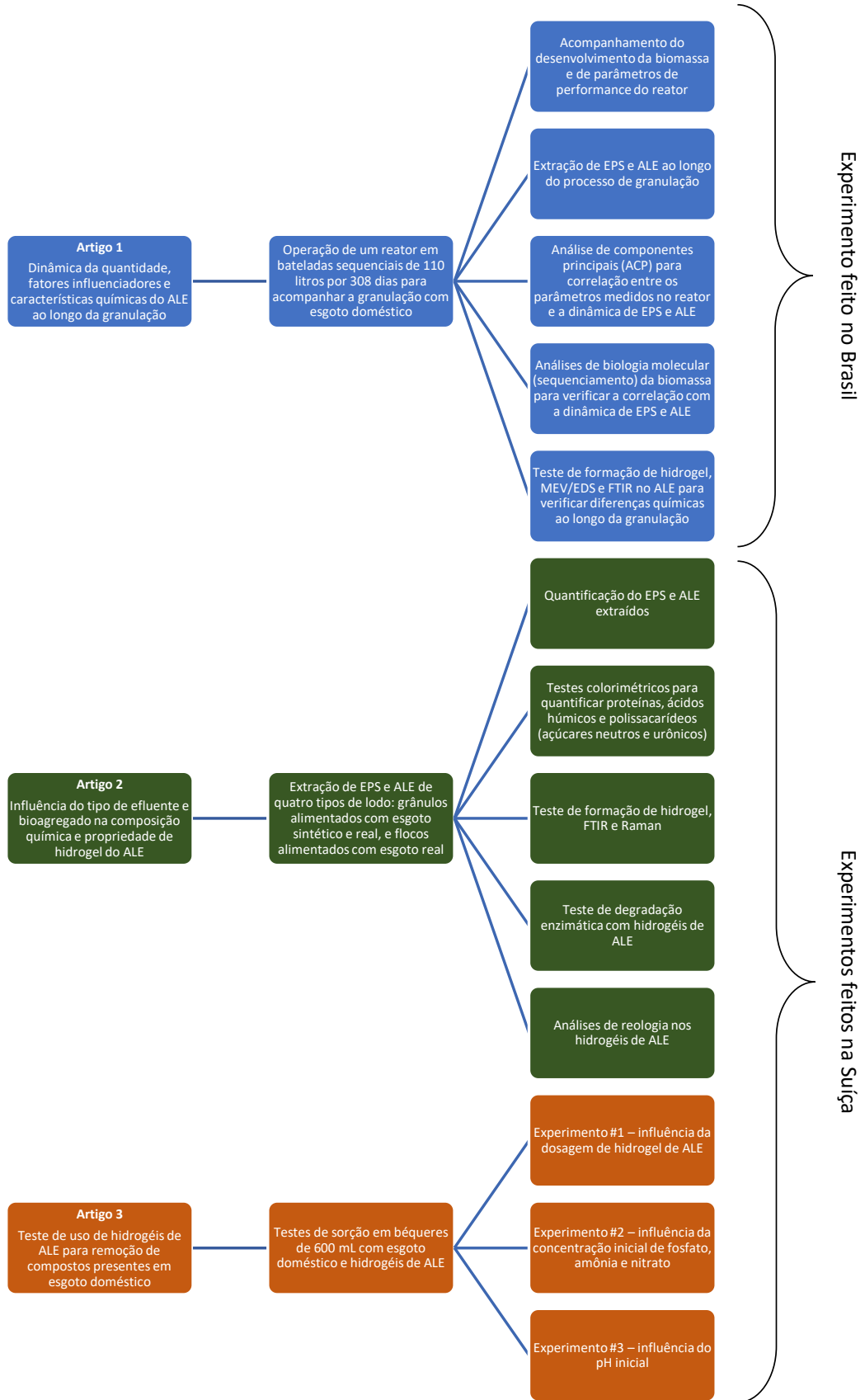
3.1 EXTRAÇÃO DE EPS E ALE

A metodologia principal da tese, que foi empregada em todos os artigos, refere-se à extração de EPS e, principalmente, de ALE a partir do lodo biológico de esgoto. A extração dos biopolímeros foi feita conforme os procedimentos estabelecidos por Felz et al. (2016) com algumas adaptações. Resumidamente, segundo esse método, o EPS é extraído (solubilizado) do lodo (biomassa) em condições alcalinas, alta temperatura e sob agitação com posterior precipitação do ALE em condições ácidas.

Uma massa conhecida de lodo foi coletada de diferentes reatores e centrifugada. O lodo centrifugado foi transferido para recipiente de vidro preenchido com água e carbonato de sódio (Na_2CO_3). Uma proporção de 3:50:0,25 de lodo, água destilada e Na_2CO_3 foi mantida em todos os experimentos. A solução foi aquecida a 80 °C e agitada por 35 minutos a aproximadamente 400 rpm. Em seguida, a solução foi centrifugada e o sobrenadante coletado, o qual correspondeu ao EPS solubilizado. O ALE foi precipitado a partir do EPS com a adição de 1 M de ácido clorídrico (HCl) sob agitação lenta de aproximadamente 100 rpm até o pH final chegar a $2,20 \pm 0,05$. Em seguida, a solução foi centrifugada, o sobrenadante descartado e o pellet coletado, o qual correspondeu ao ALE na forma ácida.

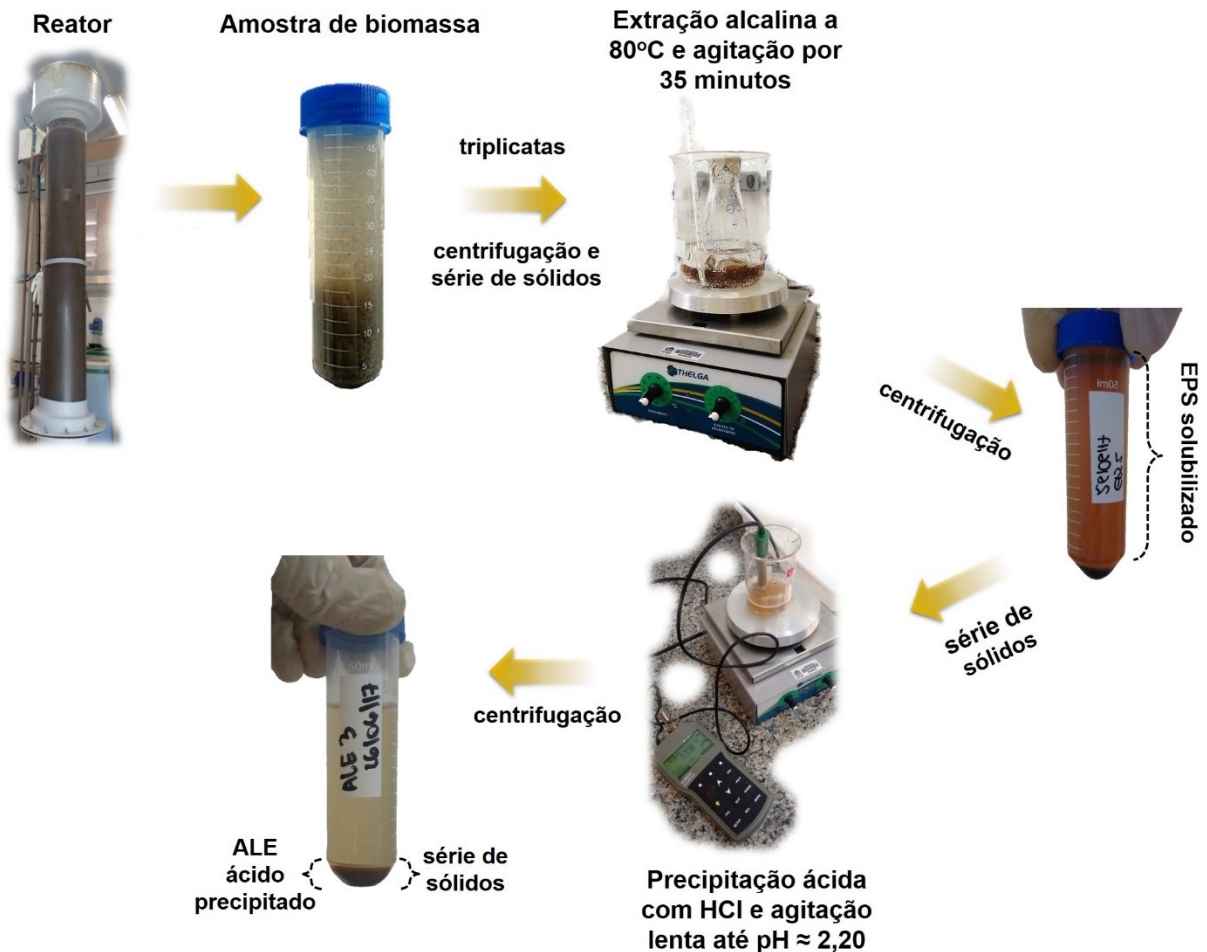
Todas as extrações foram feitas no mínimo em triplicatas e os resultados quantitativos do lodo, EPS e ALE são expressos como média em termos de sólidos totais (ST) ou sólidos voláteis (SV) medidos por métodos gravimétricos de acordo com Felz et al. (2016). A Figura 12 ilustra de modo esquemático a metodologia de extração de EPS e ALE empregada.

Figura 11 - Fluxograma metodológico geral desta tese de doutorado.



Fonte: Elaborada pelo autor (2020).

Figura 12 - Esquema do protocolo de extração de EPS e ALE utilizado nesse trabalho com base em Felz et al. (2016).



Fonte: Elaborada pelo autor (2020).

3.2 ARTIGO 1

A primeira parte experimental da tese abordou o processo de granulação e a dinâmica de quantidade de EPS e ALE durante o tratamento de esgoto doméstico. Para isso, foi utilizado um reator em bateladas sequenciais (RBS) de 110 L que tratava esgoto doméstico do município de Florianópolis. O reator estava instalado nas dependências do LABEFLU na UFSC e foi operado por 308 dias. Parâmetros operacionais do reator e de desempenho do tratamento foram acompanhados periodicamente. Concomitantemente, amostras de lodo foram coletadas para extração de EPS e ALE e, assim, avaliar a sua dinâmica de produção e relação com o processo de granulação. Análise de componentes principais (ACP) foi utilizada para verificar a relação dos parâmetros medidos no reator com a dinâmica de EPS e ALE. Sequenciamento de DNA foi

empregado para caracterização da comunidade microbiana, cuja correlação foi avaliada em relação à dinâmica de EPS e ALE. Ao fim, testes de formação de hidrogel iônico com cloreto de cálcio (CaCl_2) e análises de FTIR e MEV/EDS foram realizadas para verificar diferenças na propriedade de formação de hidrogel, nos grupos funcionais e na composição elementar do ALE ao longo do processo de granulação.

3.3 ARTIGO 2

Essa parte do trabalho buscou avaliar a influência do tipo de bioagregado (grânulos e flocos) e do tipo de efluente (sintético e real) na composição e nas propriedades de hidrogel do ALE. Para isso, EPS e ALE foram extraídos de quatro diferentes tipos de lodo (Tabela 3): (i) grânulos aeróbios que tratavam esgoto sintético composto por ácidos graxos voláteis; (ii) grânulos aeróbios que tratavam esgoto sintético composto por ácidos graxos voláteis, matéria orgânica não fermentada e matéria orgânica particulada; (iii) grânulos aeróbios que tratavam esgoto doméstico real; (iiii) e flocos de lodos ativados que tratavam esgoto doméstico real. Todos os lodos vieram de reatores instalados nas dependências da Eawag na Suíça. Os métodos colorimétricos Lowry modificado e Antrona foram empregados para quantificar as proteínas, ácidos húmicos e polissacarídeos (açúcares neutros e urônicos) respectivamente no EPS e ALE. O ALE também foi caracterizado por meio de FTIR, Raman e testes de formação de hidrogel iônico com CaCl_2 . Testes enzimáticos com quatro diferentes enzimas (alginate liase, papaína, savinase® e lisozima) foram feitos para verificar qual grupo químico/ligação estava envolvido na manutenção da estrutura de hidrogel do ALE. Por fim, as propriedades mecânicas dos hidrogéis de ALE foram avaliadas por meio de ensaios reológicos.

Tabela 3 – Detalhes dos quatro diferentes tipos de lodo usados no Artigo 2.

Tipo de lodo	Esgoto	Fonte de carbono	Reator
Lodo granular aeróbio	Simples sintético	Ácidos graxos voláteis: 1/2 acetato e 1/2 propionato (DQO solúvel)	Escala laboratorial (17 litros)
Lodo granular aeróbio	Complexo sintético	1/3 DQO: acetato e propionato (DQO solúvel) 1/3 DQO: aminoácidos e glicose (DQO solúvel) 1/3 DQO: amido e peptona (DQO particulada)	Escala laboratorial (17 litros)
Lodo granular aeróbio	Doméstico após remoção de sólidos sedimentáveis	Complexa (40% DQO solúvel, 60% DQO particulada)	Escala real (8.000 litros)
Flocos de lodos ativados	Doméstico após remoção de sólidos sedimentáveis	Complexa (40% DQO solúvel, 60% DQO particulada)	Escala real (8.000 litros)

3.4 ARTIGO 3

Esse artigo buscou avaliar a capacidade de hidrogéis de ALE em remover diferentes compostos aniônicos e catiônicos presentes em esgoto doméstico. Para isso, béqueres de 600 mL foram preenchidos com 300 mL de esgoto doméstico e hidrogéis de ALE recuperados de grânulos aeróbios que tratavam esgoto doméstico da cidade de Dübendorf (Suíça). Os béqueres foram agitados em velocidade constante e a temperatura mantida em torno de 21 °C. Três diferentes experimentos foram rodados para avaliar a influência: (i) de diferentes concentrações iniciais de hidrogéis de ALE na remoção de PO_4^{3-} , NH_4^+ , NO_3^- , NO_2^- , Cl^- , Na^+ , K^+ , Ca^{2+} e Mg^{2+} ; (ii) da composição do esgoto em termos das concentrações iniciais de PO_4^{3-} , NH_4^+ e NO_3^- ; (iii) do pH inicial do esgoto (pH 8,57, 7,00 e 6,00). Além disso, espectrometria de emissão atômica por plasma acoplado indutivamente foi utilizada para adquirir informações sobre elementos químicos específicos removidos pelos hidrogéis de ALE.

4 RESULTADOS E DISCUSSÕES

4.1 ARTIGO 1 - BIOPOLYMERS RECOVERY: DYNAMICS AND CHARACTERIZATION OF ALGINATE-LIKE EXOPOLYMERS IN AN AEROBIC GRANULAR SLUDGE SYSTEM TREATING MUNICIPAL WASTEWATER WITHOUT SLUDGE INOCULUM³

Abstract

Alginate-like exopolymers (ALE) are present in the extracellular polymeric substances (EPS) of biological sludge such as aerobic granular sludge (AGS). The recovery of ALE from excess sludge produced by wastewater treatment plants (WWTP) is a relevant approach for the recovery of valuable products of industrial interest. However, little is known about dynamics of ALE content in sludge and associated factors. Thus, this study aimed at assessing the dynamics of EPS and ALE in terms of content, some chemical properties and influencing environmental factors along granulation in a sequencing batch reactor treating municipal wastewater without sludge inoculum. Results indicated that the EPS content was not correlated with the development of AGS, while the ALE content was higher, more stable and steadily increased after granulation achievement. Overall, 236 ± 27 mg VS_{ALE}/g VS_{sludge} was recovered from AGS and 187 ± 94 mg VS_{ALE}/g VS_{sludge} from flocs. However, the lower ALE content in flocs may be compensated by the higher sludge production rate in activated sludge systems. Principal component analysis (PCA) revealed that ALE content positively correlates with the nutrient and organic substrate conversion, and with the fraction of large AGS. Microbial analyses indicated that a stable microbial community composition was associated with a higher and more stable ALE content. ALE recovered from both flocs and AGS was endowed with hydrogel property, and no clear difference in their elemental composition and functional groups

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Esse trabalho também foi aceito para *flash oral presentation* no congresso cancelado *IWA Biofilms 2019* que aconteceria em Santiago (Chile). <https://biofilms2019.org/>

was observed. Therefore, this study provides insights about quantitative and qualitative aspects of ALE which are helpful for the improvement of waste biological sludge valorization.

4.1.1 Introduction

The handling and disposal of excess sludge from wastewater treatment plants (WWTP) is a major problem. In most developing countries, waste biological sludge is simply disposed in open dumpsites or landfills (NIZAMI *et al.*, 2017). On the contrary, recent legislations in Europe now require producing energy or high-value products from excess sludge (HEALY *et al.*, 2015). In this context, the operation of WWTPs should be directed towards the recovery of products becoming itself a potential source of renewable raw materials (MOHAN *et al.*, 2016). The recovery of high-value products from excess sludge in quantities and costs compatible with current market demand and prices is growing (VAN LOOSDRECHT; BRDJANOVIC, 2014), as for example the recovery of biogas, cellulose, bioplastic, phosphorus and alginic acid (VAN DER HOEK; FOOIJ; STRUKER, 2016).

Among the technologies available for wastewater treatment, the use of biofilms in the form aerobic granular sludge (AGS) has been reported to be an effective and promising environmental biotechnology (BOLTZ *et al.*, 2017; SARMA; TAY, 2018a). AGS systems have many advantages over conventional activated sludge systems, such as high organic matter and nutrients removal rates and excellent effluent quality (DERLON *et al.*, 2016; PRONK *et al.*, 2015b). The formation of granules is governed by the microbial production of extracellular polymeric substances (EPS), holding the microorganisms together. EPS mainly consist of proteins, polysaccharides, nucleic acids, humic acids, and lipids (ADAV; LEE, 2008; BOLTZ *et al.*, 2017; LIU; LIU; TAY, 2004). Several important functions have been attributed to EPS, such as protection against environmental pressures, mechanical stability, cell adhesion to surfaces and storage of carbon or water (BOLTZ *et al.*, 2017; FREITAS; TORRES; REIS, 2017; LIU; LIU; TAY, 2004; MIAO *et al.*, 2016). However, the mechanisms of EPS production and its relationship to granulation are still unclear and remains mostly hypothetical (ADAV; LEE; TAY, 2008; DING *et al.*, 2015; NANCHARAI AH; KIRAN KUMAR REDDY, 2018). Hence, significant research efforts must be devoted to better understand the production of EPS during the formation of aerobic granules (BOLTZ *et al.*, 2017; SEVIOUR *et al.*, 2019).

A major fraction of EPS from AGS treating municipal wastewater consists of alginate-like exopolymers (ALE) (FELZ *et al.*, 2016). ALE behave as hydrogels (FELZ *et al.*, 2016; LIN *et al.*, 2010; LIN; SHARMA; VAN LOOSDRECHT, 2013) providing the granules with

strength, elasticity, hydrophobicity, and a compact structure that protects microorganisms (LIN *et al.*, 2010; SAM; DULEKGURGEN, 2015). ALE are composed of sugars, proteins and humic substances (FELZ *et al.*, 2019) and is a biomaterial that can be used in paper, medical and construction industries as well as in agriculture and horticulture (VAN LEEUWEN *et al.*, 2018). Current market conditions are favorable for ALE recovery from wastewater and this may be coupled with the implementation of WWTP using AGS (VAN DER HOEK; FOOIJ; STRUKER, 2016). Considering the recovery of biogas, cellulose, bioplastics, phosphate and ALE, the later represents more than 50% of turnover that can be generated in WWTP (VAN LEEUWEN *et al.*, 2018). In the Netherlands, it is for example expected that 85 kton of ALE can be recovered from 10 different WWTP by 2030, generating 170 million of euros (VAN LEEUWEN *et al.*, 2018). However, ALE have been also extracted from floccular sludge (LIN; SHARMA; VAN LOOSDRECHT, 2013; SAM; DULEKGURGEN, 2015). The possibility of extracting ALE from activated sludge would significantly expand the potential of recovery. While ALE represent a major constituent of both floccular and granular sludge, very little is known about ALE content dynamics, factors associated with ALE production and the changes in its chemical characteristics during the granulation with domestic wastewater. Such understanding is required to better design and manage future biorefinery implementation, especially in terms of yield and quality of the biomaterial. Consequently, the understanding of EPS and ALE dynamics can lead to improved strategies for both resource recovery and biofilm controlling in WWTP, as well as can be helpful for future modelling of AGS systems.

Thereby, this study aimed at: (1) quantifying the change in the EPS and ALE contents along granulation; (2) evaluating the correlations between the EPS/ALE contents and environmental/operational factors using principal component analyses (PCA); (3) investigating the relationship between the microbial community composition and the dynamics of EPS/ALE contents; and (4) characterizing changes in hydrogel properties, elemental composition and functional groups of the recovered ALE from flocs and granules. To achieve those objectives, AGS was cultivated for 308 days in a sequencing batch reactor (SBR) without sludge inoculum during the treatment of municipal wastewater in a subtropical climate region. Granules development, reactor's performances and the change in the EPS/ALE contents were monitored. ALE were characterised through ionic hydrogel formation test, scanning electron microscopy (SEM) coupled with energy dispersive X-ray spectroscopy (EDX) and attenuated total reflection fourier transform infrared (ATR-FTIR). The change in the microbial community

composition was analysed by high-throughput amplicon sequencing and results were correlated to the results of the EPS/ALE contents.

4.1.2 Materials and methods

4.1.2.1 Reactor operation

AGS were cultivated in a 110L SBR (2.24 m height, 0.25 m diameter) operated in variable volume mode and fed with municipal wastewater collected from the sewer system of Florianópolis (Brazil). The reactor was started without sludge inoculum and biomass developed naturally through the reactor operation. The SBR cycle was 6 hrs long and consisted of the following phases: plug-flow feeding at the reactor's bottom (1 hr), mixed anoxic phase (2x air pulse for mixing (10 s) + idleness (15 min)), aerobic phase (3h 54 min), settling (30 min) and effluent discharge (6 min). A volume exchange ratio of 65% was applied. The reactor was operated under normal seasonal variations of temperature and without pH/dissolved oxygen (DO) control. The wastewater composition was representative of a low-strength wastewater: 513 ± 283 mg/L of total chemical oxygen demand (tCOD), 212 ± 66 mg/L of soluble chemical oxygen demand (sCOD), 46.4 ± 15 mg NH₄-N/L of ammonium and 7.1 ± 1.8 mg P/L of total phosphorus (TP).

4.1.2.2 Analytical methods

Sludge was analysed on a weekly basis and always sampled at the end of the aerobic phase. Granules development was monitored by recording microscopic images using an inverted microscope (Bel Photonics, Italy). From the time when the biomass concentration reached 1 g VSS/L, sludge volumetric indexes (SVI) were measured after 5, 10 and 30 min of settling according to Standard Methods (APHA, 2005) (SVI₅, SVI₁₀ and SVI₃₀, respectively). The different size fractions of the sludge were measured by sieving at 600, 400, 300 and 212 µm using stainless steel sieves according to the method described by Laguna et al. (1999). The diameter 212 µm was used as cut-off to differentiate flocs from granules.

The monitoring of the reactor's performance was carried out also on weekly basis. Samples were taken at each phase of the SBR operation. tCOD and sCOD were analysed by dichromate oxidation method according to Standard Methods (APHA, 2005) and ammonium nitrogen (NH₄-N) was determined using standard test kits (Hach®). Total suspended solids

(TSS) and volatile suspended solids (VSS) were determined by gravimetric method (APHA, 2005) using fiber glass filters with a pore size of 0.6 μm . TP concentration was measured with the vanadomolybdophosphoric acid colorimetric method (APHA, 2005). Temperature, pH and DO were measured using a multiparameter probe (YSI 6820 V2).

4.1.2.3 Extraction of extracellular polymeric substances (EPS) and alginate-like exopolymers (ALE)

The EPS and ALE extraction from biomass was performed according to Felz et al. (2016). EPS were extracted under alkaline, high temperature and agitation conditions with subsequent precipitation of the ALE under acidic conditions. Approximately 3 g of sludge were first centrifuged at 3100 g and room temperature for 25 min, prior discharge of the supernatant. Afterwards, the pellet was transferred to 250 mL baffled flasks filled with 50 mL of demineralized water, 0.25 g of Na_2CO_3 and a magnetic stirrer. The flask was immersed in a water bath (80 °C) and stirred for 35 min at 400 rpm. In the next step, the mixed liquor was centrifuged (3100 g, room temperature, 25 min) to recover the supernatant that comprised the soluble EPS. Acidic ALE were precipitated by the addition of 1 M HCl to final pH of 2.2 ± 0.05 while stirred at approximately 100 rpm and then centrifuged (3100 g, room temperature, 25 min). Extractions were always performed in triplicate. EPS and acidic ALE were quantified by volatile solids (VS) measurement according to Felz et al. (2016). Student t-tests were performed at 95% confidence level (Statistica software, StatSoft, USA) to analyse the dependence between EPS and ALE contents as well as the average content before and after granulation achievement (until and after day 210, respectively).

4.1.2.4 Principal component analyses (PCA)

Standardized datasets (zero mean and unit standard deviation) related to the operation of the reactor were analysed through principal component analysis (PCA) (Statistica software, Statsoft, USA) to study the correlation between the change in the EPS/ALE contents and the main operating and environmental growth conditions. A preliminary data mining was performed to seek the best linear combination between variables such that the maximum variance could be extracted. The chosen key-variables were: VSS, sCOD, pH, temperature (Temp), $\text{NH}_4\text{-N}$ (Amm), TP (Phosp), fraction of flocs (D212), fraction of granules with

diameter range from 600 to 400 μm (600D400), SVI_{10} , SVI_{30} and content of EPS and ALE in sludge. The VSS, temperature, sCOD, pH, $\text{NH}_4\text{-N}$ and TP data used in PCA were obtained at the end of anaerobic phase while the sludge size fraction, SVI and EPS/ALE contents were obtained at the end of aeration phase. The PCA was performed considering that each SBR cycle was subjected to the same conditions, and then variables measured in different phases are connected due to a cause-effect relationship. Relationships were observed by retaining the first two principal components (PC1 and PC2) and plotting in two dimensions where the cases and loading were set in the biplot. Both Kaiser & Guttman criteria of eigenvalues greater than eigenvalues mean as well as scree plot inspection were done to retain the number of principal components (PC) (JACKSON, 1993).

4.1.2.5 Molecular techniques for microbial community analyses

The change in microbial community composition of the sludge was monitored along granulation. Triplicate samples were collected at regular intervals ($n=9$ time points), centrifuged and stored at $-20\text{ }^\circ\text{C}$. DNA extractions were performed using PowerSoil® DNA isolation kits (MoBio Laboratories Inc., USA). Bacterial community compositions and population dynamics were analysed by high-throughput amplicon sequencing. The amplification of the 16S rRNA V3/V4 region was carried out using the 341F (CCTACGGGGRSGCAGCAG) (WANG; QIAN, 2009) and 806R (GGACTACHVGGGTWTCTAAT) (CAPORASO *et al.*, 2012) primers. The 16S rRNA libraries were sequenced using the MiSeq Sequencing System (Illumina Inc., USA) with the V2 kit, 300 Cycles, single-end sequencing. The sequences were analysed using the Neoprosecta Microbiome Technologies's library. All the reads were individually submitted to a quality filter, based on the sum of the DNA bases probabilities errors, allowing a maximum of 1% of accumulated errors. Subsequently, the DNA sequences corresponding to the Illumina adapters were removed. The resulting sequences that presented 100% identity were clustered and used for taxonomic identification using Silva database.

4.1.2.6 Alginate-like exopolymers (ALE) characterization

4.1.2.6.1 Ionic hydrogel formation test

The property of the extracted ALE to form hydrogel was tested by dripping in a Ca^{2+} solution (FELZ *et al.*, 2016). Sodium ALE were obtained by adding 0.5 M NaOH to the

extracted acidic ALE until pH reached 8.5. The sodium ALE solution was subsequently dripped into a 2.5% (w/v) CaCl_2 solution to evaluate the formation of Ca^{2+} -ALE beads, an indicative of ionic hydrogel forming properties.

4.1.2.6.2 Scanning electron microscopy (SEM) coupled with energy dispersive X-ray spectroscopy (EDX)

A SEM equipped with EDX was used to characterize both the micro-structure and elemental composition of Ca^{2+} -ALE beads (n=6 samples) formed during the hydrogel formation tests. Ca^{2+} -ALE beads were previously frozen at $-80\text{ }^\circ\text{C}$ for 24 h (Ultrafreezer, NuAire) and later lyophilized for 48 h (Lyophilizer L101, Liotop). The lyophilized samples were placed over an aluminium support and sputtered with gold. The analyses were conducted using a JEOL JSM-6390LV equipment operating at 15 kV.

4.1.2.6.3 Attenuated total reflection Fourier transform infrared (ATR–FTIR)

The functional groups of the polymers constituting the Ca^{2+} -ALE beads were analysed by ATR–FTIR (n=6 samples) using an Agilent FTIR spectrometer (Carry 660 model) equipped with ATR zinc selenide crystal. Spectra were recorded from 4000 to 650 cm^{-1} (20 scans/samples) at a resolution of 4 cm^{-1} .

4.1.3 Results

4.1.3.1 Reactor's performance

Reactor's performance was monitored over 308 days of operation. High total COD removal of $79.6 \pm 9.5\%$ was achieved after 29 days, corresponding to an average effluent concentration of $91.4 \pm 34.4\text{ mg tCOD/L}$. An ammonium removal of $73.4 \pm 16.9\%$ was measured after 49 days of operation, corresponding to an average effluent concentration of $11.48 \pm 5.61\text{ mg NH}_4^+\text{-N/L}$. However, the total phosphorus removal was low and quite variable, with an average removal of $33.1 \pm 15.9\%$ and an effluent concentration of $4.8 \pm 1.1\text{ mg TP/L}$.

4.1.3.2 Aerobic granules formation

Granulation was followed over 308 days of operation as indicated by both visual observations (Figure 13) and by the monitoring of SVI, VSS and granules fraction (Figure 144). Two different phases in the granulation process could be distinguished: Phase #1 from day 0 to day ~182-220 corresponded to a period with no or low granulation, while Phase #2 after day ~182-220 corresponded to a period of full and stable granulation.

Phase #1 consisted of an initial accumulation of floccular biomass that resulted in an increasing in VSS concentration while high values of SVI were monitored. During this period, the sludge mainly comprised of dispersed, filamentous and floccular biomass (Figure 13), likely originated from the influent. The initial high percentage of bioaggregates with diameter larger than 212 μm is attributed to the irregular biomass retained in the biggest mesh opening of the sieves, as already reported previously (WAGNER *et al.*, 2015). During the first two months, the biomass concentration gradually increased to 2.2 g VSS/L and SVI₃₀ values of around 96 mL/gTSS were measured. After around day 60, a densification of the flocs occurred as indicated by the gradual decrease in the SVI values. However, flocs remained the dominant biomass fraction of the sludge during this phase (Figure 13 and Figure 14B) and no significant accumulation of sludge was observed (Figure 14B).

Phase #2, observed after around 6 months of operation, corresponded to a period of full granulation, indicated by visual observations, low SVI values, high accumulation of biomass, and large fraction of granules (> 80%). Visual observations indicated the formation of round and compact granules from day 220 onwards (Figure 13). During Phase #2, the SVI₅, SVI₁₀, and SVI₃₀ reached lower and more stable values of 73 ± 13 mL/gTSS, 56 ± 9 mL/gTSS and 46 ± 7 mL/gTSS, respectively. Simultaneously, high SVI₃₀/SVI₁₀ ratio of 0.8 ± 0.1 was measured. Such low SVI values (<100 mL/gTSS) and a high SVI₃₀/SVI₁₀ ratio (close to 1) are representative of AGS systems fed with domestic wastewater (DERLON *et al.*, 2016; NI *et al.*, 2009; PRONK *et al.*, 2015b). Furthermore, the VSS concentration increased from around 1.6 g VSS/L (day 182) to 4.2 g VSS/L (day 308) as a result of granulation.

Figure 13 - Images of the mixed liquor sludge over the granulation process during the 308 days (d) of SBR operation. Sludge was dominated by floccular and heterogeneous biomass during Phase #1 (day 0 to day ~182-220) and no or low fraction of granules was observed. In contrast, compact and round granules prevailed during Phase #2 (after day ~182-220). Size bar: 400 μm .

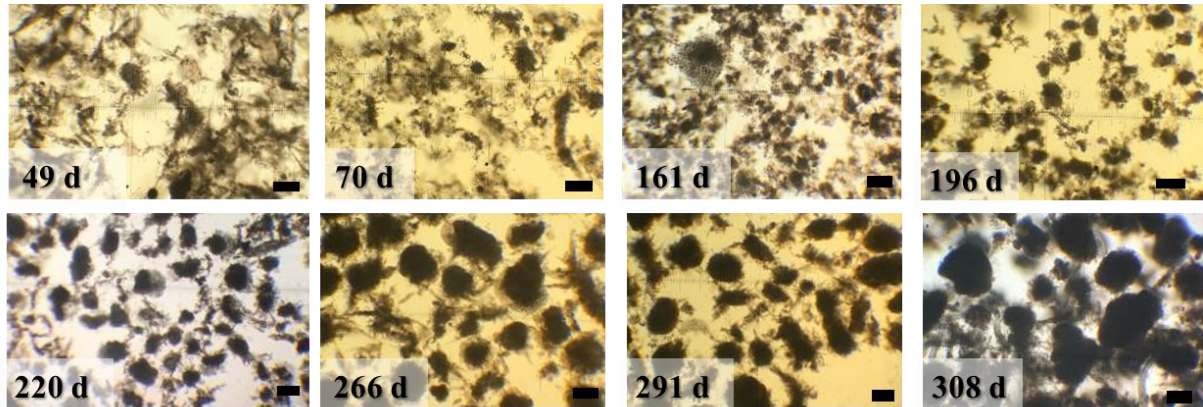
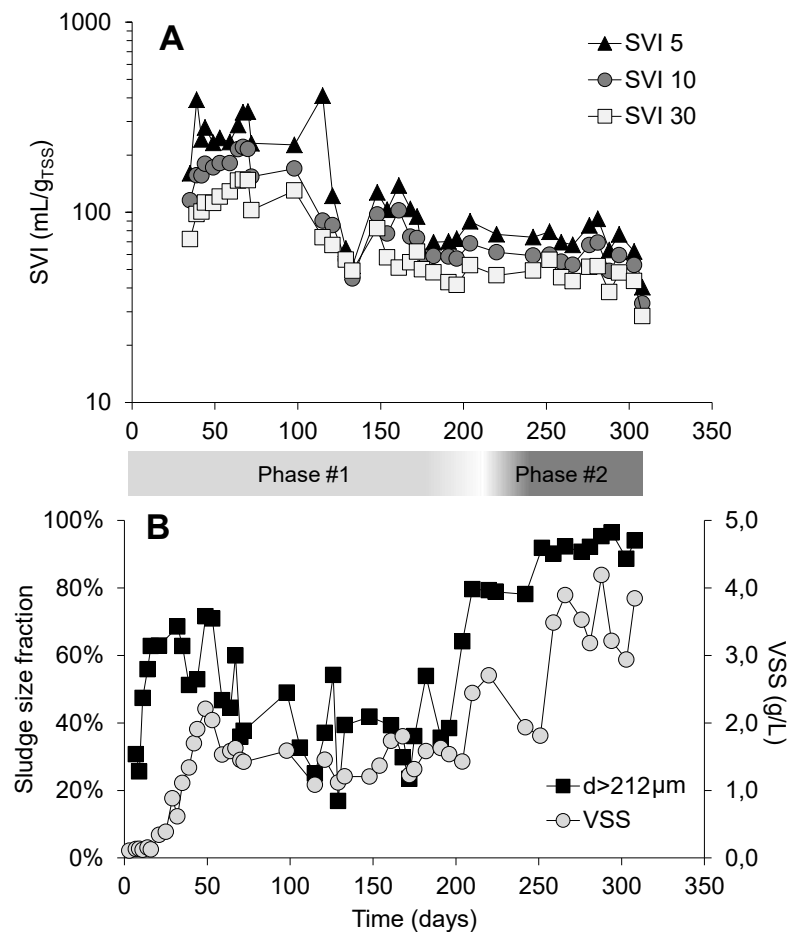


Figure 14 - (A) Change in the Sludge Volume Indexes (SVI₅, SVI₁₀ and SVI₃₀) and (B) change in the sludge fraction with diameter (d) larger than 212 μm and in the Volatile Suspended Solids (VSS) during granules formation in the SBR treating domestic wastewater. Phases #1 and #2 represent the periods of pre- and post-granulation.



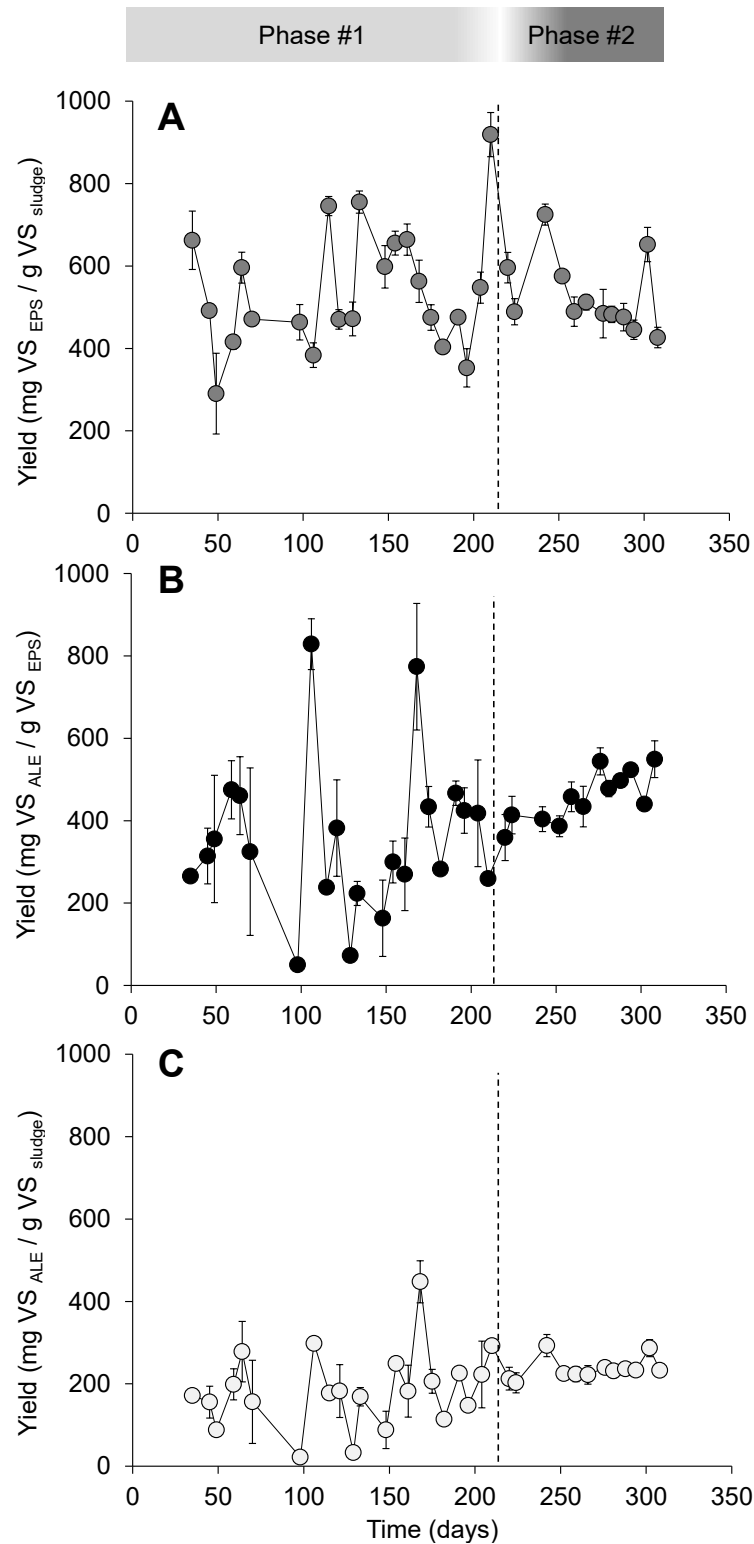
4.1.3.3 Extracellular polymeric substances (EPS) and alginate-like exopolymers (ALE) content dynamics

EPS were extracted during Phase #1 (no or low granulation) and during Phase #2 (full granulation) (Figure 15A). No evident increase in the EPS content was observed along with the formation of granules. The EPS content of the sludge was highly variable during Phase #1 while more stable once granulation was achieved (Phase #2). An average EPS content of 540 ± 150 mg VS_{EPS} /g VS_{sludge} was measured in the sludge before day 210 (before granulation, Phase #1), while 529 ± 90 mg VS_{EPS} /g VS_{sludge} was measured in the sludge as of day 210 (after granulation, Phase #2). Consequently, the EPS content measured during Phase #1 (no or low granulation) was not statistically different from the EPS content measured during Phase #2 (full granulation) ($p > 0.05$).

Significant amounts of ALE were extracted from both flocs (Phase #1) and granules (Phase #2) (Figure 15B and C), but the ALE content was larger in granules than in flocs. The formation of dense and round-shape granules resulted in a gradual increasing, more stable and higher ALE content in granules than in flocs ($p > 0.05$), especially when considering the ALE content in EPS (Figure 15B): 354 ± 186 mg VS_{ALE}/g VS_{EPS} during Phase #1 (no/low granulation) versus 457 ± 62 mg VS_{ALE}/g VS_{EPS} during Phase #2 (full granulation). Consequently, an enrichment of around 29% of ALE in granules was observed.

Statistical analysis performed to evaluate whether the ALE content changed simultaneously with the EPS content of the sludge showed that changes in EPS and ALE contents are not statistically correlated ($p < 0.05$). The weak positive correlation coefficient between EPS and ALE concentration (0.30) reveals that their direct relationship is prone to errors, such as the inverse content behaviour occurring on days 161 and 168 when EPS concentration decreased while ALE increased.

Figure 15 - Change in (A) the EPS content in the sludge, (B) the ALE content in EPS and (C) the ALE content in the sludge during granules formation in the SBR treating domestic wastewater. The EPS and ALE contents are expressed in VS. Bars indicate standard deviations between the three extractions performed for each sample. Phases #1 and #2 indicate periods of pre- and post-granulation, respectively. Dashed line defines the two groups of data (before and after day 210) used for statistics analyses.



4.1.3.4 Principal component analysis applied to the extracellular polymeric substances (EPS) and alginate-like exopolymers (ALE) content dynamics

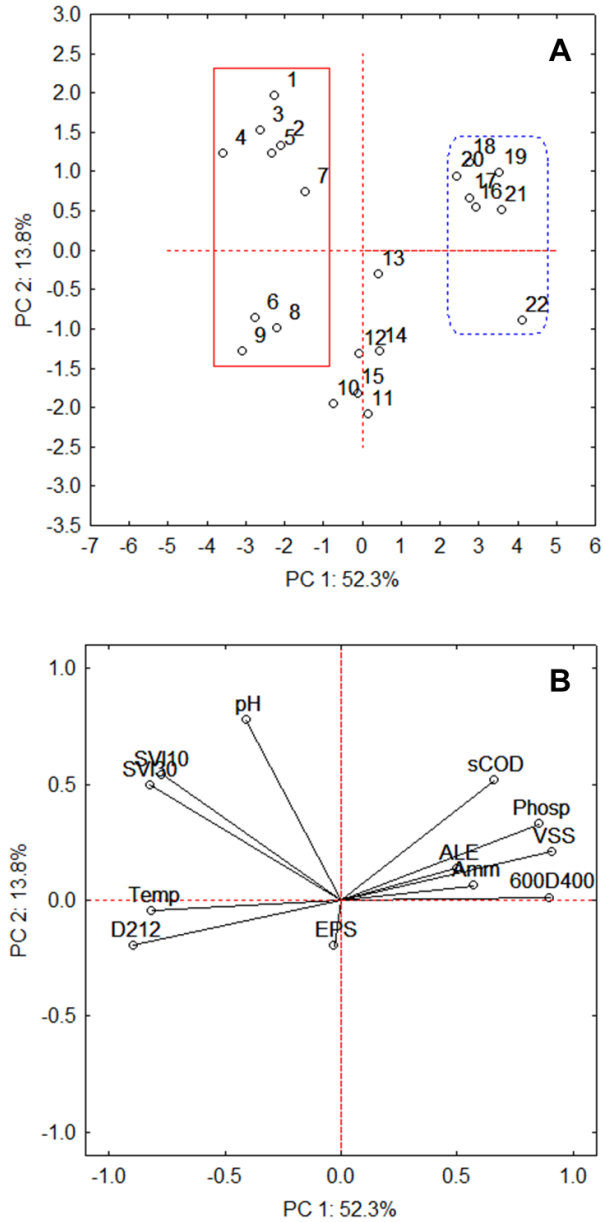
The PCA model reduced the dimensionality into 3 significant principal components (PC) that represented around 78% of the total variance (PC1 52.3%, PC2 13.8%, PC3 11.8%) (Table 4). Since the current analysis is based on the correlation matrix, results can be interpreted as the correlations of the respective variables with each PC and the behaviour of each variable is ascribed to each loading value (VASILAKI *et al.*, 2018). Based on the highest total variance of 52.3% in PC1, SVI₁₀, SVI₃₀, d<212 µm (D212) and temperature (Temp) presented the most negative loading values, whilst PC1 increased with an increase in VSS, 600>d>400 µm (600D400), TP (Phosp), sCOD, NH₄-N (Amm), and ALE. In addition, EPS had no role in explaining the variation in PC1 due to its low loading value (-0.03). Thus, the parameters in PC1 could be effectively used to describe the AGS performance. PC2 explained about 13.8% of the total variance, accounting for the next highest principal component. On the other hand, the effects of EPS and ALE on PC2 were diminished since those factors had very low absolute loadings (-0.20 and 0.14 respectively). The PC3 was largely correlated to EPS (0.79) and ALE (0.55) although presented only 11.8% of the explained variance.

Figure 16 presents the scores and loadings in the biplot model formed by PC1 and PC2. The biplot prediction should consider that both PC had an average equal to 0 (data are standardized). PC1 clustered the dataset into three groups according to the granulation process (Figure 16A): Group A distributed on the negative side is related to Phase #1 (no or low granules fraction); Group B distributed on the positive side is related to Phase #2 (fully granulated system); and Group C is a transition from Group A to Group B (mix of Phases #1 and #2). In group A, scores were more spread and revealed a biomass with poor sedimentation properties (high SVI₁₀ and SVI₃₀ values) and high proportion of small aggregates (d<212 µm), as expected during start-up. Spring-summer temperatures (19 ± 1.4 °C) supported the projection of Group A scores onto the modelled space formed by both orthogonal principal axes but with no clear influence on the ALE production. Meanwhile, all cases from Group B had positive scores in PC1, indicating higher ALE content, large granules dominating the system (i.e., more particles with diameter between 400 and 600 µm), and the biomass conversion of organic matter and nutrients (higher concentrations of sCOD, NH₄⁺-N and TP).

Table 4 - Factor loadings of PCA used to investigate the relationship between environmental/operational parameters and EPS/ALE contents. Absolute value of loadings equal or greater than 0.50 (cut-off point) is highlighted in boldface.

	PC1	PC2	PC3
EPS	-0.03	-0.20	0.79
ALE	0.50	0.14	0.55
VSS	0.91	0.21	-0.09
sCOD	0.66	0.52	-0.17
pH	-0.41	0.78	0.01
Temp	-0.81	-0.04	-0.41
Amm	0.57	0.06	-0.36
Phosp	0.85	0.33	-0.17
600D400	0.90	0.01	0.14
D212	-0.90	-0.19	-0.16
SVI ₁₀	-0.77	0.54	0.20
SVI ₃₀	-0.82	0.50	0.19
<i>Variance</i>	52.3	13.8	11.8
<i>Cumulative variance</i>	52.3	66.1	78.0

Figure 16 - Biplot model for principal component analysis of EPS and ALE contents and environmental/operational parameters: (A) score plot with group A (no/low granules fraction) and group B (granulated system) is highlighted in red-solid and blue-dashed lines respectively and (B) loading plot.



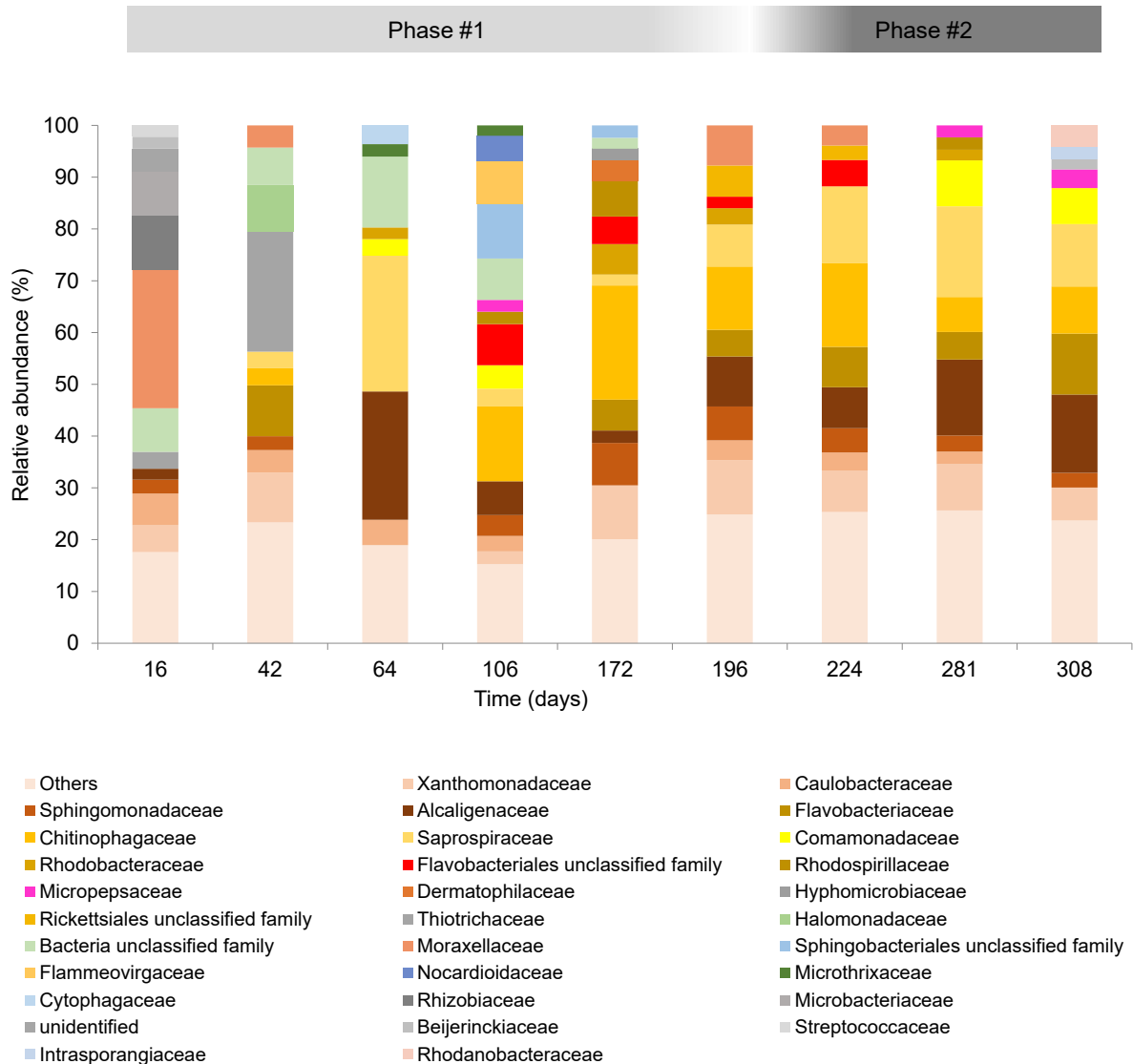
4.1.3.5 Microbial community dynamics

Significant changes in the bacterial community composition were observed over time (Figure 17). The microbial community composition was highly variable during Phase #1 (no or low granulation) while stable during Phase #2 (full granulation). The high variations in the microbial community composition observed during Phase #1 resulted in a high operational taxonomic unit (OTU) richness of $1.8 \pm 0.2\%$. Subsequently, the richness decreased and reached a lower value during Phase #2 ($1.3 \pm 0.1\%$ of OTU richness).

During the start-up of the reactor without sludge inoculum, the microbial community was dominated by microorganisms representative of municipal wastewater, such as genera *Acinetobacter* and *Thiothrix* members of *Moraxellaceae* (27%, day 16) and *Thiotrichaceae* (22%, day 42) families, respectively. Other minor populations (*Streptococcaceae*, *Microbacteriaceae* and *Rhizobiaceae*) were observed at day 16 but later were gradually outcompeted. Families such as *Xanthomonadaceae*, *Caulobacteraceae*, *Sphingomonadaceae* and *Alcaligenaceae* were also present. Interestingly, these populations along with *Flavobacteriaceae*, *Chitinophagaceae* and *Saprospiraceae* families followed a similar trend than the VSS accumulation (Figure 14B) and granulation. *Xanthomonadaceae*, *Sphingomonadaceae* and *Flavobacteriaceae* have been proposed as EPS producers in AGS (WEISSBRODT; SHANI; HOLLIGER, 2014; XIA *et al.*, 2018). In addition, *Caulobacteraceae*, *Alcaligenaceae*, *Chitinophagaceae* and *Saprospiraceae* families may be also responsible for the biopolymers production in the EPS matrix of AGS in this study.

Interestingly, some *Rhodospirillaceae* affiliates related to enhanced biological phosphorus removal (EBPR) systems represented here by *Defluvicoccus* genus (STOKHOLM-BJERREGAARD *et al.*, 2017) showed considerable abundance from day 172 (in a range of 1.5-7%). *Defluvicoccus* are glycogen accumulating organisms (GAOs) which are competitors of polyphosphate accumulating organisms (PAOs) represented here by *Tetrasphaera* from *Intrasporangiaceae* family (STOKHOLM-BJERREGAARD *et al.*, 2017) (relative abundance of 2% on day 308).

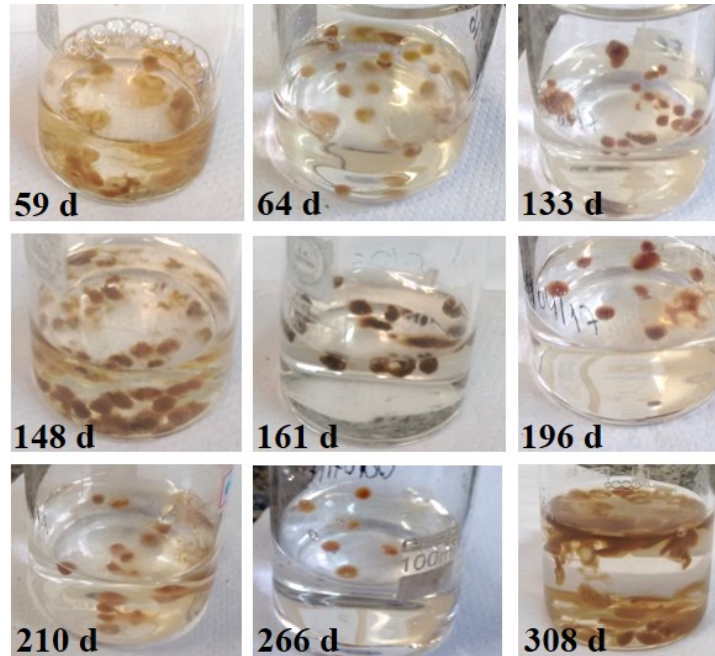
Figure 17 - Dynamics of the microbial community composition during granules formation in the SBR treating domestic wastewater over 308 days of operation. Phases #1 and #2 indicates periods of pre- and post-granulation, respectively.



4.1.3.6 Ionic hydrogel formation property of extracted alginate-like exopolymers (ALE) in $CaCl_2$

The ability of polymers from aerobic granules to form hydrogel is a key feature for the formation and mechanical stability of granules (LI *et al.*, 2014; LIN *et al.*, 2010). The hydrogel properties of polymers are also of relevance for further industrial applications (HAY *et al.*, 2013; LEE; MOONEY, 2012). In order to assess the capacity of the extracted ALE to form hydrogels, ALE were dropped in a $CaCl_2$ solution. The formation of brownish Ca^{2+} -ALE ionic hydrogels beads was observed with all ALE extracts from both floccular (Phase #1) and granular biomass (Phase #2) (Figure 18).

Figure 18 - Ionic hydrogel formation tests from samples collected in different days (d) of SBR operation from Phases #1 (pre-granulation) and #2 (post-granulation). Ca^{2+} -ALE beads were formed in the 2.5% (w/v) CaCl_2 solution.



4.1.3.7 Scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM/EDX)

SEM measurements performed on Ca^{2+} -ALE beads extracted from both Phase #1 (no/low granulation, day 161) and Phase #2 (full granulation, day 266) showed similar physical structures (Figure 19). The Ca^{2+} -ALE beads were characterized at the meso-scale by the presence of pores of different sizes, forming an apparent channel network. Moreover, an irregular, continuous and rough surface indicating a strong cross-link with Ca^{2+} was observed at the micro-scale.

EDX measurements were also performed on some Ca^{2+} -ALE beads to acquire elemental information since they are formed after EPS solubilisation (i.e., homogenous solution). The major elements of the beads were: carbon, chlorine, calcium and oxygen, with some minor traces of sodium (Figure 20). It was not overlooked the limitation of EDX in detecting hydrogen which is certainly present in ALE. Besides, no clear difference in Ca^{2+} -ALE beads was ascertained along granulation, i.e., through Phases #1 and #2.

Figure 19 - SEM images of the Ca^{2+} -ALE beads (cross-section) from samples collected in Phase #1 after 161 days (a, b and c) and in Phase #2 after 266 days (d, e and f) of SBR operation. The magnification used is identified at the bottom of each photo.

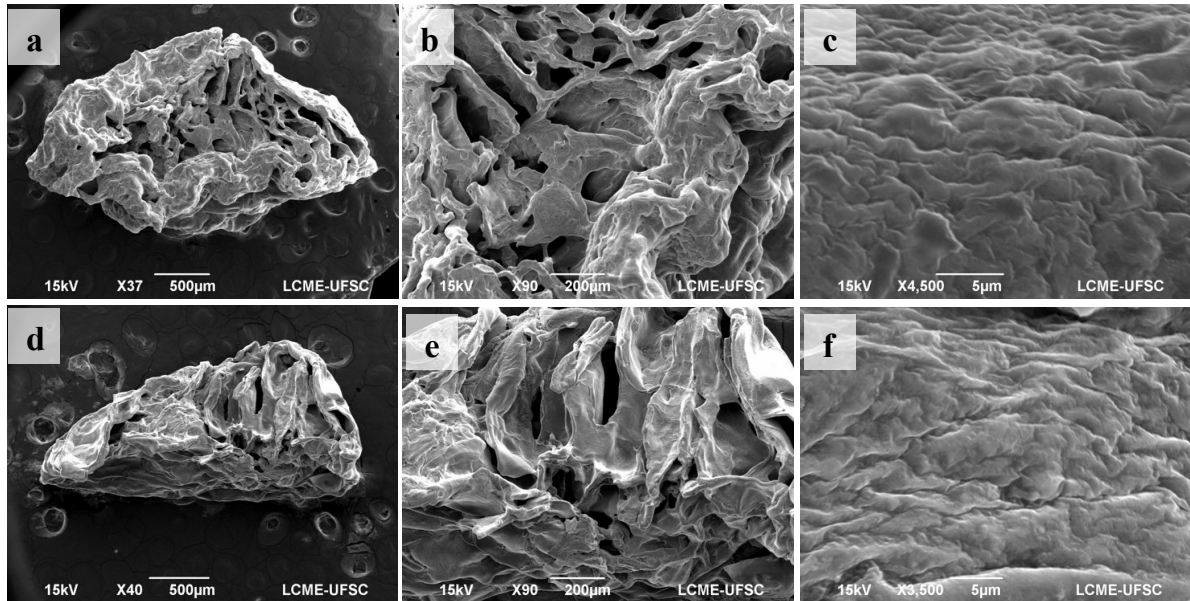
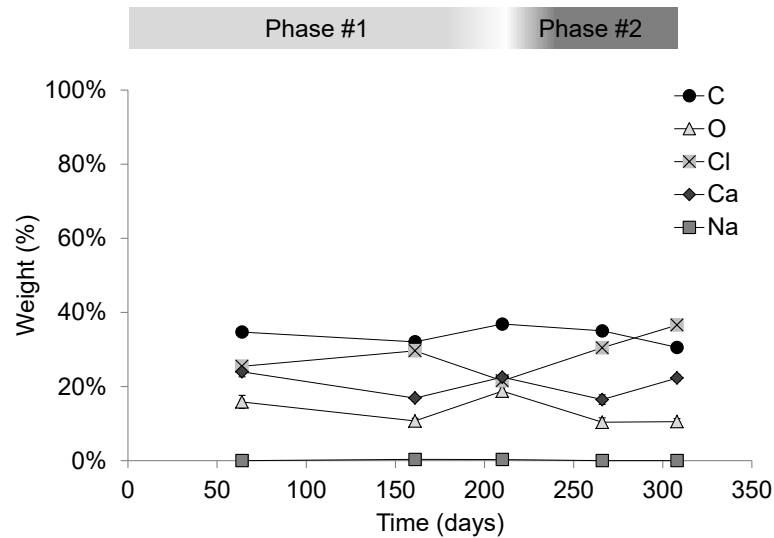


Figure 20 - Elemental composition of Ca^{2+} -ALE beads obtained from EDX analysis of samples from SBR operation from Phases #1 (pre-granulation) and #2 (post-granulation).



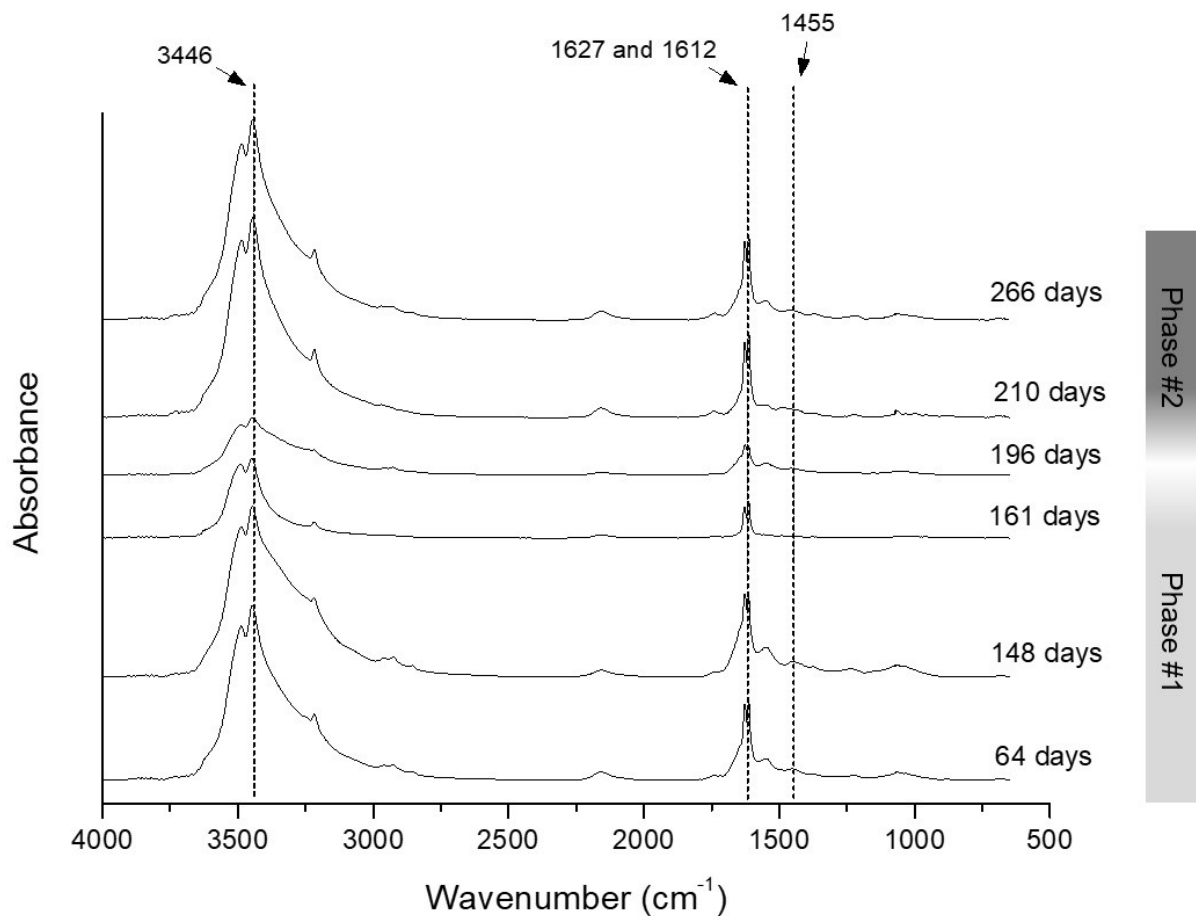
4.1.3.8 ATR-FTIR analysis

FTIR spectroscopy is useful for analysing structural changes in biopolymers (SEVIOUR *et al.*, 2012; TU *et al.*, 2012). Ca^{2+} -ALE beads exhibited similar spectra for most absorption bands and no distinguishable peak shifts throughout the operation time of reactor

(Figure 21). According to results, no evident difference between the ALE extracts from floccular (Phase #1) and granular sludge (Phase #2) was observed.

In relation to the functional groups, O–H stretching vibrations were identified above 3000 cm^{-1} (SILVERSTEIN; WEBSTER; DAVID, 2005). The strong peak at 1627 cm^{-1} can be attributed to the asymmetric stretching of O–C–O and amines (SILVERSTEIN; WEBSTER; DAVID, 2005). However, as EDX measurement did not show presence of nitrogen (section 4.1.3.7), the peak at 1627 cm^{-1} might not be attributed to nitrogen compounds. The strong asymmetric stretching bands near $1627\text{--}1612\text{ cm}^{-1}$ and the weak symmetrical stretching bands near 1455 cm^{-1} are assigned to the carboxylate C–O–C vibration and its conversion to a salt due the cross-linking with Ca^{2+} (SILVERSTEIN; WEBSTER; DAVID, 2005). The relative high intensity of the peak at 1612 cm^{-1} may result from a considerable content in guluronic acid residues (RAMOS *et al.*, 2018) which are believed to crosslink with Ca^{2+} (LEE; MOONEY, 2012), conferring stiffness and strength to the formed hydrogel (HAY *et al.*, 2013).

Figure 21 - ATR-FTIR spectra of Ca^{2+} -ALE beads extracted from sludge in different days of SBR operation in Phases #1 (pre-granulation) and #2 (post-granulation).



4.1.4 Discussion

4.1.4.1 Granulation: a phenomenon that enriches ALE in sludge but does not influence the EPS content

Granulation was successfully obtained in the system without any sludge inoculum addition, where AGS developed naturally. ALE were recovered in high amounts from the biomass dominated by both flocs and granules. However, ALE content after granulation was higher, more stable and gradually increasing in comparison to the predominant floccular biomass (Phase #1). ALE contents two times higher in granules than in flocs have already been reported with municipal sewage sludge (LIN; SHARMA; VAN LOOSDRECHT, 2013). Those evidences confirm that ALE content increases with granulation. Gelation is an important key mechanism for formation and stability of AGS (LI *et al.*, 2014; LIN *et al.*, 2010), where hydrogels are key components for granulation (YANG *et al.*, 2014). Granules have been characterized as hydrogels (SEVIOUR; DONOSE; PIJUAN, 2010). Moreover, the increase in ALE content has been reported as a prerequisite for the formation of mature granules, but a higher ALE content alone does not necessarily result in granulation (YANG *et al.*, 2014), as also demonstrated in this study by some high ALE content measured in flocs (e.g., days 106 and 168). Therefore, the more stable and higher average ALE content in AGS may be an indicative parameter of granulation.

Regarding EPS, the absence of differences in the EPS content before and after full granulation was observed. This similarity in EPS content along granulation is distinct of the usual mechanistic models proposed in the literature where biofilm development is considered to rely on stimulus of EPS production (ADAV; LEE; LAI, 2009; LIU; LIU; TAY, 2004; NANCHARAI AH; KIRAN KUMAR REDDY, 2018; NI *et al.*, 2009; WANG *et al.*, 2014; WHITELEY; LEE, 2015; ZHU *et al.*, 2015). Consequently, lower EPS content is expected in flocs than granules (ADAV; LEE; TAY, 2008; MIAO *et al.*, 2016; WAN *et al.*, 2013). However, contradictory results have been also reported and indicated a reduction in the EPS content along granulation (LI *et al.*, 2017a; TU *et al.*, 2012). A high biomass concentration, as presented in AGS compared to flocs, does not necessarily lead to more favourable EPS production (FREITAS; TORRES; REIS, 2017). The excess of EPS has been shown to be detrimental for AGS stability due to the clogging of the pores that limits the diffusion of oxygen, organic matter and nutrients (CORSINO *et al.*, 2016). Moreover, the microbial aggregation of activated sludge was shown to be controlled not only by the total amount of EPS, but also by

the different chemical functions and structure of EPS (GUO; WANG; LIU, 2016). Those evidences along with results of this study suggest that EPS content is driven by others factors than only bioaggregates' form. Hence, granules development does not necessarily lead to a higher EPS content in comparison to flocs.

4.1.4.2 Environmental and operational factors influence on extracellular polymeric substances (EPS) and alginate-like exopolymers (ALE)

Environmental and operational factors can potentially affect ALE content in sludge. ALE content in EPS during the post-granulation period (Phase #2) was $46 \pm 6\%$, which is a lower yield than the 63% obtained by Felz et al. (2016) using similar extraction conditions. However, such high yield was measured for sieved AGS while in this study yields are reported for the mixed liquor samples (flocs and granules). For this reason, the higher ALE content measured by Felz et al. (2016) can be attributed to the selectivity of the sieving process as well as to differences in the operating conditions and wastewater composition. Pronk et al. (2017) used the same protocol of extraction developed by Felz et al. (2016) to extract the ALE from granules fed with acetate cultivated under mesophilic conditions and obtained a much lower yield (1.4%) than granules fed with real wastewater (17.8%). Altering the composition of wastewater can lead to different values of EPS content when other operational conditions are kept (LI *et al.*, 2017a). Therefore, operational conditions and wastewater composition may affect the ALE content.

The conversion of organic matter and nutrients and a stable microbial community composition in the granulated system resulted in a steadier and higher production of ALE. The correlation of higher ALE content after granulation to the granules fraction and to the conversion of COD, ammonium and phosphorus was indicated by PCA results (Section 4.1.3.4). A direct association between ALE content and COD concentration has been already reported before with AGS treating synthetic wastewater, where high loads of organic matter led to higher ALE content in granules (YANG *et al.*, 2014). The increase in COD concentration stresses cells that then start to synthesize exopolymers, including ALE (YANG *et al.*, 2014). Besides, high EPS and ALE contents were measured over the entire reactor operation, i.e. before and after granulation (Figure 15), while the microbial composition was variable before granulation and more stable in the granulated system (Figure 17). This change in the composition of the microbial community between flocs and AGS has been observed previously

(LV *et al.*, 2014). The higher ALE content measured during Phase #2 (post-granulation) correlated with a more stable microbial community composition. Hence, after granulation, more steady-state conditions in the system may provide balanced ecological niches for the microbial community development what in turn supports the establishment of biochemical pathways for carbon and nutrients uptake. Consequently, carbon, nitrogen and phosphorus conversions together with the stability of the microbial community in the granulated system lead to a more stable and higher ALE production.

Microbial community composition (section 4.1.3.5) can also provide information about microorganisms involved in ALE production. Bacterial alginate can be obtained from *Pseudomonas* and *Azotobacter* (LEE; MOONEY, 2012), and the phylum *Proteobacteria* and the genus *Pseudomonas* have been associated with ALE production (MENG *et al.*, 2019; ZHANG *et al.*, 2019b). The most abundant families found in this study belong the phylum *Proteobacteria*, as the families *Xanthomonadaceae*, *Caulobacteraceae*, *Sphingomonadaceae* and *Alcaligenaceae*. Consequently, these organisms can be associated to the ALE production in the floccular and granular sludge. Moreover, PAO and GAO groups found after granulation (Figure 17) are likely to be associated to stable granulation and ALE content in the present study. Therefore, a wider range of microorganisms may be involved in ALE production.

Specifically in relation to phosphorus, it was not overlooked that the system showed relatively low phosphorus removal efficiency. However, PCA showed a correlation between phosphorus and ALE content, and there was the presence of PAO after granulation, the same phase when ALE content was higher. Hence, further studies should be done to evaluate to what extent and how phosphorus conversion impacts ALE content.

Regarding EPS, statistical analyses showed that EPS dynamics is not followed by the same trend in ALE (Section 4.1.3.3). This independency denotes that different factors contribute for EPS and ALE production even though ALE are a fraction of EPS. According to the PCA results (Section 4.1.3.4), the high loading of EPS in PC3 and low loadings in the first two principal components suggest that EPS content is more resistant to the environmental and operational factors acting in a real treatment system. The lack of correlation between EPS content and some environmental and operational factors like granules size and organic loading rate has been already reported previously (RUSANOWSKA *et al.*, 2019). Several factors can influence EPS as operational configuration, wastewater characteristics, bioaggregates type, complexity of the microbial community and the protocol of extraction (ADAV; LEE, 2008; DING *et al.*, 2015; MCSWAIN *et al.*, 2005; NI *et al.*, 2015; TU *et al.*, 2012; YANG *et al.*, 2019, 2014; ZHAO *et al.*, 2016). Therefore, EPS dynamics seems to be a more complex

phenomenon occurring in a real treatment system where the synergetic effect of several different variables acts on EPS production.

4.1.4.3 Alginate-like exopolymers (ALE) chemical characteristics along granulation

Tracking changes in the properties of ALE along granulation during the treatment of real wastewater is relevant to better understand the dynamics of qualitative characteristics aiming future industrial applications. In general, results about ALE characterization showed no clear difference between Ca^{2+} -ALE formed both from flocs and granules. Regarding the hydrogel properties presented by both flocs and granules, gel-forming polymers may be present in relevant concentrations in both types of bioaggregates. Results from literature are contradictory with regard of the ability of ALE from floccular biomass to form hydrogels. In a review, Seviour et al. (2012) reported that the main difference between flocs and granules is the presence of exopolysaccharides with gel-forming properties. In this context, Lin et al. (2013) obtained denser and stronger Ca^{2+} -ALE beads from granular sludge while fluffy beads were formed from floccular sludge. Differences between results of this study and those of Lin et al. (2013) could be due to differences in operational conditions and/or wastewater composition of each system. In the study of Lin et al. (2013), ALE were extracted from granules fed with a mixture of municipal wastewater (75%) and slaughterhouse wastewater (25%). This specific influent composition might lead to differences in the composition of the EPS and ALE. Nonetheless, other results from literature also indicate that EPS extracted from floccular biomass have hydrogel properties similarly to commercial alginate and AGS (SAM; DULEKGURGEN, 2015; YANG *et al.*, 2014). Hence, based on results of this study and results from literature, the hydrogel formation capability may not be a specific attribute of EPS isolated from granules.

Moreover, FTIR and EDX results provided some information about not only the similarity of Ca^{2+} -ALE extracted from flocs and granules but also about ALE composition. The gel-forming property of EPS in AGS is probably associated with high molecular weight polysaccharides (SEVIOUR; DONOSE; PIJUAN, 2010) whose fingerprint region in FTIR spectrum is $950\text{-}750\text{ cm}^{-1}$ (SEVIOUR *et al.*, 2012). However, in this study Ca^{2+} -ALE peaks were weaker at the fingerprint region below 1500 cm^{-1} when compared to spectra of non-cross-linked ALE (LIN *et al.*, 2010), commercial alginate (SARTORI; FINCH; RALPH, 1997) and cross-linked commercial alginate (RAMOS *et al.*, 2018). This difference is herein attributed to

the replacement of sodium ions by calcium ions during the cross-linkage, what changes the charge density, the radius and the atomic weight of the cation, creating a new environment (LI *et al.*, 2014; SARTORI; FINCH; RALPH, 1997). In addition, ALE have other constituents than polysaccharides in its composition, as proteins and humic acids, giving rise to a more complex biopolymer (FELZ *et al.*, 2019), what ultimately impacts the FTIR spectrum. In terms of elemental composition, Ca^{2+} beads derived from commercial sodium alginates have substantial higher oxygen content (range of 45-36% weight) than carbon content (range of 16-27%) (RAMOS *et al.*, 2018) in comparison to Ca^{2+} -ALE of this study. As observed in FTIR, this difference is possibly attributed to the different raw materials used to form the beads, since ALE extracted in this study come from a much more complex source of organic matter (proteins, polysaccharides, humic substances, etc.) than commercial alginate (polysaccharide), what ultimately may influence its composition. Hence, although similarities between ALE extracted from flocs/granules and commercial alginate have already been reported (SAM; DULEKGURGEN, 2015), ALE may be a complex polymeric mixture with other constituents besides alginate (MENG *et al.*, 2019).

4.1.4.4 Practical implications: alginate-like exopolymers (ALE) recovery from wastewater treatment plants

ALE are one of the most promising bioresources to be recovered from WWTP (VAN LEEUWEN *et al.*, 2018) and that is why the estimate of the amount that can be recovered is valuable. Results about ALE content along granulation (Figure 15) provide a relevant basis for quantifying the recovery potential. Considering wet sludge with 90-95% of water content (based on results of this work), ALE made up $1.12 \pm 0.68\%$ in flocs and $2.05 \pm 0.19\%$ in granules (VS based). Those values can be used as parameters for future economic and quantitative estimation analyses for ALE recovery facilities. Roughly, considering a robust WWTP with generation of 1 ton of wet sludge per day, nearly 20.5 ± 1.9 kg of VS_{ALE} can be recovered from granules and 11.2 ± 2.8 kg VS_{ALE} can be recovered from flocs, which are values that may be increased by improving the dewatering. In terms of volatile solids in the sludge, 236 ± 27 mg $\text{VS}_{\text{ALE}}/\text{g VS}_{\text{sludge}}$ can be recovered from granules and 187 ± 94 mg $\text{VS}_{\text{ALE}}/\text{g VS}_{\text{sludge}}$ from flocs.

In addition to that, results demonstrate that a more stable environment in terms of granulation, microbial community composition, and organic matter and nutrients removal provides a higher and more stable ALE content in granules. Those characteristics encourage

ALE recovery from WWTP using AGS that have already passed the start-up phase. However, AGS systems have a lower sludge production and consequently removal (SARMA; TAY, 2018a; WAN; BESSIÈRE; SPÉRANDIO, 2009) when compared to activated sludge systems. In activated sludge systems, as results demonstrated, ALE yield is lower and variable, but this might be compensated by the low solid retention time and by the subsequent higher sludge production that results from the higher biomass conversion yield. Moreover, the lack of pronounced differences in ALE along granulation in terms of functional groups, elemental composition and hydrogel properties together with the considerable content recovered from flocs may enlarge the possibilities of ALE recovery also from activated sludge systems. Therefore, cost benefit studies and deeper characterization of ALE recovered from flocs and granules are encouraged in order to give a clearer economic value to this bioproduct according to the source of sludge. This may expand the recovery of ALE to WWTP using different technologies contributing to a broader implementation of wastewater resource recovery facilities.

4.1.5 Conclusions

1. Alginate-like exopolymers (ALE) were important constituents of the polymers extracted from both floccular and granular sludge. Despite ALE could be found in significant amounts in flocs, ALE content was higher and more stable in granules and steadily increased over time once granulation was reached. Hence, stable and higher ALE content was an evidence for granulation establishment. Nevertheless, granulation was not necessarily followed by an increase in EPS content.
2. Higher and more stable ALE content in the sludge was associated with the granulation achievement, a stable microbial community composition, and conversions yields of organic matter and nutrients.
3. Although a higher amount of ALE can be recovered from AGS systems, the recovery potential of ALE from activated sludge treatment plants cannot be overlooked. Recovered ALE seemed to be a complex biopolymer whose hydrogel properties, elemental composition and functional groups were similar regardless of being extracted from flocs or granules. Therefore, further economic and characterization studies about recovery of ALE from flocculent sludge are necessary to support the expansion of resource recovery in WWTP.

4.2 ARTIGO 2 - CHEMICAL AND PHYSICAL PROPERTIES OF ALGINATE-LIKE EXOPOLYMERS OF AEROBIC GRANULES AND FLOCS PRODUCED FROM DIFFERENT WASTEWATERS ⁴

Abstract

This study aimed at better understanding how the wastewater composition in terms of organic substrates and the type of bioaggregates (floccular vs. aerobic granular sludge - AGS) influence: (1) the content and physico-chemical properties of Alginate-Like Exopolymers (ALE), and in turn (2) the formation of hydrogels and their rheological properties. ALE were extracted from various granular and flocculent sludge fed with different wastewaters. The chemical composition and mechanical properties of the different ALE extracts was characterized by complementary techniques: colorimetric assays, FTIR and Raman spectroscopy, rheometric assays and enzymatic tests. Our results showed that ALE are a complex mixture of proteins, humic acids and polysaccharides. Overall, rather similar ALE content/composition was observed for the different types of sludge. Only the AGS fed with acetate/propionate yielded significantly larger amount of ALE ($261 \pm 33 \text{ mg VS}_{\text{ALE}}/\text{g VS}_{\text{sludge}}$, +49%) and of uronic sugars in ALE ($254 \pm 32 \text{ mg}_{\text{glucuronic acid}}/\text{g VS}_{\text{ALE}}$, +62%) than the other types of bioaggregates (fed with no/very little volatile fatty acids). The elasticity of the different ALE hydrogels changed as a result of the type/origin of the bioaggregates. The elasticity of ALE extracted from AGS was always higher than the ones from flocs when fed with real wastewater. For hydrogels with the same dry matter content, ALE extracted from AGS fed with acetate/propionate formed the most elastic hydrogels. Enzymatic tests confirmed that mannuronic acids are involved in the cohesion of the hydrogels, while proteins have no clear effect. Those findings suggest that different types of sludge impact the composition and ultimately the properties of the recovered ALE, which is particularly relevant for further industrial applications.

⁴ Publicado com pequenas modificações como: SCHAMBECK, Cássio Moraes; GIRBAL-NEUHAUSER, Elisabeth; BÖNI, Lukas; FISCHER, Peter; BESSIÈRE, Yolaine; PAUL, Etienne; COSTA, Rejane Helena Ribeiro da; DERLON, Nicolas. Chemical and physical properties of alginate-like exopolymers of aerobic granules and flocs produced from different wastewaters. **Bioresource Technology**, v. 312, 123632, 2020. <http://dx.doi.org/10.1016/j.biortech.2020.123632>.

Esse trabalho também foi apresentado oralmente na 3rd *IWA Resource Recovery Conference* em Veneza (Itália). <https://www.iwarr2019.org/>

4.2.1 Introduction

Resource recovery from wastewater represents a relevant approach to reduce energy consumption, greenhouse gases emissions and waste generation from wastewater treatment plants (WWTP) (WANG *et al.*, 2015). In a near future, many conventional WWTPs will be converted into water resource recovery facilities (WRRF). These WRRFs will potentially represent a net source of valuable bioproducts, such as alginate-like exopolymers (ALE), bioplastics, cellulose, phosphorus or biogas (VAN DER HOEK; FOUIJ; STRUKER, 2016). Current market conditions are especially favorable for the recovery of ALE from granular sludge. In Netherlands, 85 ktons of ALE are expected to be recovered from 10 different WWTP by 2030, generating 170 million of euros (VAN LEEUWEN *et al.*, 2018). However, industrializing the recovery of ALE requires to better understand several key aspects. A key question is specially to understand how the composition and hydrogel properties of ALE change in response to the wastewater composition or the type of bioaggregates (flocs or granules). Acquiring more knowledge about the composition and hydrogel properties of ALE can help developing applications in the industry, for instance in the chemical fields and as rheology conditioner/emulsion stabilizer in the field of formulations (LOTTI *et al.*, 2019).

ALE are a mixture of biopolymers extracted from the extracellular polymeric substances (EPS) matrix of bacterial aggregates under alkaline conditions, and further recovered by acidic precipitation (FELZ *et al.*, 2016). Recent studies suggest that ALE contain proteins, polysaccharides including polyuronic ones, humic acids and lipids (FELZ *et al.*, 2019; LIN *et al.*, 2015). However, the origin of those different biopolymers in the ALE extracts is unclear. Several factors were proposed to explain EPS secretion in biological systems, such as the reactor's operating conditions, the wastewater composition or the microbial community composition (ADAV; LEE, 2008; DING *et al.*, 2015; LIU; LIU; TAY, 2004; MCSWAIN *et al.*, 2005; TU *et al.*, 2012; YANG *et al.*, 2019). However, little information is actually available on the factors and mechanisms that govern the ALE composition and content. ALE were first extracted from aerobic granular sludge (AGS) cultivated with a mixture of municipal and slaughterhouse wastewater (LIN *et al.*, 2010). ALE have further been reported to be key structural polymers of AGS (FELZ *et al.*, 2016; YANG *et al.*, 2014). However, ALE have also been extracted from activated sludge (LIN; SHARMA; VAN LOOSDRECHT, 2013; SAM; DULEKGURGEN, 2015). The amount of ALE recovered from granules can be twice as large as that recovered from flocs: 160 ± 4 mg/g (VSS ratio) and 72 ± 6 mg/g (VSS ratio), respectively

(LIN; SHARMA; VAN LOOSDRECHT, 2013). Lin et al. (2013) also reported some differences in the chemical structure of ALE recovered from flocs and AGS, which ultimately influence their hydrogel and mechanical properties. On the other hand, other studies reported hydrogel properties for ALE extracted from flocculent sludge, similarly to commercial alginate or to ALE extracted from AGS (SAM; DULEKGURGEN, 2015; SCHAMBECK *et al.*, 2020a). Thus, it remains rather unclear to what extent ALE isolated from activated sludge or from granules have similar or distinct composition/hydrogel properties.

Also, those contradictory observations about ALE content in activated/granular sludge were obtained for bioaggregates cultivated with different wastewaters: mixture of slaughterhouse and municipal (LIN; SHARMA; VAN LOOSDRECHT, 2013), brewery (SAM; DULEKGURGEN, 2015) or municipal (SCHAMBECK *et al.*, 2020a). The influent composition may affect the microbial community, and in turn the production of biopolymers such as ALE. To what extent ALE composition and gelling properties is governed by the types of bioaggregates (flocs vs. granules) or by the wastewater composition must be identified. High concentration in organic substrates were correlated with high ALE contents (more than 100 mg ALE/g VSS) in granular sludge (YANG *et al.*, 2014), but very variable ALE contents were in fact reported for AGS fed with different wastewater. For instance, AGS fed with synthetic saline wastewater yielded very little ALE, 5% w/w (VSS based) (LI *et al.*, 2017a; MENG *et al.*, 2019), AGS fed with propionate yielded 10% w/w (VSS based) (YANG *et al.*, 2014), while AGS fed with real wastewater yielded around 16% w/w (VSS based) (LIN *et al.*, 2010; LIN; SHARMA; VAN LOOSDRECHT, 2013). Hence, the comparison of those values suggests that wastewater composition might significantly influences the content and possibly also the composition and hydrogel properties of ALE. For further industrialization of ALE recovery, it is essential to better understand if ALE in sludge is influenced by the type of bioaggregates and/or by the wastewater composition.

Therefore, the current study aims at (1) better understanding how the wastewater composition and type of bioaggregate influence the content and composition of ALE, and (2) getting insights about the link between the ALE composition and its hydrogel properties. To answer those questions, EPS and ALE were extracted from different types of bioaggregates (flocs vs. granules) and from granules fed with different wastewaters in terms of organic substrates. The EPS/ALE content were quantified by gravimetric methods. The chemical composition of the different ALE extracts was characterized by several different and complementary analytical methods: biochemical colorimetric assays (polysaccharides, proteins and humic substances), FTIR, Raman spectroscopy and enzymatic tests. The mechanical

properties of hydrogels were quantified via rheometric tests, and correlated with the composition of the ALE extracts.

4.2.2 Materials and methods

4.2.2.1 Cultivation of aerobic granules and flocs

EPS and ALE were extracted from different microbial aggregates (flocs and granules) fed with different types of wastewater in terms of organic substrate (Table 5) (LAYER *et al.*, 2019). Flocs were cultivated with a real primary effluent wastewater, composed of 40% of soluble COD and 60% of particulate COD. Granules were cultivated with different synthetic or municipal wastewater: (1) simple synthetic substrate, (2) complex synthetic substrate, and (3) real primary effluent wastewater. The simple synthetic wastewater comprised of acetate and propionate (at equal COD fraction). The complex synthetic wastewater comprised of acetate/propionate (1/3 of the total COD), non-fermented soluble COD (amino acids and glucose, 1/3 COD) and particulate COD (starch and peptone, 1/3 COD). The real primary effluent used for the cultivation of the AGS was similar to the one used for the cultivation of the flocs. More details about the different wastewaters composition and the properties of the resulting AGS can be found in Layer *et al.* (2019).

Table 5 - Details of the different types of bioaggregates and wastewater used in this study.

Sample	Type of bioaggregate (sludge)	Feed wastewater	Carbon source
AGS _{simple synthetic}	Aerobic granular sludge	Simple synthetic wastewater	Volatile fatty acids (VFA): 1/2 acetate and 1/2 propionate (soluble COD)
AGS _{complex synthetic}	Aerobic granular sludge	Complex synthetic wastewater	1/3 COD: Acetate and propionate (soluble COD) 1/3 COD: amino acids and glucose (soluble COD) 1/3 COD: starch and peptone (particulate COD)
AGS _{real}	Aerobic granular sludge	Primary effluent from municipal wastewater	Complex (40% soluble COD, 60% particulate COD)
AS _{real}	Flocs from activated sludge	Primary effluent from municipal wastewater	Complex (40% soluble COD, 60% particulate COD)

4.2.2.2 Extraction of EPS and ALE

The extraction of biopolymers was performed according to the protocol proposed by Felz et al. (2016). A known mass of wet sludge was centrifuged (3100 g, room temperature, 25 min) prior discharging the supernatant. The pellet was then transferred to a 250 mL baffled flask filled with demineralized water, Na₂CO₃ and equipped with a magnetic stirrer. A ratio of 3:50:0.25 (sludge mass (g), demineralized water (mL) and Na₂CO₃ (g)) was kept during the extractions. The flask was immersed in a water bath (80 °C) and stirred for 35 min at 400 rpm. The mixed liquor was then centrifuged (3500 g, room temperature, 25 min) to recover the supernatant that comprised the solubilized EPS. Acidic ALE were extracted from EPS by acidic precipitation: 1M HCl was added to the soluble EPS solution to reach a final pH of 2.20 ± 0.05 while stirred at approximately 100 rpm. Extractions were made in triplicate. Results are expressed in terms of volatile solids (VS) according to Felz et al. (2016). Student t-tests were performed to evaluate differences in the EPS and ALE contents from the four different types of sludge.

4.2.2.3 EPS and ALE chemical composition

4.2.2.3.1 Humic acids, proteins and polysaccharides content in EPS and Na⁺-ALE

Proteins and humic acids in EPS and Na⁺-ALE were determined using the modified Lowry method (FROLUND; T. GRIEBE; NIELSEN, 1995), with bovine serum albumin (BSA) and humic acid sodium salt (both from 0 to 1000 mg/L) as standards. Colorimetric assays are not absolute quantitative methods (LE; KUNACHEVA; STUCKEY, 2016; LE; STUCKEY, 2016), but those methods provide relevant insights for comparing the biochemical composition of the extracts. All measurements were performed in triplicate. 200 µL of solution either with or without CuSO₄ was added to 40 µL of sample in 48-well microplates and shaken for 30 s. After 10 min incubation at room temperature, 20 µL of Folin-Ciocalteu Phenol Reagent 1 N was added to each well. The microplate was then incubated and shaken for 30 min at room temperature before the absorbance was measured at 720 nm. Concentrations of protein and humic acids were calculated based on the following three equations (FROLUND; T. GRIEBE; NIELSEN, 1995):

$$A_{total} = A_{protein} + A_{humic} \quad (1)$$

$$A_{protein} = 1.25(A_{total} - A_{blind}) \quad (2)$$

$$A_{humic} = A_{blind} - 0.2A_{protein} \quad (3)$$

Where A_{total} is the total absorbance with CuSO_4 , A_{blind} is the total absorbance without CuSO_4 , $A_{protein}$ is the absorbance due to proteins and A_{humic} is the absorbance due to humic acids. Using standard curves, $A_{protein}$ and A_{humic} were then converted to concentrations expressed in mg equivalent BSA or mg equivalent humic acids per g VS, respectively.

Polysaccharide content in EPS and Na^+ -ALE was measured after acid hydrolysis and reaction of the osidic monomers with the anthrone reagent. The classical Anthrone method was adapted with a double absorbance detection (620 nm and 560 nm) in order to discriminate the quantification of uronic from neutral sugars (RONDEL; MARCATO-ROMAIN; GIRBAL-NEUHAUSER, 2013). Indeed, uronic monomers exhibit higher absorbance at 560 nm compared to 620 nm while it is the opposite for the neutral monomers. Double absorbance monitoring of EPS and Na^+ -ALE samples in addition to calibration of the method with glucose (Glc) and glucuronic acid (Gla) as standards (0 to 100 mg/L and 0 to 400 mg/L, respectively) allows quantifying the neutral and uronic sugars separately in a simultaneous assay.

All measurements were performed in triplicate. 2 g/L anthrone reagent was prepared by dissolving anthrone in 98% sulphuric acid. 200 μL of 2 g/L anthrone reagent was added to 100 μL of sample or standards dispensed in 48-well microplates. After 30 min incubation at 60°C, absorbance of standards and samples at 560 and 620 nm were recorded at room temperature. Neutral and uronic sugars were quantified according to the following equation (RONDEL; MARCATO-ROMAIN; GIRBAL-NEUHAUSER, 2013):

$$[\text{Glc}] = \frac{(A_{560} \times b_{620} - A_{620} \times b_{560})}{(a_{560} \times b_{620} - a_{620} \times b_{560})} \quad (4)$$

$$[\text{Gla}] = \frac{(A_{560} \times a_{620} - A_{620} \times a_{560})}{(a_{620} \times b_{560} - a_{560} \times b_{620})} \quad (5)$$

Where $[\text{Glc}]$ is the concentration of neutral sugars expressed in equivalent Glc, $[\text{Gla}]$ is the concentration of uronic sugars expressed in equivalent Gla, A_i is the absorbance of sample at i nm (either 560 nm or 620 nm), a_i is the slope of glucose calibration curve at i nm, and b_i is

the slope of glucuronic acid calibration curve at i nm. Concentration of polysaccharides in EPS and Na^+ -ALE are expressed in terms of mg of equivalent Glu or Gla per g VS.

4.2.2.3.2 Fourier transform infrared spectroscopy (FTIR)

Spectra were recorded on a Biorad FTS 575C instrument equipped with a mercury cadmium telluride (MCT) detector and a 9-reflection diamond ATR unit with KRS-5 optics (SensIR Technologies, 15 Great Pasture Road, Danbury, CT 06810-9931). The diameter of the round diamond disk was 4 mm. Scans from 4000 to 400 cm^{-1} were collected at 2 cm^{-1} resolution versus the appropriate background. Single beam background spectra were collected with the cleaned, uncoated diamond disk. The diamond ATR disk was then coated with Na^+ -ALE sample over the disk. The solutions were gently dried under a N_2 stream and absorbance spectra were collected several times to assure that the formed layer of organic material was dry. Results are presented as an average of three measurements.

4.2.2.3.3 Raman spectroscopy

Raman spectra of Na^+ -ALE samples were measured with a Bruker SENTERRA Raman Microscope over the spectral range 100-3500 cm^{-1} . Profiles were acquired with a 785 nm laser wavelength and a 50x objective lens. The following parameters were used: 100 mW of laser power, 5 sweeps and 10 seconds of integration time.

4.2.2.4 ALE hydrogels

4.2.2.4.1 Ionic hydrogel formation test

Gelation tests were performed to verify the hydrogel properties of the different ALE extracts according to Felz et al. (2016). Soluble Na^+ -ALE were first obtained by the addition of 0.5 M NaOH to the precipitated acidic ALE until pH reached 8.5. Afterwards, Na^+ -ALE were dripped into a 2.5 % (w/v) CaCl_2 solution to evaluate the formation of drop-shaped Ca^{2+} -ALE beads, which indicates the ionic hydrogel forming property.

4.2.2.4.2 Enzymatic degradation of Ca²⁺-ALE beads

Enzymatic degradation tests were performed to evaluate what chemical group/bond was related to the hydrogel properties of ALE. Different enzymes, which attack specific chemical groups/bonds were tested to assess the contribution of the targeted bonds to the mechanical stability of the gels. Those tests were performed only for the Ca²⁺-ALE recovered from AGS_{real}. The different enzymatic digestion tests were performed in 48-well microplates (24 h, 24°C, moderate agitation). Pictures of the hydrogel beads were then recorded after enzymatic attack using a stereomicroscope (SZX10, Olympus, Japan). Papain from papaya latex (EC 3.4.22.2, Sigma-Aldrich, molecular weight 21 kDa) was used for cleaving amide type peptide bonds of basic amino acids, leucine and glycine, and to hydrolyze esters and amides. Lysozyme from chicken egg white (EC 3.2.1.17, Sigma-Aldrich, molecular weight 14.4 kDa) was used for the digestion of β (1 \rightarrow 4) linkage between N-acetylmuramic acid and N-acetyl-D-glucosamine in N-acetyl amino glycans. Protease from *Bacillus* sp. (EC 232-752-2, Sigma-Aldrich, 20 to 27 kDa) (Savinase®) was applied for the digestion of amide type linkages in proteins and peptides. Finally, alginate lyase (poly β -D-mannuronate lyase, EC 4.2.2.3, Sigma-Aldrich, 38 kDa) was employed for the digestion of osidic linkages found in uronic sugars as alginate. All enzymatic solutions were prepared with distilled water. Controls with denatured enzymes (previously heated up to 90 °C for 15 min) and without enzymes addition were also performed. The following enzymes concentrations were used for the digestion tests: 12 U/mL for papain, 420,000 U/mL for lysozyme, 10% (v/v) for savinase and 100 U/mL for alginate lyase. The unit of enzymatic activity (U) was defined according to the supplier's definition.

4.2.2.5 Rheological property

The rheological properties of both Na⁺-ALE samples (before gelation) and Ca²⁺-ALE samples (after gelation) were analyzed. The rheological properties of the samples were monitored both at their original dry matter content (as obtained by the extraction protocol) and at normalized dry matter content (2.3% of dry matter). Na⁺-ALE samples were heated up to 40 °C and then 5 mL were poured in crystal-grade polystyrene dishes (35 x 10 mm). After cooling down to 4 °C, 2.5% (w/v) CaCl₂ solution was sprayed in excess over the Na⁺-ALE samples and at least 24 hours were waited to trigger hydrogel formation. Rheological measurements were performed on a MCR 501 rheometer (Anton Paar, Austria). The Na⁺-ALE samples were

measured with a cone-plate (CP50, Anton Paar, Austria) geometry and the Ca^{2+} -ALE samples were measured in parallel plate mode, using sandblasted 25 mm diameter stainless steel plates (PP25 S Anton Paar, Austria) and a gap size of 1 mm. Amplitude sweeps ($\gamma = 0.01 - 100\%$, $f = 0.5$ Hz) were performed to ensure the linear viscoelastic (LVE) region for the subsequent frequency sweeps ($f = 0.1 - 1$ Hz, $\gamma = 0.1\%$). For sample loading, the Na^+ -ALE samples were transferred from the tubes to the lower measuring plate using a 1 mL micropipette. The Ca^{2+} -ALE samples were gently loaded using a spatula and the upper plate was slowly lowered onto the ALE. A solvent trap containing moist sponges was placed over the sample. All measurements were performed under temperature controlled at 22 °C.

4.2.3 Results

4.2.3.1 EPS and acidic ALE content

The four different types of sludge were surprisingly characterized by very similar extracted EPS and acidic ALE contents (Table 6). Only the granules fed with simple synthetic wastewater (acetate/propionate) yielded substantially more extracted EPS and acidic ALE than the flocs/granules fed with synthetic complex or real wastewater: 598 ± 37 mg $\text{VS}_{\text{EPS}}/\text{g VS}_{\text{sludge}}$, 449 ± 70 mg $\text{VS}_{\text{ALE}}/\text{g VS}_{\text{EPS}}$, and 261 ± 33 mg $\text{VS}_{\text{ALE}}/\text{g VS}_{\text{sludge}}$, compared to 472-507 mg $\text{VS}_{\text{EPS}}/\text{g VS}_{\text{sludge}}$, 333-391 $\text{VS}_{\text{ALE}}/\text{g VS}_{\text{EPS}}$, 165-184 mg $\text{VS}_{\text{ALE}}/\text{g VS}_{\text{sludge}}$, respectively ($n=8-9$ measurements). Consequently, the $\text{AGS}_{\text{simple synthetic}}$ contained, in average, 22% more EPS in sludge, 21% more ALE in EPS and especially 49% more ALE in sludge than the other samples. Also, the type of bioaggregate did not clearly influence the extracted EPS and acidic ALE contents (Table 6). Flocs and granules fed with the same real wastewater indeed had similar EPS and acidic ALE content.

Table 6 - EPS content in sludge, acidic ALE content in EPS and acidic ALE content in sludge in terms of VS in each sludge sample ($\text{AGS}_{\text{real}} n=8$, $\text{AS}_{\text{real}} n=9$, $\text{AGS}_{\text{complex synthetic}} n=8$, $\text{AGS}_{\text{simple synthetic}} n=8$, where n means the number of measurements).

	EPS content in sludge (mg $\text{VS}_{\text{EPS}}/\text{g VS}_{\text{sludge}}$)	Acidic ALE content in EPS (mg $\text{VS}_{\text{ALE}}/\text{g VS}_{\text{EPS}}$)	Acidic ALE content in sludge (mg $\text{VS}_{\text{ALE}}/\text{g VS}_{\text{sludge}}$)
$\text{AGS}_{\text{simple synthetic}}$	598 ± 37	449 ± 70	261 ± 33
$\text{AGS}_{\text{complex synthetic}}$	$507 \pm 40^{**}$	$333 \pm 86^*$	$165 \pm 32^{***}$
AGS_{real}	$495 \pm 45^{**}$	388 ± 65	$184 \pm 18^{***}$
AS_{real}	$472 \pm 31^{***}$	$391 \pm 26^*$	$176 \pm 12^{***}$

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. $\text{AGS}_{\text{simple synthetic}}$ (Student t-tests performed at 95% confidence level).

4.2.3.2 EPS and ALE composition

4.2.3.2.1 Polysaccharide, protein and humic acid content

All EPS extracts were characterized by a very complex composition in terms of sugars, proteins, and humic acids, as indicated by the results of the colorimetric assays (Figure 22A). While differences in the EPS composition of the different types of aggregates were expected, results from the colorimetric assays proved rather similar. The EPS_{AS real} had the lowest proteins (137 ± 28 mg_{BSA}/g VS_{sludge}) and sugars contents (18 ± 3 mg_{glucose}/g VS_{sludge} and 48 ± 13 mg_{glucuronic acid}/g VS_{sludge}) while the EPS_{AGS simple synthetic} presented the highest contents (309 ± 31 mg_{BSA}/g VS_{sludge}, 64 ± 12 mg_{glucose}/g VS_{sludge} and 153 ± 40 mg_{glucuronic acid}/g VS_{sludge}). The AGS fed with simple synthetic wastewater (acetate/propionate) thus yielded up to 255% and 219% more neutral and uronic sugars than the aggregates fed with complex or real wastewater. In addition, the EPS_{AGS real} yielded the highest humic acid content (181 ± 18 mg_{humic acids}/g VS_{sludge}).

Colorimetric assays also confirmed the complex composition of the Na⁺-ALE extracts, with sugars, proteins and humic acids (Figure 22B and C). The Na⁺-ALE extracts from AGS_{simple synthetic} had higher neutral sugar, uronic sugar and protein contents than the other types of sludge. AGS_{simple synthetic} especially contained a large amount of uronic sugars (254 ± 32 mg_{glucuronic acid}/g VS_{ALE}), around 62% more than in the other sludges. Neutral sugars content was relatively low in all Na⁺-ALE extracts, ranging from 34 ± 5 mg_{glucose}/g VS_{ALE} in AGS_{complex synthetic} to 53 ± 1 mg_{glucose}/g VS_{ALE} in AGS_{simple synthetic}. An enrichment of humic substances was observed in all the Na⁺-ALE extracts compared to the EPS extracts, e.g. up to 10% in Na⁺-ALE_{AS real} (Figure 23). For real wastewater, a protein to humic acids ratio of around 1 was observed for the Na⁺-ALE extracts of both the flocs and granules. A much higher protein to humic acids ratio was measured for the AGS_{simple synthetic}. No clear influence of the type of bioaggregates on the composition of the Na⁺-ALE extracts was noticed.

Figure 22 - Effect of type of bioaggregate and wastewater composition on the humic acids, proteins (BSA equivalent), neutral (glucose equivalent) and uronic sugars (glucuronic acid equivalent) contents in (A) the EPS extracts (mg/g VS_{sludge}), (B) the Na⁺-ALE extracts (mg/g VS_{sludge}), and in (C) the Na⁺-ALE extracts (mg/g VS_{ALE}) in each sludge sample. The error bars illustrate the standard deviation of the analytical replicates (n=3).

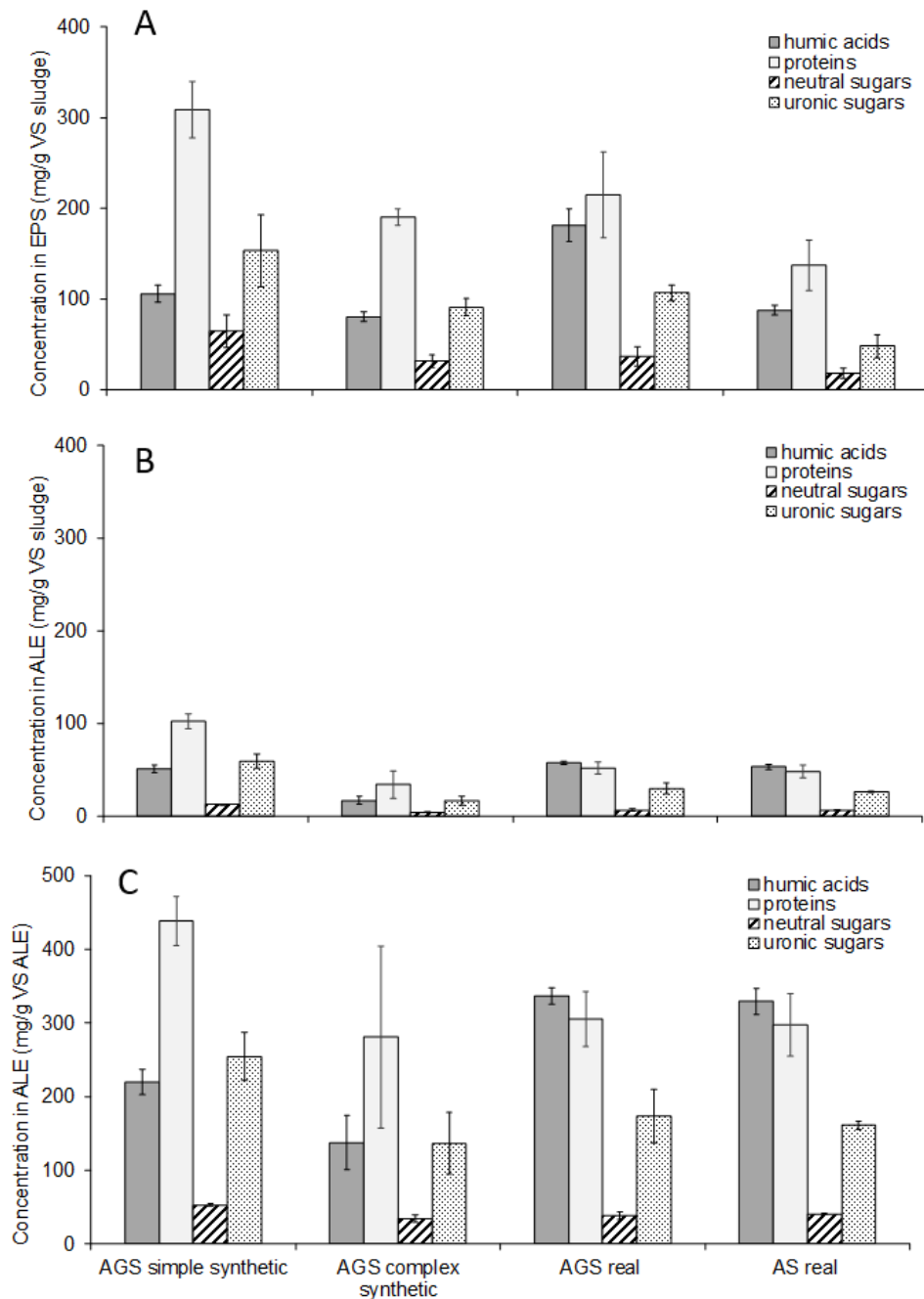
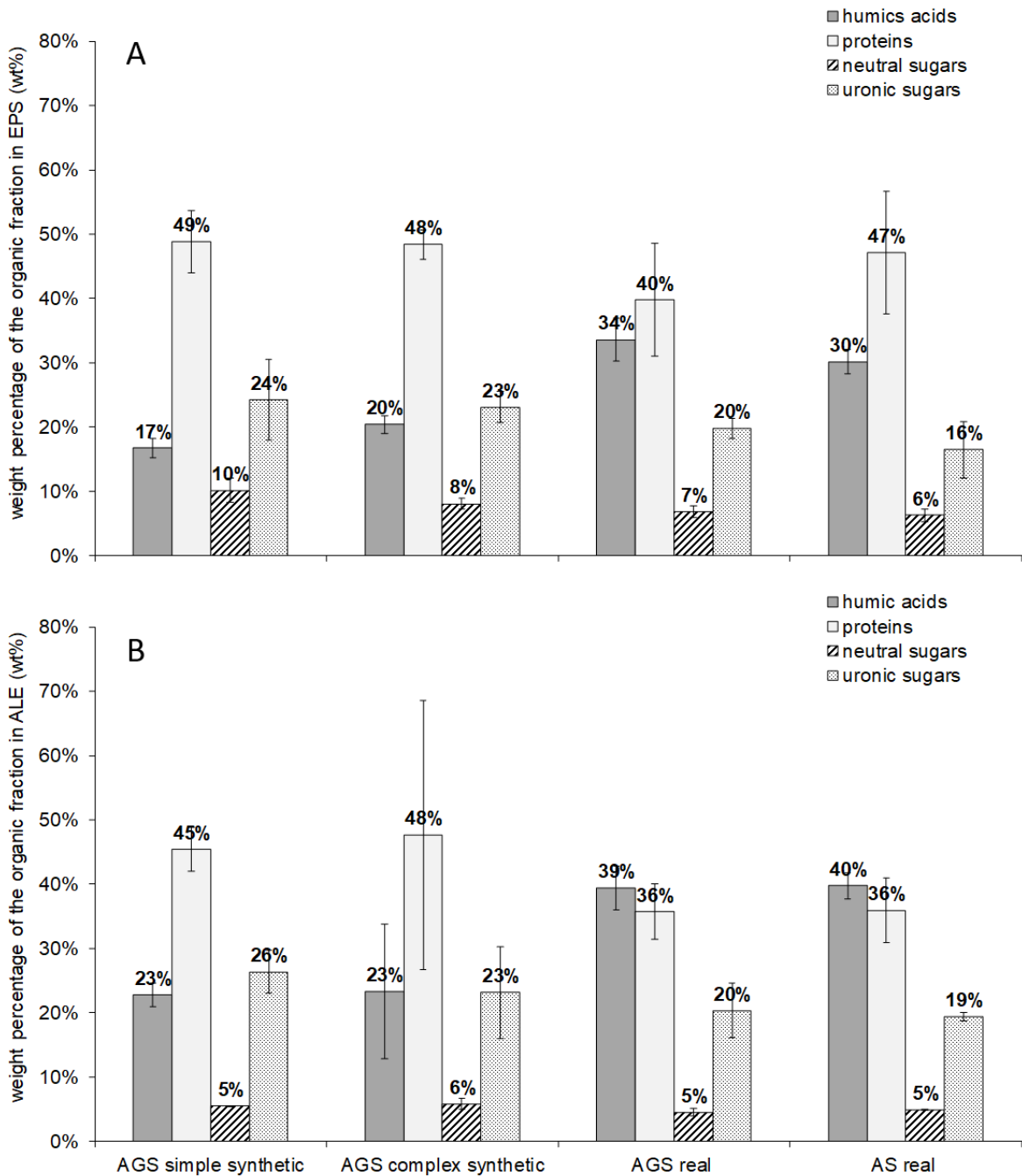


Figure 23 - Overall composition of (A) EPS and (B) Na⁺-ALE presented in weight percentage of the organic fraction. Compounds were measured by colorimetric methods and are presented in equivalents of the standards used for the corresponding measurement. The error bars illustrate the standard deviation of the measurements (n=3).



4.2.3.2.2 Na⁺-ALE extracts characterization using FTIR

FTIR analyses confirmed the complex composition of the Na⁺-ALE extracts, as already suggested by the results of the colorimetric assays. Several peaks observed on the FTIR spectra can be attributed to different chemical groups such as polysaccharides, proteins and humic acids (Figure 24 and Table 7).

Overall, the FTIR spectra of the different Na⁺-ALE proved rather similar to each other. Only very small differences in the presence/absence of some peaks were observed. The Na⁺-ALE_{AGS simple synthetic} and Na⁺-ALE_{AGS complex synthetic} presented a weak peak at 826 and 818 cm⁻¹ that can be attributed to mannuronic acid residues (SAM; DULEKGURGEN, 2015; SARTORI; FINCH; RALPH, 1997; SEVIOUR *et al.*, 2012). This peak was not observed for Na⁺-ALE extracted from aggregates fed with real wastewater (both flocs and granules). Moreover, the FTIR spectra of Na⁺-ALE_{AGS complex synthetic}, Na⁺-ALE_{AGS real} and Na⁺-ALE_{AS real} have similar spectra in the 1397-1232 cm⁻¹ region with the spectrum of ALE enriched in guluronic acids blocks obtained by Lin et al. (2013). Therefore, the ALE extracted from granules cultivated with VFA-rich wastewater may be richer in mannuronic acid blocks (M) than guluronic acid blocks (G). But overall differences in the composition of the Na⁺-ALE extracts remain minor. In addition, no specific influence of the type of bioaggregates could be observed.

Figure 24 - (A) FTIR spectra of Na⁺-ALE extracted from the four different samples of sludge and (B) a zoom in the 1800-500 cm⁻¹ region.

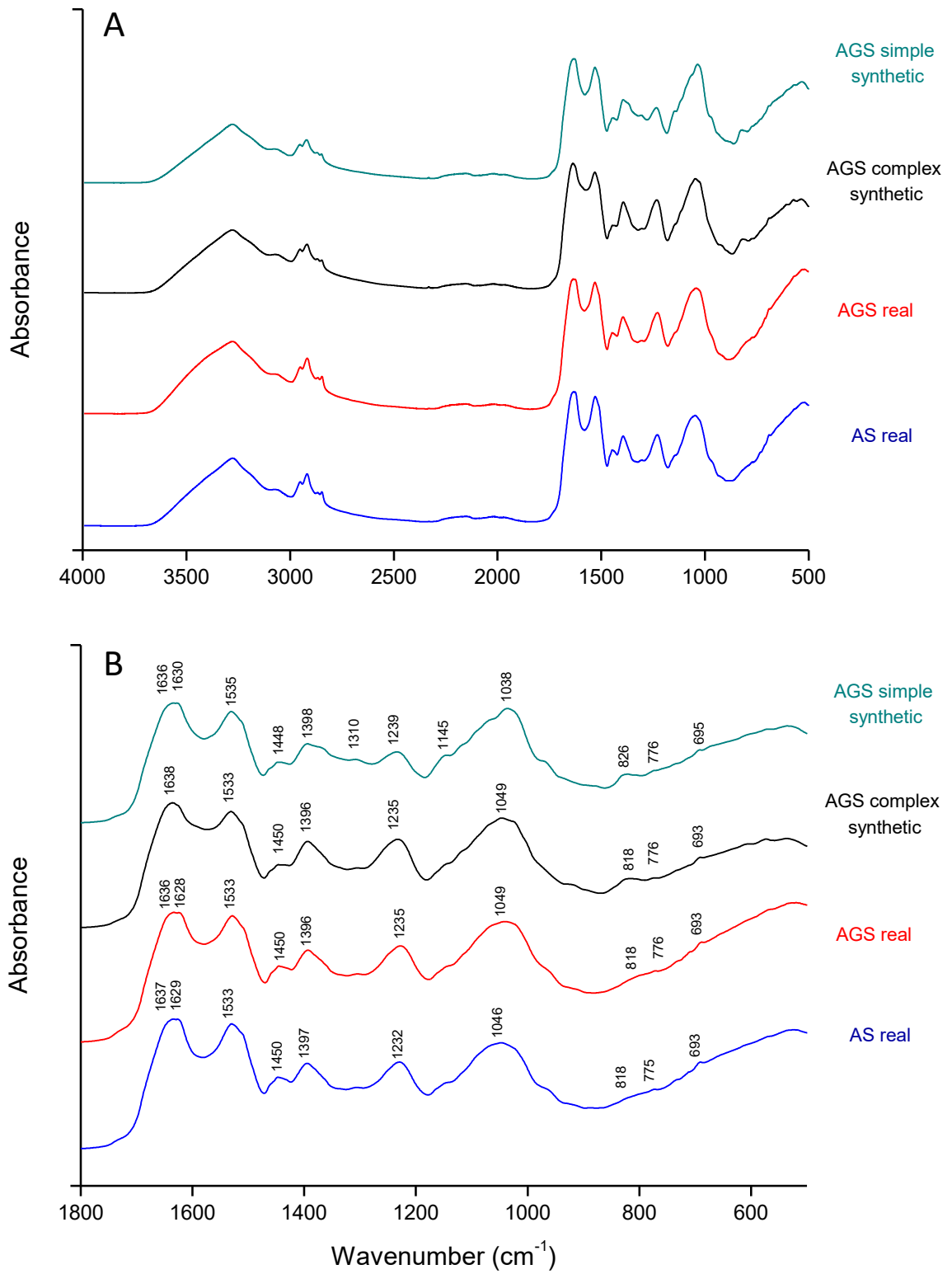


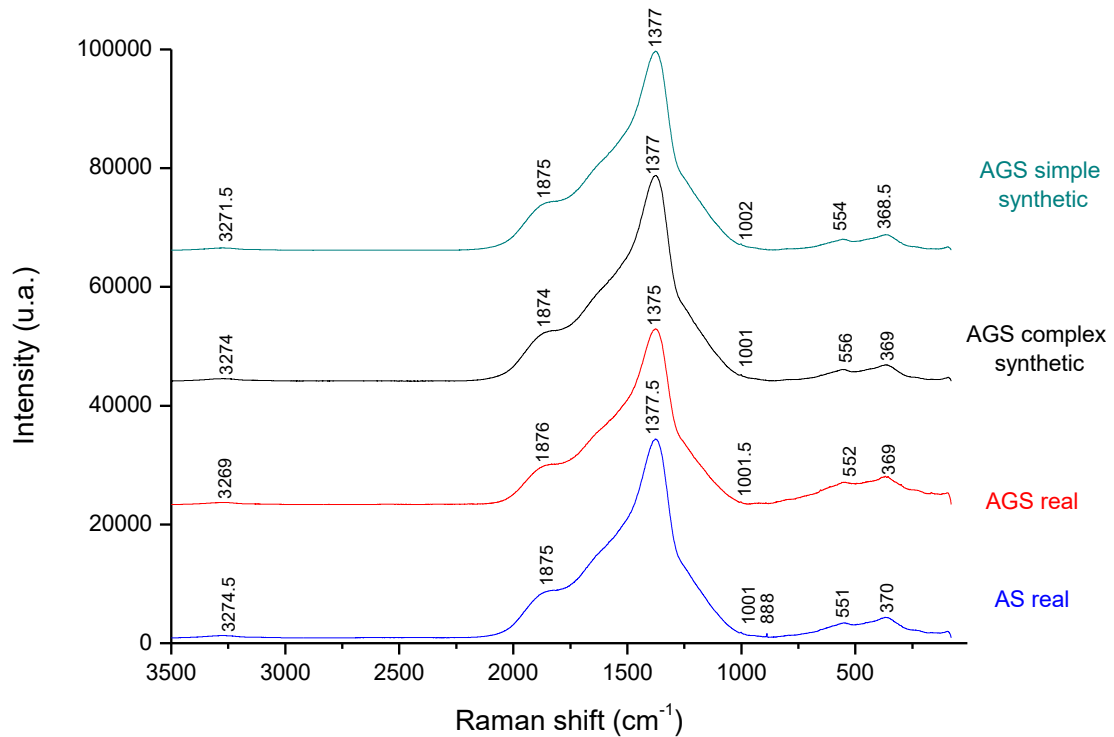
Table 7 - The main absorbance bands in FTIR spectra and their assignments.

Wavenumber (cm ⁻¹)	Assignment	Reference
>3000	O-H stretching vibrations, N-H stretching vibrations of sodium salts of amino acids	Silverstein et al. (2005)
3073-3082	C-H from aromatic amino acids	Lin et al. (2018)
2875-2962	C-H stretching vibration of aliphatic structures	Silverstein et al. (2005); Tu et al. (2012)
1638-1628	asymmetric stretching of O-C-O, stretching vibration of C(N)=O groups in amides I, β -sheets structure of secondary proteins	Lin et al. (2018); Silverstein et al. (2005); Zhao et al. (2016)
1396-1398	C-N stretching band of primary amides, carboxylate ions	Silverstein et al. (2005)
1533-1535	N-H deformation, C=N stretching of amides II	Tu et al. (2012)
1448-1450	O-H of phenols of humic acids, CH ₂ groups of aliphatic chains, C=O symmetric stretching vibration in deprotonated carboxyl group of amino acids	Amir et al. (2010); Zhao et al. (2016)
1396-1398 and 1038-1049	O-C-O groups stretching and C-O symmetric vibration of uronic acids	Sam and Dulekgurgen (2015)
1239-1232	O-acetyl ester for bacterial alginates, amide III and aromatic ethers C-O-C in humic acids	Amir et al. (2010); Kazy et al. (2002)

4.2.3.2.3 Na⁺-ALE extracts characterization using Raman spectroscopy

Raman spectroscopy also confirmed the complex composition of all the Na⁺-ALE, with signals assigned to polysaccharides, proteins and humic acids (Figure 25). In addition, all Raman spectra of Na⁺-ALE were very similar to each other. The broad peak at near 1377 cm⁻¹ can be ascribed to amorphous carbon and to humic-like substances in biofilms (CHAO; ZHANG, 2012; IVLEVA *et al.*, 2009), to polyanionic polysaccharides and amino acids (IVLEVA *et al.*, 2008), and to indole ring of the amino acid tryptophan (LIN *et al.*, 2018).

Figure 25 - Raman spectra of Na⁺-ALE extracted from the four different samples of sludge.

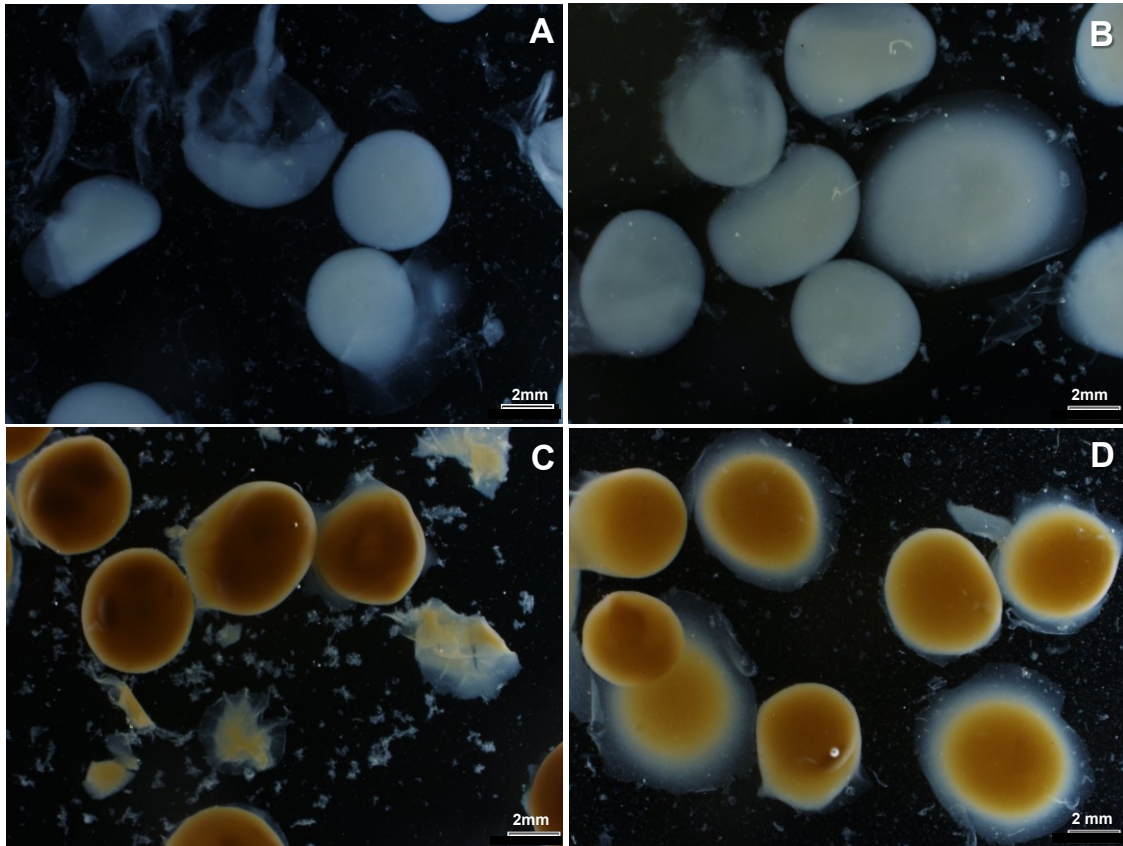


4.2.3.3 ALE hydrogels

4.2.3.3.1 Hydrogel formation

All four Na⁺-ALE extracts formed hydrogel beads when dropped in a CaCl₂ solution (Figure 26). Visually and based on touching, Na⁺-ALE_{AGS simple synthetic} formed the weakest and more unstable Ca²⁺-ALE beads (Figure 26A), while Na⁺-ALE_{AGS real} formed the strongest ones (Figure 26C). After approximately one month of storage in the fridge, only Ca²⁺-ALE beads from AGS_{simple synthetic} disintegrated (data not shown). In addition, the colors of the beads were similar to the color of the sludge source.

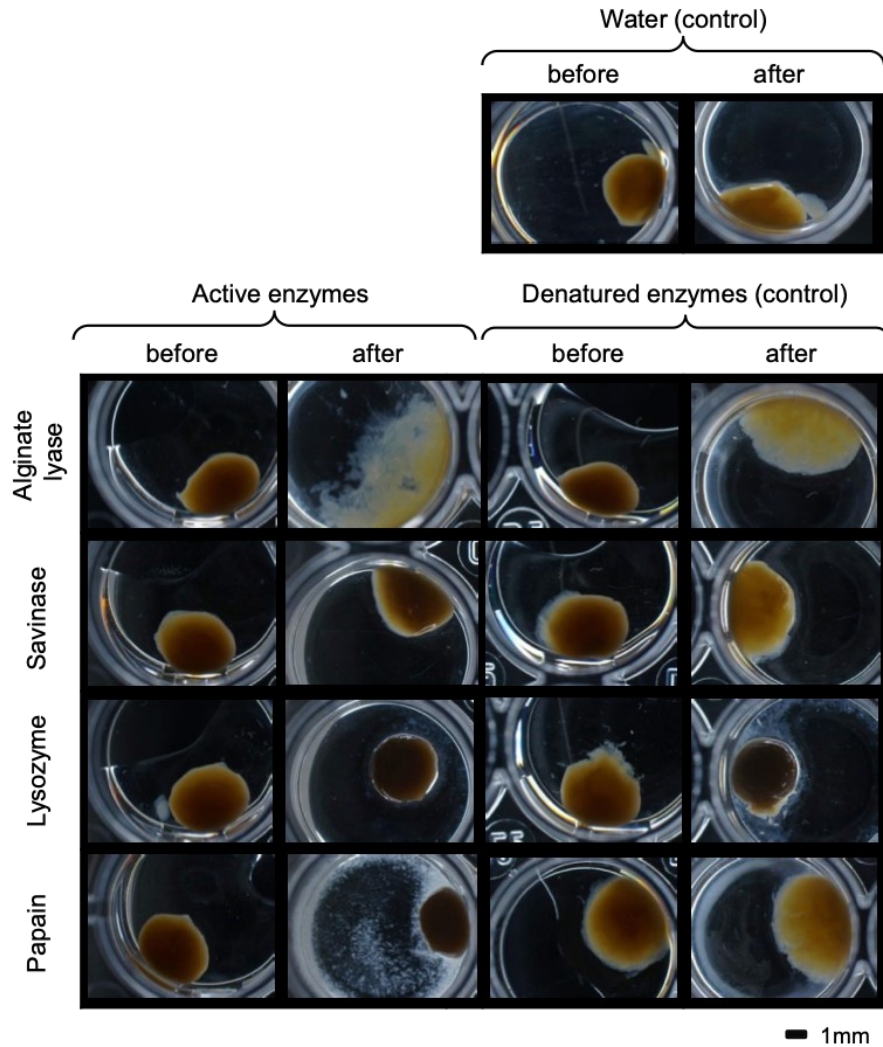
Figure 26 - Stereomicroscopic images of Ca^{2+} -ALE beads from (A) AGS_{simple} synthetic, (B) AGS_{complex} synthetic, (C) AGS_{real} and (D) AS_{real} sludge samples. Ca^{2+} -ALE beads were formed in the 2.5% (w/v) CaCl_2 solution.



4.2.3.3.2 Enzymatic tests

Enzymatic tests were performed to verify what biopolymers/chemical bonds were involved in the hydrogel property of Ca^{2+} -ALE beads (Figure 27). Alginate lyase was the only enzyme able to clearly disintegrate Ca^{2+} -ALE beads. On the contrary, Ca^{2+} -ALE beads after the enzymatic treatment with active savinase, lysozyme and papain visually maintained their structure.

Figure 27 - Stereomicroscopic images of Ca^{2+} -ALE beads extracted from AGS_{real} along the enzymatic degradation test (24 h, room temperature) using the active enzymes alginate lyase, savinase, lysozyme and papain. Controls were run simultaneously with denatured enzymes (heated up to 90 °C for 15 min) and no enzymes (distilled water).



4.2.3.3.3 Rheological properties of the Ca^{2+} -ALE hydrogels

The rheological properties of gel-like viscoelastic materials were characterized by measuring the frequency dependence of storage modulus G' and loss modulus G'' . The influence of the different ALE extracts on the rheological properties of the hydrogels was first studied at a similar dry matter content of 2.3%. Here, the composition of wastewater and bioaggregate type influenced the rheological properties of Na^+ -ALE (left frequency sweep in Figure 28A). Na^+ -ALE_{AGS real} and Na^+ -ALE_{AS real} showed similar values of G' and G'' over the tested frequency regime, indicating a viscoelastic material directly at the gel-point (WINTER;

CHAMBON, 1986). The Na^+ -ALE_{AGS simple synthetic} and Na^+ -ALE_{AGS complex synthetic} form an elastic dominated gel, indicated by G' always larger than G'' . The addition of CaCl_2 leads to an increase and frequency independent behavior of both moduli, due to the formation of a cross-linked gel-like structure of the sample (rubber elastic plateau) (right frequency sweeps in Figure 28A). Ca^{2+} -ALE_{AGS simple synthetic} formed the most elastic hydrogels, followed by Ca^{2+} -ALE_{AGS real}, Ca^{2+} -ALE_{AGS complex synthetic} and Ca^{2+} -ALE_{AS real}. Amplitude sweeps of the CaCl_2 crosslinked gels reveals a comfortable linear viscoelastic regime and a strain-thinning behavior at a deformation of about 10 % (Figure 29). Pictures of the different hydrogels also reveal their rheological properties and follows the rheological properties with the largest G' for Ca^{2+} -ALE_{AGS simple synthetic} followed by Ca^{2+} -ALE_{AGS real}, Ca^{2+} -ALE_{AGS complex synthetic}, and Ca^{2+} -ALE_{AS real} (Figure 30).

The rheological properties of the four Ca^{2+} -ALE hydrogels formed at their original dry matter content were also evaluated (Figure 28B). The extraction of ALE from the different types of sludge resulted in different dry matter contents: 4.2% for AGS_{real}, 3.8% for AS_{real}, 2.4% for AGS_{complex synthetic} and 2.1% for AGS_{simple synthetic}. The different resulting hydrogels were in turn associated with different rheological properties than those measured at normalized dry matter content. At their original dry matter content, the Ca^{2+} -ALE extracted from AGS_{real} formed the most elastic gels, while AGS_{simple synthetic} formed the least elastic gel (Figure 28B). Before gelation, G' was slightly larger than G'' for all Na^+ -ALE samples across the tested frequency range. This suggests that all Na^+ -ALE samples are at the gel-point showing a slope of around 0.5 in the double-logarithmic representation and are shear thinning with the presence of the yield stress. After gelation, G' exceeded G'' , indicating a gel-like sample with a rubber-elastic plateau. Due to instrument and sample inertia the upturn of the G' values in Figure 28 right to the blue dashed line should not be considered as a rheological property of the samples.

Figure 28 - Frequency dependence of G' (solid symbols) and G'' (open symbols) of the four ALE samples before (left) and after (right) hydrogel formation (A) with a controlled dry matter content of 2.3 % (B) with the original dry matter content obtained with the protocol of extraction applied: 4.2% for AGS_{real} , 3.8% for AS_{real} , 2.4% for $AGS_{complex\ synthetic}$ and 2.1% for $AGS_{simple\ synthetic}$. The inertia boundary (dashed blue line) stands for the sensitivity limit for the measurements.

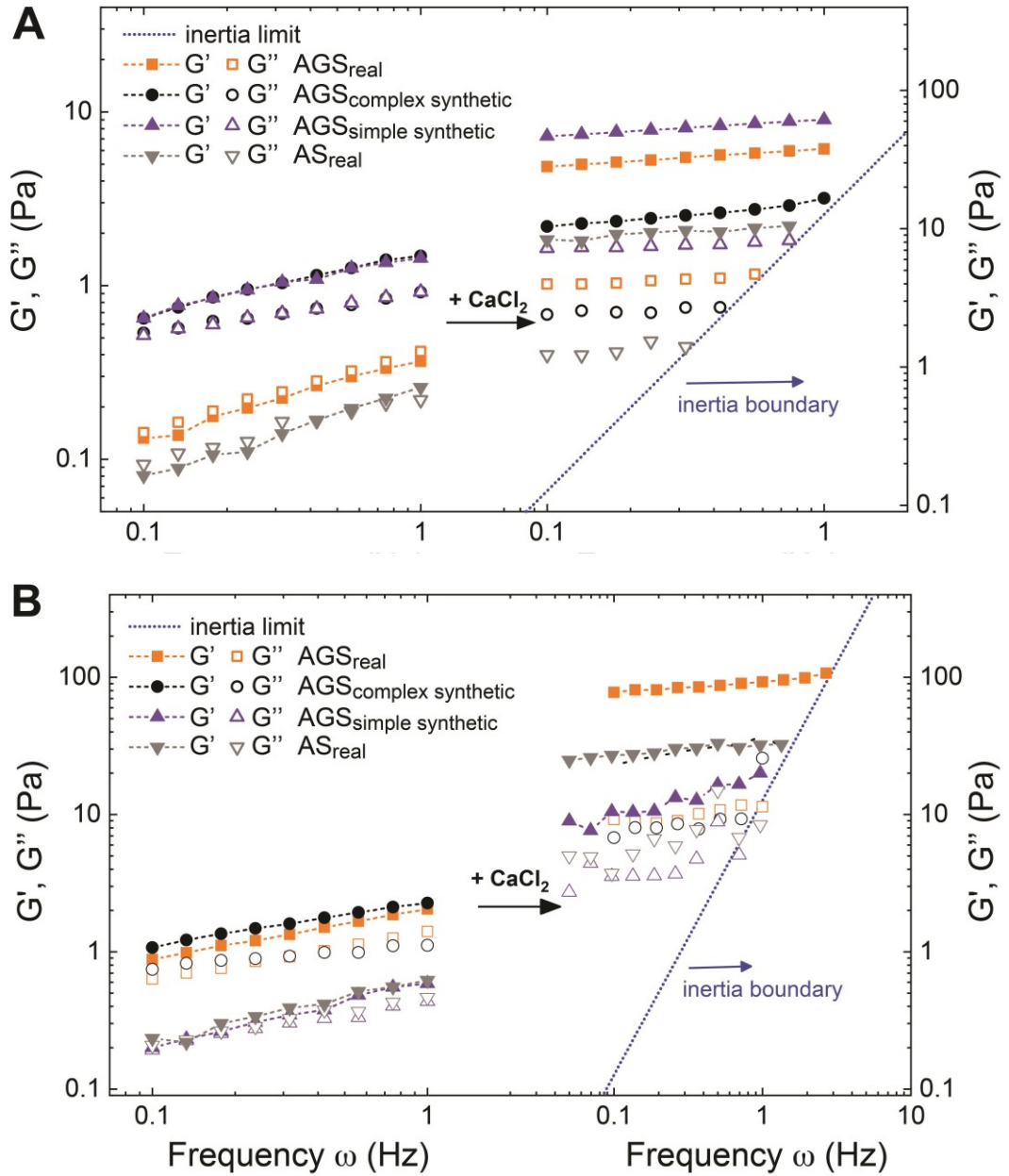


Figure 29 - Amplitude sweep of the Ca^{2+} -ALE samples with a controlled dry matter content of 2.3 %.

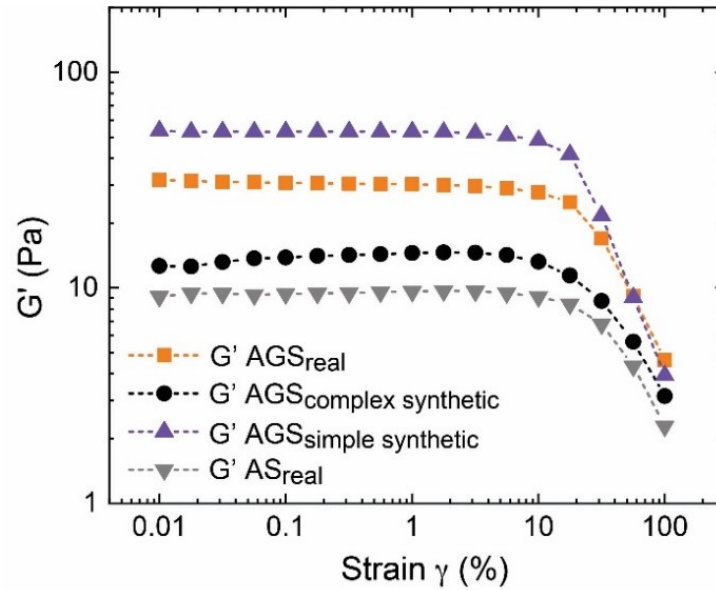


Figure 30 - Image of the Ca^{2+} -ALE samples with a controlled dry matter content of 2.3% (from left to right: AGS_{simple synthetic}, AGS_{real}, AGS_{complex synthetic} and AS_{real}).



4.2.4 Discussion

4.2.4.1 ALE composition

4.2.4.1.1 ALE extracts are a complex mixture of polysaccharides, proteins and humic acids

A main result of this study is that Na^+ -ALE are a very complex mixture of polysaccharides, proteins and humic acids. While ALE extracted from granules are often presented as extracts enriched in polysaccharides (LIN *et al.*, 2010; SAM; DULEKGURGEN, 2015), results herein also demonstrate the significant presence of proteins and humic

substances. The complex composition of Na⁺-ALE was confirmed by using several complementary methods (colorimetric assays, FTIR and Raman) applied to various sludge samples. Although colorimetric assays are only semi-quantitative analytical methods (LE; KUNACHEVA; STUCKEY, 2016; LE; STUCKEY, 2016), these methods provided relevant insights about the composition of Na⁺-ALE extracts. For the analysis of sugars, the modified anthrone method based on a double calibration and on a double absorbance monitoring of total and uronic sugars at 560 and 620 nm was used, respectively. Such analytical method helps quantifying poly-uronic among total carbohydrates, since the uronic sugars are generally not discriminated in other colorimetric assays (DREYWOOD, 1946; DUBOIS *et al.*, 1956).

Results from colorimetric assays indicated that uronic sugars were the most abundant polysaccharides found in the Na⁺-ALE extracts, particularly in Na⁺-ALE_{AGS simple synthetic}, while a low amount of neutral sugars was detected (Figure 22). Results from FTIR, Raman spectroscopy and enzymatic tests also support those observations. FTIR spectra of the Na⁺-ALE extracts from synthetic wastewater further suggested the presence of mannuronic acids, especially in Na⁺-ALE_{AGS simple synthetic}. The presence of mannuronic acids blocks in ALE have been reported for AGS fed with propionate (YANG *et al.*, 2014). Herein, the presence of mannuronic acids in ALE was also confirmed by the results of enzymatic tests. Overall, results support recent findings indicating that ALE are composed of different polysaccharides, divided in both neutral and uronic ones (FELZ *et al.*, 2019).

Another interesting result is the significant presence of proteins in all ALE extracts, confirmed by colorimetric assays, FTIR and Raman analyses. Results from colorimetric assays suggested proteins contents of ~300 to 440 mg_{BSA}/mg VS_{ALE}. Based on the FTIR analyses, a typical protein signature was observed for all Na⁺-ALE spectra, with two clear peaks associated to amide I (1628 to 1638 cm⁻¹) and amide II (1533 to 1535 cm⁻¹). Besides, sodium salts of amino acids show the N-H stretching vibrations at 3400-3200 cm⁻¹ and the characteristic carboxylate ions band appears near 1400 cm⁻¹ (Silverstein *et al.*, 2005), as also observed in Na⁺-ALE spectra. Finally, the Na⁺-ALE Raman spectra were similar to the spectrum of AGS dominated by ammonium-oxidizing bacteria, for which the intense peak at 1365 cm⁻¹ was attributed to the indole ring of the amino acid tryptophan (Lin *et al.*, 2018). Thus, it is evident that ALE extracts do not only contained sugars, but also proteins in (likely) non-negligible quantities.

Humic acids also represented a major fraction of ALE extracts. The Na⁺-ALE spectra obtained with FTIR were very similar to the ones of humic acids isolated from composted activated sludge (AMIR *et al.*, 2010). Humic acids are a mixture of compounds mainly

composed of phenolic and carboxylic acid in majority, but can also contain enolic, quinone, ether functional groups, sugars and peptides (DE MELO; MOTTA; SANTANA, 2016). They are abundant in various terrestrial and aquatic environments and originate from the humification process of several compounds such as amino acids, lignin, pectins and carbohydrates (PEÑA-MÉNDEZ; HAVEL; PATOČKA, 2005). Humic substances are resistant to complete biodegradation (FLEMMING; WINGENDER, 2010). Therefore, it is likely to find them in real wastewater and ultimately in the EPS matrix of flocs and granules, like indicated by the results (Figure 22, Figure 24 and Figure 25). A noticeable content in humic substances was also detected in the extracts of the sludge fed with synthetic wastewater and might come from the groundwater used for preparation of the feed solution. Overall, results clearly indicate the presence of humic acids in Na⁺-ALE extracts.

Hence, the results obtained from different and complementary analytical methods prove that Na⁺-ALE extracts are a complex mixture of polysaccharides, proteins and humic-like substances. A main question is thus to understand what factors (e.g., wastewater composition and bioaggregates type) possibly influence the composition of the Na⁺-ALE extracts.

4.2.4.1.2 Influence of wastewater composition and the type of the bioaggregate on ALE content and composition

The results indicate that the compositions and contents of the different Na⁺-ALE extracts were surprisingly similar. In fact, major differences were expected, due to the very diverse types of the bioaggregates that were analyzed. But only the AGS_{simple synthetic} fed with acetate and propionate presented noticeable differences in their ALE composition/content.

AGS_{simple synthetic} yielded the highest EPS and Na⁺-ALE contents and had the highest contents in proteins and uronic sugars (Table 6 and Figure 22). Overall, the AGS fed with acetate/propionate yielded 49% more ALE (mg VS_{ALE}/g VS_{sludge}, Table 6, n=9 measurements) and 62% more uronic sugars (mg_{glucuronic acid}/g VS_{ALE}, Figure 22, n=3 measurements) than the other sludges. On the contrary, sludge fed with more complex wastewaters, which contains large amount of non-diffusible organic substrates and almost no/little volatile fatty acids, yielded lower amounts of proteins and uronic sugars. The high contents in uronic sugars and proteins in the ALE_{simple synthetic} can be explained by the high availability of volatile fatty acids in the wastewater. The physical structure and microbial community composition of the AGS used in this study was analyzed in details by Layer et al. (2019). High concentrations of VFAs

in the influent resulted in the development of a very distinct microbial community composition and in the formation of large and dense granules (LAYER *et al.*, 2019). It is suggested that the high ALE and uronic sugar contents found in the AGS_{simple synthetic} results from the specific microbial and physical structure of those granules. Storing microorganisms such as *Ca. Accumulibacter* (classical polyphosphate accumulating organisms - PAO) and *Ca. Competibacter* or CPB C22&F32 (classical glycogen accumulating organisms - GAO) represented the dominant populations in the AGS_{simple synthetic} (LAYER *et al.*, 2019). On the contrary, the fermentative PAO *Tetrasphaera* and the fermentative GAO *Micropruina* dominated the AGS fed with complex/real wastewater that were characterized by lower ALE and uronic acid contents. Microbial populations that utilize VFA might have a higher capability to produce specific polysaccharides such as uronic sugars. Pronk *et al.* (2017) also observed that granules fed with acetate are associated with very specific microbial populations and in turn contain very specific polymers (acid-soluble). In addition, the production of polysaccharides and proteins in the AGS_{simple synthetic} might also be enhanced by the formation of ecological niches within the granules. The large diameter and the high concentration gradients favor anoxic growth, i.e., the growth of denitrifying microorganisms and decay in the deep layers of the granules (LAYER *et al.*, 2020). A high decay rate ultimately promotes the production of proteins, originating from the membrane of the bacterial cells. Such mechanism might explain the large proteins content found in the ALE extracted from AGS_{simple synthetic}.

The results also indicate that the type of bioaggregate (granules versus flocs) does not really influence the EPS and ALE composition and content (Table 6, Figure 22, Figure 24 and Figure 25). Herein, similar EPS and ALE contents were indeed measured for both flocs and granules. This observation is in agreement with the findings Sam and Dulekgurgen (2015) but in contradiction with those of Lin *et al.* (2013). Lin *et al.* (2013) reported ALE contents in AGS nearly two times larger than in flocs. Qualitatively, no clear differences in the results of the colorimetric assays, FTIR and Raman of ALE extracts from AGS_{real} and AS_{real} were observed. Besides, ALE extracted from both AGS and AS were able to form hydrogels (Figure 26). This absence of pronounced differences in the qualitative characteristics of ALE recovered from flocs and granules fed with the same wastewater has been reported (SCHAMBECK *et al.*, 2020a). Hence, it remains rather unclear to what extent the composition and content of ALE really changes between flocs and granules. Likely, other mechanisms such as abiotic ones (e.g., extraction method) also influence the composition of ALE and contributes for the similarity in composition of flocs and granules.

4.2.4.1.3 Influence of ALE composition by the extraction protocol

The composition and concentration of every EPS extracts is significantly influenced by the extraction/purification protocol (HONG *et al.*, 2017; MCSWAIN *et al.*, 2005; YANG *et al.*, 2019). ALE extracts were enriched in proteins and humic-like substances. Humic substances are usually extracted using alkaline soaking, prior precipitation at low pH (DE MELO; MOTTA; SANTANA, 2016; PEÑA-MÉNDEZ; HAVEL; PATOČKA, 2005). Hence, the extraction protocol used in this study might selectively enrich the ALE extracts in those substances (Figure 23). The combined high-temperature and alkaline conditions might also generate some cell lysis, and in turn a dilution of the EPS extracts with intracellular proteins (MCSWAIN *et al.*, 2005; YANG *et al.*, 2019). While harsh extraction methods can lead to an overestimation of the EPS content (HONG *et al.*, 2017), those conditions are required for an efficient recovery and representative EPS analysis (PRONK *et al.*, 2017). Therefore, the high temperature and alkaline conditions used for EPS extraction can ultimately dilute the EPS composition in a way that differences in the content/composition are less detectable.

The results in fact suggest that polymers found in ALE extracts are likely not solely extracellular. Although, at first, the EPS solubilization from the biomass is necessary, it is not certain that polymers originate solely from the extracellular polymeric matrix. This is true for likely all studies in which ALE was recovered with harsh extraction methods. Hence, the term “extracellular polymeric substances - EPS” is not an appropriate terminology when alkaline, high-temperature and mixing conditions are applied for biopolymers recovery. Under those conditions, there may be microbial polymeric extracts that encompasses the broth of internal and external polymers that contributes to ALE composition. The term alginate-like polymers (AGP) would fit better to describe this gel polymer recovered from biological sludge.

4.2.4.2 ALE hydrogel property

4.2.4.2.1 Mannuronic sugars are involved in ALE's hydrogel property

Enzymatic tests were performed to verify what biopolymers/chemical bonds were involved in the hydrogel property of Ca²⁺-ALE beads. Alginate lyase was the only enzyme able to clearly disintegrate Ca²⁺-ALE beads (Figure 27). On the contrary, Ca²⁺-ALE beads after the enzymatic treatment with active savinase, lysozyme and papain visually maintained their

structure. All those enzymes had a molecular weight lower than 40000 Da and were thus able to penetrate the hydrogels. The stability of the Ca^{2+} -ALE_{AGS real} beads structure after the enzymatic treatment with active savinase, lysozyme and papain suggests that (i) either peptide and peptidoglycan bonds have been broken down by savinase and papain (proteases), and lysozyme (glycoside hydrolase), but with no clear effect in the gel structure stability (ii) or those bonds were not present. However, results of colorimetric assays, FTIR and Raman show that peptide and peptidoglycans bonds may in fact be present. Hence, peptide and peptidoglycan bonds are not involved in the maintenance of the hydrogel structure. The disintegration of Ca^{2+} -ALE_{AGS real} exposed to alginate lyase confirms both the presence of mannuronic acids (uronic sugars) and their role in the formation of ALE hydrogels. Alginate lyase cleaves the β -(1-4)-D-mannuronic bonds in the homopolymeric regions of mannuronic residues. However, it is not clear whether alginate lyase cleaves only MM (mannuronic-mannuronic) or also MG (mannuronic-guluronic) blocks.

So far, there is no consensus in literature about the role of polysaccharides and proteins in the gel properties of bioaggregates. Granule cohesion has been proposed to depend on the formation of calcium bridges between anionic proteins, thus creating an intercellular cement (CAUDAN *et al.*, 2014). Proteins are believed to have an important role in bonding EPS due to their high affinity for cations (ZHU *et al.*, 2015). However, other studies ascribed the gelling properties of EPS to high molecular weight polysaccharides (SEVIOUR; DONOSE; PIJUAN, 2010), although the hydrophobicity of the proteins can be helpful for hydrogel formation (LI *et al.*, 2014). Herein, enzymatic tests indicated that alginate lyase was the sole enzyme able to disintegrate the Ca^{2+} -ALE_{AGS real} beads. Therefore, peptide and peptidoglycans bonds may not play a role on hydrogel property of ALE while uronic carbohydrates containing mannuronic acids are key molecules involved in the cohesion of ALE hydrogels. Possibly, mannuronic acids are also important structural gelling polymers in bioaggregates.

4.2.4.2.2 Influence of the wastewater composition and type of bioaggregate on ALE hydrogel property

Another key question is whether the different ALE extracts result in the formation of hydrogels with similar or distinct rheological properties. Such aspect is especially important for the scale-up of ALE recovery and its further industrial applications. The results demonstrate that the elasticity of the different hydrogels was very variable (Figure 28). The following

general trends are however suggested: (1) the elasticity of ALE extracted from AGS is always higher than the one from flocs when fed with real wastewater and (2) for hydrogels with the same dry matter content, the highest elasticity is observed for the ALE recovered from AGS fed with VFA-rich wastewater.

The results indicate that Ca^{2+} -ALE_{AGS real} were more elastic than Ca^{2+} -ALE_{AS real} (Figure 28). This result demonstrates the influence of the bioaggregates type on the hydrogel properties of ALE. Similarly, Lin et al. (2013) conducted stress-relation tests and concluded that Ca^{2+} -ALE extracted from granules were more elastic than flocs. Differences in the chemical composition of ALE can explain the different rheological and mechanical properties between Ca^{2+} -ALE_{AGS real} and Ca^{2+} -ALE_{AS real}. Alginate is composed of both mannuronic (M) and guluronic (G) acids, which can form three different blocks: MM, MG and GG blocks (Lee and Mooney, 2012). ALE recovered from AGS is enriched in GG blocks, while ALE extracted from flocs have more MM blocks (LIN; SHARMA; VAN LOOSDRECHT, 2013). This may explain the stronger gelling properties of ALE recovered from AGS since GG blocks are believed to be responsible for stiffness and strength in hydrogels (HAY *et al.*, 2013). Low mannuronic/guluronic acids ratio can produce stronger alginate hydrogels (RAMOS *et al.*, 2018). FTIR, Raman and colorimetric analyses indicated a very similar composition of ALE isolated from both flocs and granules. Nevertheless, more specific differences in the chemical composition in ALE may be linked to the composition of uronic sugars (i.e., mannuronic/guluronic acids ratio) that govern the gel-forming and mechanical properties in flocs and granules.

Moreover, the results give some evidence about the influence of wastewater composition on the rheological properties of ALE. At the original dry matter content, Na^+ -ALE extracts with the highest uronic sugar content ($\text{mg}_{\text{glucuronic acid/g}} \text{VS}_{\text{ALE}}$) were, in descending order: $\text{AGS}_{\text{simple synthetic}} > \text{AGS}_{\text{real}} > \text{AS}_{\text{real}} > \text{AGS}_{\text{complex synthetic}}$ (Figure 22). At similar conditions, the elasticity of Ca^{2+} -ALE hydrogels decreased like $\text{AGS}_{\text{real}} > \text{AGS}_{\text{complex synthetic}} \approx \text{AS}_{\text{real}} > \text{AGS}_{\text{simple synthetic}}$ (Figure 28B). Thus, at the original dry matter content obtained from the protocol of extraction, ALE recovered from VFA-rich wastewater formed the least elastic hydrogels while having the highest uronic sugar content. This is possibly due to the higher content of mannuronic acids in ALE_{AGS simple synthetic} as suggested by FITR results (section 4.2.3.2.2). Higher contents of mannuronic acids indeed form weaker hydrogels (HAY *et al.*, 2013). Nonetheless, at normalized dry matter content, ALE recovered from VFA-rich wastewater formed the most elastic hydrogels (Figure 28A). Hence, probably it is not the content of uronic sugars itself that governs the elasticity of ALE hydrogels, but also the type of

uronic sugars. Further studies are encouraged to confirm to what extent the different types of uronic sugars impact the rheological properties of ALE hydrogels.

4.2.4.3 Practical implications: impact of ALE composition and different sludges sources on ALE recovery

The results clearly demonstrate that ALE are a complex mixture of different biopolymers such as polysaccharides, proteins and humic acids. The hydrogel property of ALE is governed by the uronic sugars. Nevertheless, the presence of other compounds in the ALE extracts, such as proteins or humic acids, is inevitable in practice and may provide additional properties and extra applications to this biomaterial (LIN *et al.*, 2015). Hence, the synergetic effect of the different constituents in ALE should be evaluated according to the use and required degree of purity.

The hydrogel properties and organic nature of ALE encourages its use for encasement and release of different substances, as fertilizers in soil. The presence of humic acids might broaden the range of applications, e.g., in agriculture, industry, environment and biomedicine (PEÑA-MÉNDEZ; HAVEL; PATOČKA, 2005). Humic acids have an amphiphilic character, finding application as surfactant and metals chelating agent (DE MELO; MOTTA; SANTANA, 2016). If a higher stiffness is required for ALE hydrogels, the mechanical properties can be tailored by (i) increasing the uronic sugar content (e.g., by the treatment with enzymes) (ii) cross-linkage to cations with higher affinity, as copper or zinc (FELZ *et al.*, 2020b), or (iii) changing the length and proportion of guluronic and mannuronic blocks (HAY *et al.*, 2013). Low guluronic acids block content in commercial alginate generates more porous hydrogels (RAMOS *et al.*, 2018), what in turn can impact future uses of ALE adsorbents.

Another main result is that the rheological properties of the ALE hydrogels are dependent on type/origin of the bioaggregate. This result has significant implications for the full-scale recovery of ALE. Recovering ALE of different compositions would allow forming hydrogels with distinct rheological properties, which can help broadening the scope of industrial applications. However, recovering ALE at each WWTP site might not be relevant from an economical point of view. Likely, it would be more relevant to recover ALE at a single centralized site that would receive excess sludge from different satellite WWTPs. Recovering ALE from a blend of sludge would represent a relevant approach to control the composition

and overall ALE content, and consequently to govern ALE properties, which in turn represent a key advantage for further industrial applications.

4.2.5 Conclusions

1. ALE is a complex mixture of polysaccharides, proteins and humic acids. The content and composition of ALE extracted from different types of sludge is rather similar. Only the presence of volatile fatty acids in the wastewater significantly impacts the ALE content and composition. However, the type of bioaggregate (flocs vs. granules) does not clearly influence the composition and overall ALE content.
2. The higher the volatile fatty acids content in the wastewater, the higher the overall ALE and uronic sugars contents of the sludge. Aerobic granular sludge fed with acetate/propionate yielded in average 49% more ALE and up to 62% more uronic sugars in ALE than other bioaggregates.
3. Mannuronic acids are involved in the hydrogel property of ALE. Besides, the elasticity of the hydrogels greatly varies in response to the type/origin of the sludge from where ALE is extracted. The hydrogels formed with ALE extracted from aerobic granular sludge fed with real wastewater were consistently more elastic than the hydrogels formed with ALE extracted from flocs.
4. The presence of proteins (from lysis of bacterial cells) and humic substances (due to the enrichment of the precipitation step) in the ALE extracts is increased by the robust extraction protocol and by the precipitation step. Nonetheless, the presence of proteins and humic substances in the extract does not affect the hydrogel formation property of ALE and thus further applications.
5. Due to the fact that the forming polymers of ALE may come from the intracellular media of cells and also from external sources (e.g., humic substances), the term alginate-like polymers (ALP) would suit better to describe this gel polymer recovered from biological sludge.

4.3 ARTIGO 3 - PHOSPHATE REMOVAL FROM MUNICIPAL WASTEWATER BY ALGINATE-LIKE EXOPOLYMERS HYDROGELS RECOVERED FROM AEROBIC GRANULAR SLUDGE

Abstract

Alginate-like exopolymers (ALE) can be recovered from waste biological sludge such as aerobic granular sludge (AGS). Among the several different industrial uses proposed for ALE, the formation of hydrogels to be used as adsorbents is a relevant approach. In this context, this work aimed at assessing the potential of Ca^{2+} -ALE hydrogels in removing different compounds commonly present in domestic wastewater. Batch sorption experiments were performed with Ca^{2+} -ALE hydrogels recovered from AGS which treated domestic wastewater. Different environmental and operating conditions were tested by varying hydrogel dosage, pH, and PO_4^{3-} , NH_4^+ and NO_3^- concentrations. The results showed that ALE hydrogels were able to effectively remove only phosphate with removals of up to 90.8%. However, ALE hydrogels largely contributed to the increase in COD, total nitrogen and total phosphorus in the bulk liquid. Thus, chemical changes in the composition of the ALE must be made to tailor its mechanical stability and avoid the release of other compounds. Phosphate removal was not impacted neither by higher ammonium nor nitrate concentrations. Moreover, neutral to acidic pH values strongly impaired the phosphate removal ($28.9 \pm 0.8\%$ at $\text{pH}=6$), while basic pH at around 8.5 was the most favorable. Therefore, our study demonstrates that a recovered resource (ALE) has a promising potential to be used for resource recovery (i.e., phosphorus).

4.3.1 Introduction

The environmental sustainability of wastewater treatment plants (WWTP) can be deeply enhanced by an integrated approach based on the resource recovery from these systems (WANG *et al.*, 2015). The main objective is the valorization and recovery of valuable materials from wastewater, and thus minimizing the need for a final disposal site. Biogas, heat, phosphorus, nitrogen, sulfur, cellulose, bioplastics and EPS (extracellular polymeric substances) can, for example, be recovered (SOLON *et al.*, 2019). In this context, the reuse of waste biological sludge is a relevant approach. The application of waste biological sludge as a raw material for manufacturing high value-added products such as adsorbents, flocculants and construction materials is promising (SHI *et al.*, 2018). Given the increasing amount of aerobic

granular sludge (AGS) produced around the world, there has been a great interest in assessing their EPS recovery potential (FREITAS; TORRES; REIS, 2017).

Alginate-like Expolymers (ALE) recovery from waste biological sludge is an alternative route for sludge management (NANCHARAIAH; KIRAN KUMAR REDDY, 2018). ALE are a group of structural biopolymers that has been successfully recovered mainly from the EPS of AGS applied to the treatment of municipal wastewater (LIN *et al.*, 2010; SCHAMBECK *et al.*, 2020a). ALE can form ionic hydrogels and have other similar characteristics to the commercial polysaccharide alginate (FELZ *et al.*, 2016; LIN *et al.*, 2010; SCHAMBECK *et al.*, 2020a). The current market conditions are promising for ALE recovery since ALE is regarded as the most valued resource to be recovered during the treatment of domestic wastewater (VAN DER HOEK; DE FOOIJ; STRUKER, 2016). Studies have been demonstrating interesting uses of ALE such as a coating material to provide waterproofness and flame retardancy to surfaces, and as a potential biosorbent for dyes and phosphate (DALL'AGNOL *et al.*, 2020; KIM *et al.*, 2020; LADNORG *et al.*, 2019; LIN *et al.*, 2015). Thus, there is an increase need for recovery techniques, characterization methodologies and studies focusing on the applications of ALE (LIN *et al.*, 2015).

ALE are a complex group of biopolymers comprised by different constituents such proteins, humic substances, sugars (neutral, uronic, alcoholic and amino ones), glycoconjugates and lipids (FELZ *et al.*, 2019, 2020a; MENG *et al.*, 2019; SCHAMBECK *et al.*, 2020b). ALE composition is more complex than alginate, thus several functional groups can be involved in the hydrogel formation (FELZ *et al.*, 2020b). The complex composition of ALE can endow it with potential chemical properties, such as adsorbent properties for different compounds. For instance, ALE recovered from AGS fed with domestic wastewater has been showed to have a much higher content of humic substances and proteins than neutral and uronic sugars (SCHAMBECK *et al.*, 2020b). Humic acids are rich in phenols and carboxylic acids, and then can be employed as chelating agents (DE MELO; MOTTA; SANTANA, 2016). The ability of humic acids and proteins in binding with cationic metals and in turn to form complexes (CAUDAN *et al.*, 2014; DE MELO; MOTTA; SANTANA, 2016) may make them useful for different adsorbent applications. Indeed, ALE can form ionic hydrogels by cross-linking with different cations (FELZ *et al.*, 2016, 2020b). Therefore, this complex cross-linked network of ALE hydrogels have miscellaneous polymers, which may provide sorption sites for different compounds in solutions. Besides, gathering information about what compounds/elements can be sorbed by ALE can improve the knowledge about ALE composition which ultimately can impact industrial uses.

Hydrogel polymers have been successfully employed as adsorbents for cationic elements from domestic wastewater, such as ammonium, magnesium, calcium and potassium (CRUZ *et al.*, 2018). Similarly, one interesting possibility is to test ALE hydrogels as an adsorbent for wastewater pollutants. Dall’Agnol *et al.* (2020) have showed that Ca^{2+} -ALE beads have high affinity for phosphorus sorption with removals up to 72.5%. Moreover, Ca^{2+} -ALE hydrogels have been able to remove up to 69% of the dye methylene blue (LADNORG *et al.*, 2019). However, both studies were performed using synthetic solutions. In natural waters or wastewaters, the presence of several ions can hinder the adsorption process due to the different ionic strengths and thus present different results in relation to synthetic solutions (BASSIN *et al.*, 2011; HU *et al.*, 2015; KUMAR *et al.*, 2018; SIWEK *et al.*, 2016; YANG *et al.*, 2006a; ZHENG; XIE; WANG, 2012). Consequently, it is relevant to test the sorption process by using real wastewater to have more realistic results that ultimately may impact up-scale applications (KUMAR *et al.*, 2019; WANG *et al.*, 2018).

Therefore, this work aimed at assessing the potential of ALE hydrogels in removing different cationic and anionic compounds commonly present in domestic wastewater. Batch sorption experiments were performed with different dosages of ionic crosslinked ALE hydrogels recovered from AGS which treated domestic wastewater. Different environmental and operating conditions were tested to assess: (1) what wastewater soluble compounds can be removed (i.e., PO_4^{3-} , NH_4^+ , NO_3^- , NO_2^- , Cl^- , Na^+ , K^+ , Ca^{2+} and Mg^{2+}); (2) the influence of the wastewater composition in terms of initial concentrations of PO_4^{3-} , NH_4^+ and NO_3^- ; and (3) the influence of the wastewater pH.

4.3.2 Materials and methods

4.3.2.1 Alginate-like exopolymers (ALE) hydrogels preparation

ALE was extracted from AGS cultivated in a sequencing batch reactor which treated municipal wastewater from the city of Dübendorf, Switzerland. Details about the reactor operation can be found in Layer *et al.* (2019). ALE was recovered from AGS under hot, alkaline and mixing conditions according to the protocol described by Felz *et al.* (2016). Briefly, a known mass of wet sludge was centrifuged (4000 g, 4°C, 20 min) prior discharging the supernatant. The pellet was then transferred to a 1 L beaker filled with demineralized water, Na_2CO_3 and equipped with a magnetic stirrer. A ratio of 3:50:0.25 (sludge mass (g), demineralized water (mL) and Na_2CO_3 (g)) was kept during the extractions. The solution was

heated up to (80 °C) and stirred for 35 min at 400 rpm. The mixed liquor was then centrifuged (4000 g, 4 °C, 20 min) to recover the supernatant that comprised the solubilized EPS. Acidic ALE were extracted from EPS by acidic precipitation: 1M HCl was added to the soluble EPS solution to reach a final pH of 2.20 ± 0.05 while stirred at approximately 100 rpm. Soluble Na^+ -ALE were then obtained by the addition of 0.5 M NaOH to the precipitated acidic ALE until pH reached 8.5.

Ca^{2+} -ALE hydrogels were formed by immersing and pipetting continuously (10 mL micropipette tip) Na^+ -ALE into a 5% (w/v) CaCl_2 solution. Hydrogels strips were thus formed without control of their shape (Figure 31). After 24 hours, the hydrogels were washed 5 times with tap water and reserved in a 1 L beaker filled distilled water for at least 24 hours before being used for the sorption tests. ALE were quantified as total solids (TS) according to Felz et al. (2016).

Figure 31 - ALE hydrogels stripes used for the sorption tests.



4.3.2.2 Wastewater characterization

Fresh primary effluent municipal wastewater from the WWTP of Eawag (Dübendorf, Switzerland) was collected and immediately frozen to avoid microbial conversion, and thus keeping a similar composition for the different sorption tests. Prior to the experimental runs, the wastewater was brought to ambient temperatures (~ 21 °C). The same initial collected wastewater was used for every set of experiments with characterization as shown in Table 8.

Table 8 - Characterization of the primary effluent municipal wastewater used in the experiments. Error estimates are standard deviation from five measurements (n=5).

Parameter	Value
Soluble COD (sCOD) (mg/L)	341 ± 13
Total COD (tCOD) (mg/L)	571 ± 21
Temperature (°C)	20.5 ± 1.0
pH	8.67 ± 0.20
PO ₄ ³⁻ (mg PO ₄ ³⁻ -P/L)	2.7 ± 0.2
Total phosphorus (TP) (mg/L)	6.2 ± 0.7
NH ₄ ⁺ (mg NH ₄ ⁺ -N/L)	21.4 ± 3.3
NO ₃ ⁻ (mg NO ₃ ⁻ -N/L)	1.3 ± 0.1
NO ₂ ⁻ (mg NO ₂ ⁻ -N /L)	0.3 ± 0.1
Total nitrogen (TN) (mg/L)	40.2 ± 4.6
Cl ⁻ (mg/L)	129 ± 23
Na ⁺ (mg/L)	111 ± 2
K ⁺ (mg/L)	31.8 ± 21.0
Ca ²⁺ (mg/L)	83 ± 6
Mg ²⁺ (mg/L)	19.7 ± 0.7

4.3.2.3 Sorption tests

Three sets of 4-hour batch experimental runs (triplicates – three batches, ~21°C) were conducted to: (i) assess the influence of the initial mass of ALE hydrogels on the sorption capability of several ions under normal wastewater conditions; (ii) to investigate separately the effect of the PO₄³⁻, NH₄⁺ and NO₃⁻ initial concentrations on the overall sorption capacity of ALE hydrogels; (iii) to determine the impact of the wastewater pH on the sorption performance of several ions by ALE hydrogels (Table 9).

Experiments were carried out in 600 mL beaker reactors using 300 mL of primary effluent municipal wastewater. Hydrogels were sieved prior being added to the adsorption tests beakers to remove the supernatant. The beakers were placed on a horizontal shaking incubator and shaken at a moderate speed to allow fully mixed conditions without breaking of hydrogels. Aliquots of 5 mL were collected from the settled liquid at t = 5, 10, 25, 60 min and 2, 3 and 4 hrs.

The parameters measured in the experiments are shown in Table 9. PO₄³⁻-P concentrations were measured at t = 0, 5, 10, 25, 60 min and 2, 3 and 4 hrs. The remaining parameters were analysed in the beginning (t = 0, corresponding to primary wastewater without hydrogel addition) and end of the experiments (t = 4 hrs). Since the volume of wet hydrogels added to the batches was significant in comparison to the volume of wastewater, the dilution factor must be considered for the concentrations decay at the beginning of the tests (i.e., at t = 0 min). Therefore, all the initial concentration values (i.e., at t = 0 min) were corrected

considering the dilution factor. The dilution factor was calculated considering the volume of hydrogel added to the volume of wastewater. Thus, a dilution factor of 1.19, 1.16, 1.13 and 1.10 was applied for the dosage of 3.75, 3.13, 2.50 and 1.88 g of TS_{ALE}/L, respectively. The practical loading was calculated and regarded as the sorption capacity that will be realized in practice for a given set of conditions as the ones used in the present study (KUMAR *et al.*, 2019). The practical loading (q) and removal efficiency (R) were calculated as follows:

$$q = \frac{(C_0 - C_e)V}{W} \quad (1)$$

$$R(\%) = \frac{(C_0 - C_e)}{C_0} \times 100 \quad (2)$$

Where q (mg/g) is the amount of ion sorbed per unit weight of the sorbent, V (L) is the volume of the solution, and W (g) is the weight of the ALE hydrogel. C_0 and C_e (mg/L) are the initial and final concentrations of ion in the solution, respectively.

Table 9 – Summary of the sorption tests performed.

	Experiment #1	Experiment #2	Experiment #3
	Influence of the ALE hydrogel dosage	Influence wastewater composition in terms of PO ₄ ³⁻ , NH ₄ ⁺ and NO ₃ ⁻ initial concentration	Influence of the wastewater pH
ALE hydrogel dosage (g TS _{ALE} /L)	3.75, 3.13, 2.50 and 1.88	3.75	3.75
PO ₄ ³⁻ , NH ₄ ⁺ and NO ₃ ⁻ initial concentration	Original values from the primary effluent municipal wastewater	8.7 mg PO ₄ ³⁻ -P/L 98.4 mg NH ₄ ⁺ -N/L 20.9 mg NO ₃ ⁻ -N/L (by the addition of KH ₂ PO ₄ , NH ₄ Cl and NaNO ₃ - ACS reagents, Sigma Aldrich USA)	Original values from the primary effluent municipal wastewater
pH	Original values from the primary effluent municipal wastewater	Original values from the primary effluent municipal wastewater	6.00, 7.00 and 8.57 (by adjusting the pH of the primary effluent municipal wastewater with HCl)
Parameters measured	tCOD, sCOD, TN, TP, PO ₄ ³⁻ -P, NH ₄ ⁺ -N, NO ₃ ⁻ -N, NO ₂ ⁻ -N, Cl ⁻ , Na ⁺ , K ⁺ , Ca ²⁺ and Mg ²⁺		

4.3.2.4 Wastewater chemical analysis

Samples were analysed for total COD (tCOD), total nitrogen (TN) and total phosphorus (TP) using photochemical tests (Hach Lange, Germany, LCK 114, 314, 338, 238,

348, 349). Soluble COD (sCOD) was measured after filtration at 0.45 μm (Macherey Nagel, Nanocolor Chromafil membrane filter GF/PET 0.45 μm , Germany). $\text{NH}_4^+\text{-N}$ was measured using flow injection analysis (Foss, FIA star flow injection 5000 analyser, Denmark). $\text{NO}_3^-\text{-N}$, $\text{NO}_2^-\text{-N}$, $\text{PO}_4^{3-}\text{-P}$ Cl^- , Na^+ , K^+ , Ca^{2+} and Mg^{2+} were measured by anion chromatography (Methrom, 881 compact IC, Switzerland). The pH was monitored using a handheld pH probe (Thermo Fisher, USA).

4.3.2.5 Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES)

ALE hydrogel samples before and after the sorption tests were collected, frozen and lyophilized. Then, samples were digested in 4ml HNO_3 and 500 μL H_2O_2 at 240 $^\circ\text{C}$ and 120 bar of pressure for 20 minutes before being analyzed via inductively coupled plasma optical emission spectrometry (ICP-OES). This analysis aimed at acquiring information about specific elements sorption by ALE hydrogels.

4.3.3 Results

4.3.3.1 How is the influence of the different ALE hydrogels dosages in removing soluble compounds from wastewater (experiment #1)?

The effectiveness of different dosages of ALE hydrogels to sorb different soluble compounds from wastewater was investigated. ALE hydrogels were capable to efficiently remove PO_4^{3-} ions from the wastewater, while no effect on other soluble compounds were observed (Figure 32 and Table 10). Since ALE hydrogels were only able to efficiently remove PO_4^{3-} from the domestic wastewater, the analysis from now on is focused specifically on this ion. Interestingly, a direct relationship was not observed between the dosage of ALE hydrogels and phosphate removal, practical loading or kinetics (Table 11 and Figure 33A). The highest removal of $90.8 \pm 0.0\%$ was obtained when the highest dosage of 3.75 g $\text{TS}_{\text{ALE}}/\text{L}$ was used, while the lowest removal of $52.4 \pm 2.0\%$ occurred for the second highest dosage of 3.13 g $\text{TS}_{\text{ALE}}/\text{L}$. In terms of mass of phosphate sorbed by ALE, the highest value of 0.85 ± 0.02 mg $\text{PO}_4^{3-}\text{-P}/\text{g}$ TS_{ALE} was obtained for the lowest dosage of 1.88 g $\text{TS}_{\text{ALE}}/\text{L}$. Moreover, the least practical loading of 0.28 ± 0.01 mg $\text{PO}_4^{3-}\text{-P}/\text{g}$ TS_{ALE} occurred for the second highest dosage of 3.13 g $\text{TS}_{\text{ALE}}/\text{L}$. Therefore, although the highest dosage of ALE tested presented the highest phosphate removal efficiency, it did not lead to the highest practical loading of the hydrogels.

On the contrary, a relatively high removal ($75.6 \pm 1.5\%$) and the highest practical loading were obtained by the lowest dosage of ALE hydrogels added to the batches ($1.88 \text{ g TS}_{\text{ALE}}/\text{L}$). The lowest dosage of ALE was also beneficial for the lower release of COD and TN, and enabled TP removal in addition to phosphate (Figure 32).

Organic matter and nitrogen compounds were largely released from ALE hydrogels, as indicated by an increase up to $87.1 \pm 1.2\%$ of tCOD, $117.6 \pm 1.5\%$ of sCOD, and $118.0 \pm 3.4\%$ of TN when $3.13 \text{ g TS}_{\text{ALE}}/\text{L}$ was employed (Figure 32B, C and D). Even though the PO_4^{3-} removal by ALE hydrogels, TP increased up to $42.5 \pm 3.5\%$ in the experiments with $3.75 \text{ g TS}_{\text{ALE}}/\text{L}$ (Figure 32E). TP concentration lowered following the PO_4^{3-} removal only when a lower mass of ALE was used ($1.88 \text{ g TS}_{\text{ALE}}/\text{L}$). Ca^{2+} and Cl^- ions concentrations significantly rose as a result of the residual solution used for cross-linkage (CaCl_2) still present on ALE hydrogels, even after the several steps of washing (Table 10). No clear influence of ALE hydrogels could be ascertained in relation to the concentration of the ions NO_3^- , NO_2^- , Na^+ , Mg^{2+} and K^+ , despite a considerable K^+ removal of $82.9 \pm 0.6\%$ occurred in the experiment with $1.88 \text{ g TS}_{\text{ALE}}/\text{L}$. Additionally, in the end of the experiments, pH tended to stabilize in the range of 8.63-8.78.

Figure 32 - Wastewater parameters measured during the sorption tests with different dosages of ALE hydrogels (experiment #1). (A) $\text{PO}_4^{3-}\text{-P}$, (B) tCOD (C) sCOD, (D) TN and (E) TP measured in the wastewater at the beginning (initial, $t = 0$ min, light gray) and end (final, $t = 240$ min, dark gray) of the sorption tests. Conditions: initial pH 8.67 ± 0.20 , temperature 21 ± 1 °C, duration 240 minutes, triplicates.

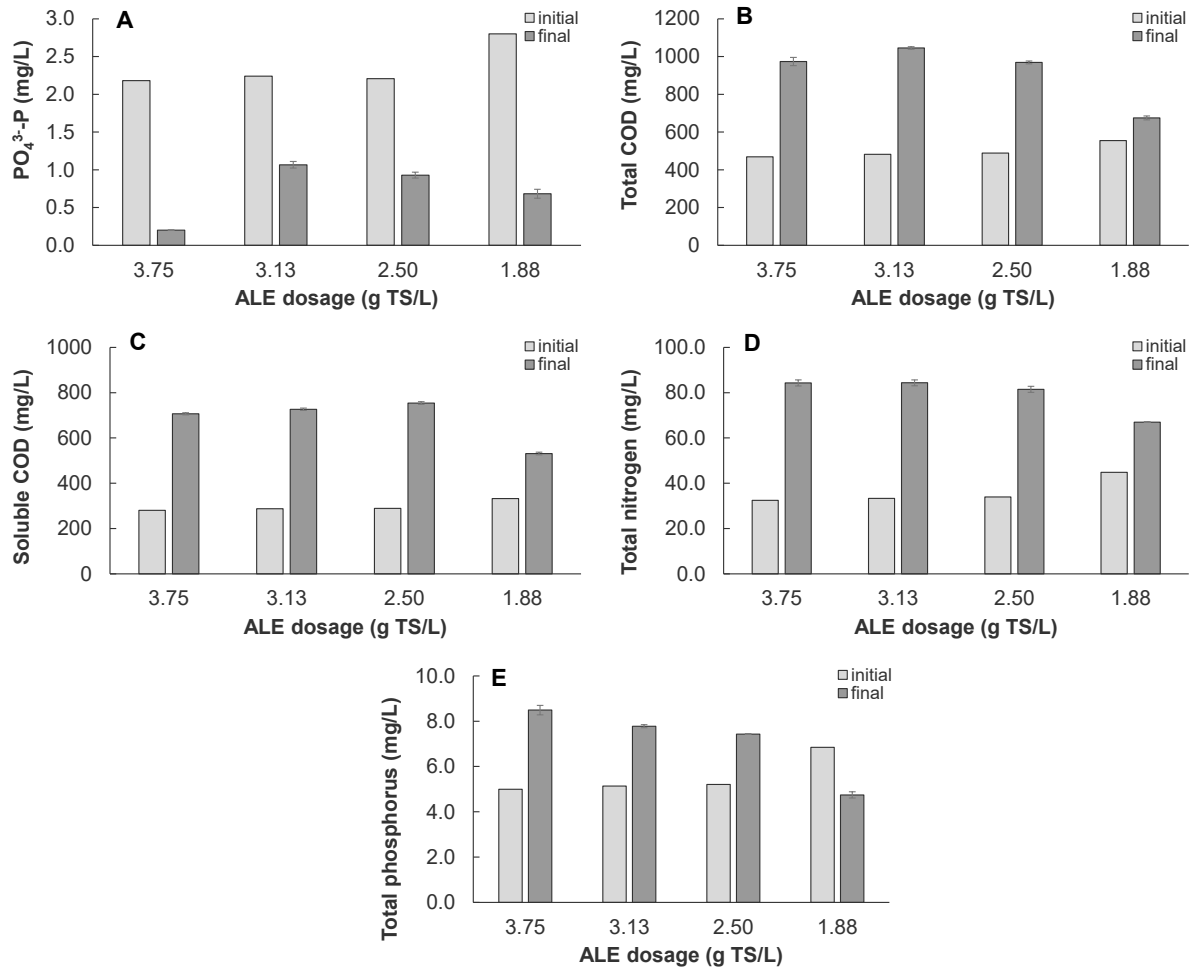


Table 10 - Wastewater parameters measured in the sorption tests with different masses of ALE hydrogels (experiment #1). Conditions: initial pH 8.67 ± 0.20 , temperature 21 ± 1 °C, duration 240 minutes, triplicates.

ALE (g TS/L)	Time	pH	NH ₄ ⁺ -N (mg/L)	NO ₃ ⁻ -N (mg/L)	NO ₂ ⁻ -N (mg/L)	Cl ⁻ (mg/L)	Na ⁺ (mg/L)	K ⁺ (mg/L)	Ca ²⁺ (mg/L)	Mg ²⁺ (mg/L)
3.75	t = 0 ^a	8.57	16.4	1.09	0.17	92	91	14.7	67	16.2
	t = 4 hrs ^b	8.76 ± 0.01	14.3 ± 0.2	1.30 ± 0.07	0.20 ± 0.00	711 ± 29	108 ± 1	11 ± 1	401 ± 20	18 ± 3
3.13	t = 0 ^a	8.91	16.8	1.12	0.17	95	94	15	69	17
	t = 4 hrs ^b	8.63 ± 0.00	15.7 ± 0.2	1.33 ± 0.04	0.26 ± 0.01	273 ± 11	102 ± 3	16 ± 1	200 ± 0	16 ± 0
2.50	t = 0 ^a	8.52	17.3	0.97	0.44	107	102	17	78	19
	t = 4 hrs ^b	8.78 ± 0.01	15.9 ± 0.1	1.20 ± 0.00	0.20 ± 0.00	198 ± 4	109 ± 2	16 ± 0	150 ± 2	16 ± 0
1.88	t = 0 ^a	8.90	25.5	1.09	0.18	157	102	66	67	17
	t = 4 hrs ^b	8.65 ± 0.01	21.8 ± 0.3	1.20 ± 0.00	0.20 ± 0.00	221 ± 14	105 ± 1	11 ± 0	154 ± 10	16 ± 0

Note: ^a corresponds to the primary wastewater after the addition of ALE (including the dilution factor), except for the pH that corresponds to the valued measured for the primary wastewater. ^b corresponds to the wastewater in the end of the experiments (triplicate).

Figure 33 - Kinetics of sorption. (A) $\text{PO}_4^{3-}\text{-P}$ concentration in the wastewater as a function of time after the addition of different initial dosages of ALE hydrogels. (B) $\text{PO}_4^{3-}\text{-P}$, (C) $\text{NH}_4^+\text{-N}$ and (D) $\text{NO}_3^-\text{-N}$ concentration in the wastewater as a function of time after the addition of KH_2PO_4 , NH_4Cl and NaNO_3 , respectively. (E) $\text{PO}_4^{3-}\text{-P}$ concentration in the wastewater as a function of time at different pH. pH at 8.57 corresponds to raw wastewater (without amendments). Error bars in all graphs show the standard deviation from triplicate tests.

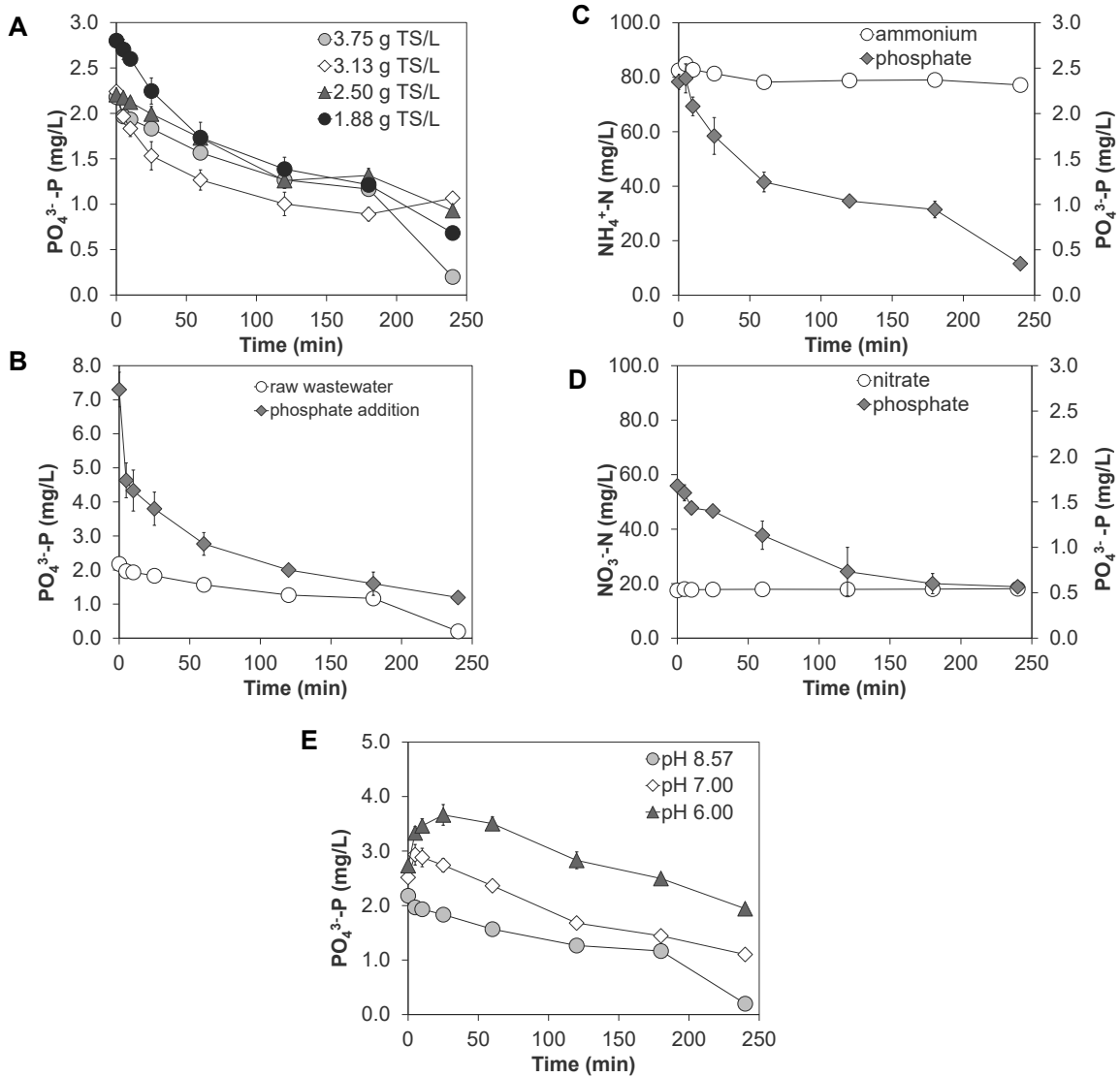


Table 11 - PO₄³⁻-P removal and practical loading from wastewater by different initial dosages of ALE hydrogels. Conditions: initial pH 8.67 ± 0.20, temperature 21±1 °C, duration 240 minutes, triplicates.

ALE concentration (g TS/L)	PO ₄ ³⁻ -P removal (%)	Practical loading (mg PO ₄ ³⁻ -P/g TS _{ALE})
3.75	90.8 ± 0.0	0.40 ± 0.00
3.13	52.4 ± 2.0	0.28 ± 0.01
2.50	57.9 ± 1.8	0.38 ± 0.01
1.88	75.6 ± 1.5	0.85 ± 0.02

4.3.3.2 How is the influence of wastewater composition in terms of initial concentrations of PO₄³⁻, NH₄⁺ and NO₃⁻ (experiment #2)?

Since sorption is also a concentration-dependent phenomenon, the effect of high initial concentrations of the ions PO₄³⁻, NH₄⁺ and NO₃⁻ separately on ALE hydrogels sorption behaviour was also evaluated. Even when the adsorption tests were performed at high concentrations of ions, ALE hydrogels removed only PO₄³⁻ from wastewater (Figure 33B, C and D and Table 12). The higher PO₄³⁻ initial concentration of 7.3 mg PO₄³⁻-P/L considerably increased the practical loading more than three times when compared to concentration at 2.2 mg PO₄³⁻-P/L (from 0.40 ± 0.00 mg PO₄³⁻-P/g TS_{ALE} to 1.22 ± 0.07 mg PO₄³⁻-P/g TS_{ALE}), although the removal efficiency has slightly decreased (from 90.8 ± 0.00 to 83.6 ± 4.7) (Table 13). Furthermore, a sharp drop on PO₄³⁻ concentration from 7.3 mg PO₄³⁻-P/L to 4.6 mg PO₄³⁻-P/L occurred during the first 5 minutes of experiment for the higher initial PO₄³⁻ concentration (Figure 33B). The lower concentration of organic phosphorus in relation to phosphates due to KH₂PO₄ addition also led to an overall TP removal of 34.7 ± 2.7% (15.45 ± 0.63 mg TP/L) (Figure 34E).

Higher initial NH₄⁺-N and NO₃⁻-N concentrations did not result in the removal of these ions by ALE hydrogels (Figure 33C and D and Table 12). Besides, higher initial NH₄⁺-N concentrations did not impact significantly PO₄³⁻-P removal and sorption. At initial concentration of 82.5 mg NH₄⁺-N/L, 85.2 ± 3.9% of PO₄³⁻-P was removed and the practical loading was 0.40 ± 0.00 mg PO₄³⁻-P/g TS_{ALE} (Table 13). Also, no significant release of TP occurred (Figure 34E). For the initial higher concentration of 17.5 mg NO₃⁻-N/L, there was both lower removal of 66.2 ± 6.6% of PO₄³⁻-P and practical loading of 0.22 ± 0.02 mg PO₄³⁻-P/g TS_{ALE} apart from an increase of 39.9 ± 2.3% of TP.

As observed for the previous experiments, a high release of COD and TN occurred for all the tests with higher PO_4^{3-} , NH_4^+ and NO_3^- concentrations and no clear effect on Cl^- , Na^+ , K^+ , Ca^{2+} and Mg^{2+} concentrations was ascertained (Figure 34 and Table 12).

Figure 34 - Wastewater parameters measured during the sorption tests with different initial concentrations of PO_4^{3-} , NH_4^+ and NO_3^- in wastewater (experiment #2). Phosphate concentration at 2.2 mg $\text{PO}_4^{3-}\text{-P}$ /L corresponds to raw wastewater (without amendments) (A) $\text{PO}_4^{3-}\text{-P}$, (B) tCOD (C) sCOD, (D) TN and (E) TP measured in the wastewater at the beginning (initial, t = 0 min, light gray) and end (final, t = 240 min, dark gray) of the sorption tests. Conditions: dosage 3.75 g $\text{TS}_{\text{SALE}}/\text{L}$, temperature 21 ± 1 °C, duration 240 minutes, triplicates.

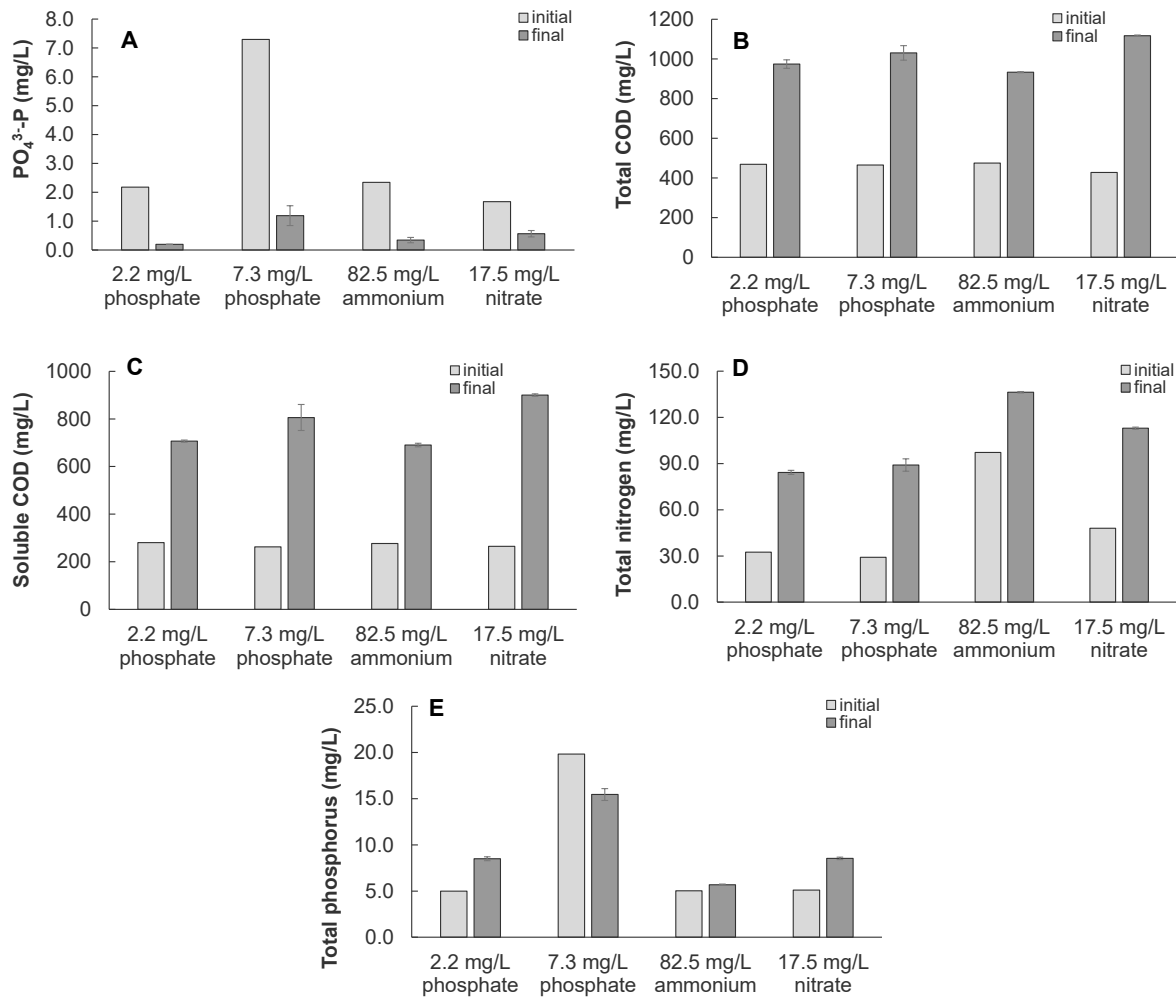


Table 12 - Wastewater parameters measured in the sorption tests with different initial concentrations of PO_4^{3-} , NH_4^+ and NO_3^- in wastewater (experiment #2). Conditions: dosage 3.75 g $\text{TS}_{\text{ALE}}/\text{L}$, temperature 21 ± 1 °C, duration 240 minutes, triplicates, KH_2PO_4 , NH_4Cl and NaNO_3 were added to increase the initial PO_4^{3-} , NH_4^+ and NO_3^- concentrations, respectively.

Initial ion concentration	Time	pH	$\text{NH}_4^+\text{-N}$ (mg/L)	$\text{NO}_3^-\text{-N}$ (mg/L)	$\text{NO}_2^-\text{-N}$ (mg/L)	Cl^- (mg/L)	Na^+ (mg/L)	K^+ (mg/L)	Ca^{2+} (mg/L)	Mg^{2+} (mg/L)
2.2 mg $\text{PO}_4^{3-}\text{-P/L}$	t = 0 ^a	8.57	16.4	1.09	0.17	92	91	14.7	67	16.2
	t = 4 hrs ^b	8.76 ± 0.01	14.3 ± 0.2	1.30 ± 0.07	0.20 ± 0.00	711 ± 29	108 ± 1	11 ± 1	401 ± 20	18 ± 3
7.3 mg $\text{PO}_4^{3-}\text{-P/L}$	t = 0 ^a	8.41	17.1	0.92	0.17	101	87	30	68	14
	t = 4 hrs ^b	8.44 ± 0.03	16.2 ± 0.12	1.33 ± 0.07	0.20 ± 0.00	599 ± 146	103 ± 5	29 ± 1	345 ± 81	14 ± 1
82.5 mg $\text{NH}_4^+\text{-N/L}$	t = 0 ^a	8.71	82.5	1.09	0.42	274	95	16	85	17
	t = 4 hrs ^b	8.89 ± 0.00	77.2 ± 0.29	1.47 ± 0.16	0.50 ± 0.00	984 ± 46	122 ± 14	17 ± 0	484 ± 26	17 ± 1
17.5 mg $\text{NO}_3^-\text{-N/L}$	t = 0 ^a	9.04	16.9	17.5	0.17	99	115	14	73	15
	t = 4 hrs ^b	8.79 ± 0.01	15.1 ± 0.18	18.2 ± 0.0	0.20 ± 0.00	340 ± 24	120 ± 2	13 ± 0.9	245 ± 15	14 ± 1

Note: ^a corresponds to the primary wastewater after the addition of ALE (including the dilution factor), except for the pH that corresponds to the valued measured for the primary wastewater. ^b corresponds to the wastewater in the end of the experiments (triplicate).

Table 13 - PO₄³⁻-P removal and practical loading from wastewater by ALE hydrogels at different initial ions concentrations. Conditions: initial pH 8.68 ± 0.23, dosage 3.75 g TS_{ALE}/L, temperature 21±1 °C, duration 240 minutes, triplicates.

Initial ion concentration in wastewater	PO ₄ ³⁻ -P removal (%)	Practical loading (mg PO ₄ ³⁻ -P/g TS _{ALE})
2.2 mg PO ₄ ³⁻ -P/L*	90.8 ± 0.0	0.40 ± 0.00
7.3 mg PO ₄ ³⁻ -P/L	83.6 ± 4.7	1.22 ± 0.07
82.5 mg NH ₄ ⁺ -N/L	85.2 ± 3.9	0.40 ± 0.02
17.5 mg NO ₃ ⁻ -N/L	66.2 ± 6.6	0.22 ± 0.02

* Raw wastewater (without amendments)

4.3.3.3 How is the influence of wastewater pH (experiment #3)?

During the sorption tests, pH considerably affected the phosphate concentration dynamics by ALE hydrogels for the three pH values tested of 6.00, 7.00 and 8.57 (Figures 33E and 33 and Tables 14 and 15). Interestingly, according to the kinetics (Figure 33E), an initial increase in PO₄³⁻ concentration occurred at pH 7.00 and even more pronounced at pH 6.00, which is a tendency not observed at pH 8.57. For pH 7.00, the PO₄³⁻ concentration rose until t=5 min and then declined. In opposite, at pH 6.00 the increase in PO₄³⁻ concentration lasted at least until t=120 min. Decreasing pH also tended to reduce both the removal efficiency and practical loading of PO₄³⁻ by ALE during the timeframe of the experiments (Table 15). pH 6.00 significantly decreased the PO₄³⁻ removal efficiency down to 28.9 ± 0.8% and the practical loading to 0.16 ± 0.00 mg PO₄³⁻-P/g TS_{ALE} compared to the other pH values tested. Moreover, the pH in the end of all the experiments tended to rise (Table 14). The lower the initial pH, the higher was the increasing in the final pH. For instance, for the initial pH 6.00, the solution pH was 8.06 at t=240 min. For all the values of pH tested occurred a high release of COD and TN with no clear influence of pH (Figure 35B, C and D). There was no significant release of TP at pH 7 and 6, while for pH 8.57 there was a raise of 42.5 ± 3.5% (Figure 35E). Finally, no clear pH influence was observed for the other parameters analyzed (Table 14).

Figure 35 - Wastewater parameters measured during the sorption tests at different initial pH values (experiment #3). (A) PO₄³⁻, (B) tCOD (C) sCOD, (D) TN and (E) TP measured in the wastewater at the beginning (initial, t = 0 min, light gray) and end (final, t = 240 min, dark gray) of the sorption tests. Conditions: dosage 3.75 g T_{SALE}/L, temperature 21±1 °C, duration 240 minutes, triplicates. pH at 8.57 corresponds to raw wastewater (without amendments).

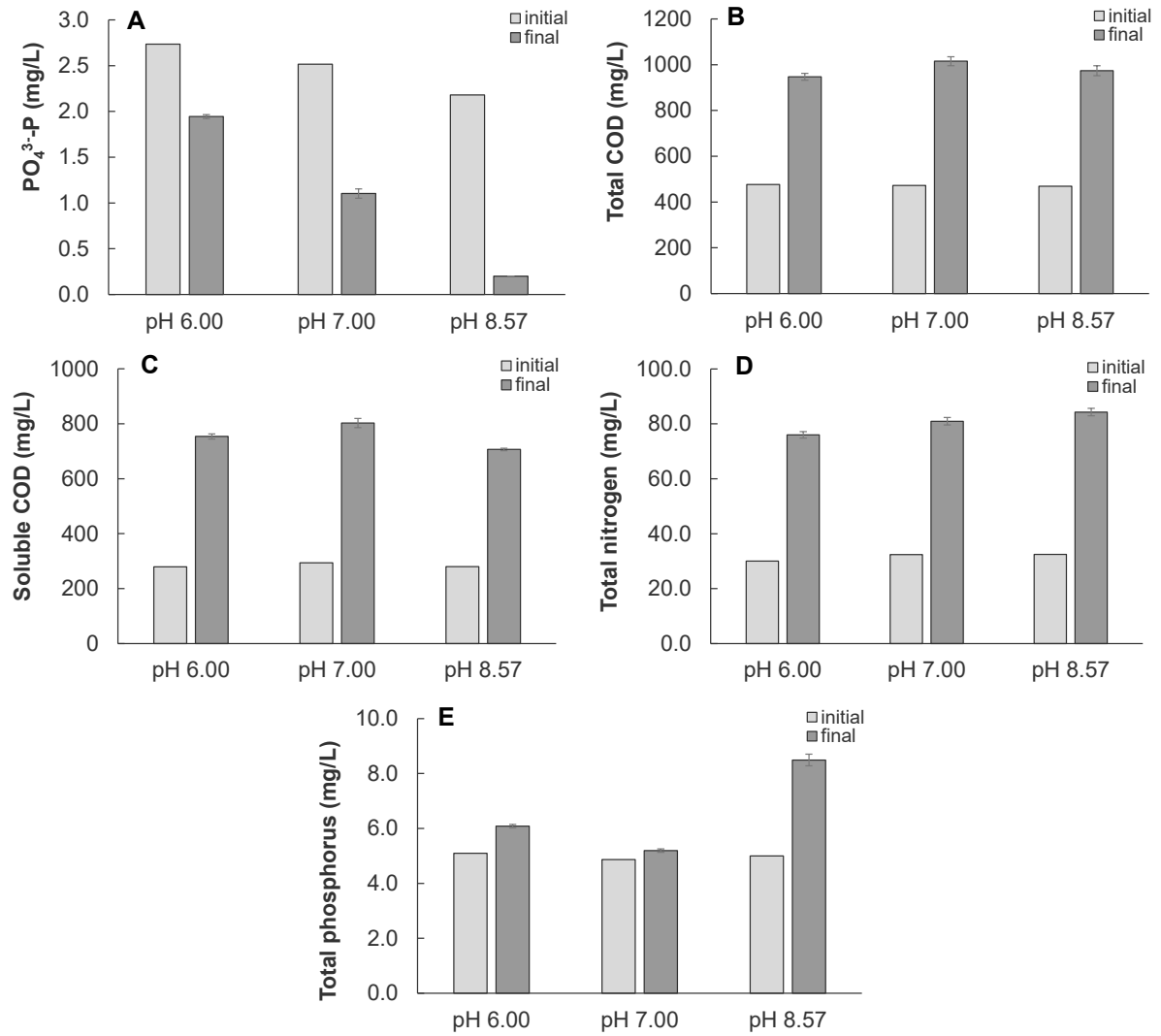


Table 14 – Wastewater parameters measured in the sorption tests at different initial pH (experiment #3). pH at 8.57 corresponds to raw wastewater (without amendments). Conditions: dosage 3.75 g TS_{ALE}/L, temperature 21±1 °C, duration 240 minutes, triplicates.

Initial wastewater pH	Time	pH	NH ₄ ⁺ -N (mg/L)	NO ₃ ⁻ -N (mg/L)	NO ₂ ⁻ -N (mg/L)	Cl ⁻ (mg/L)	Na ⁺ (mg/L)	K ⁺ (mg/L)	Ca ²⁺ (mg/L)	Mg ²⁺ (mg/L)
8.57	t = 0 ^a	8.57	16.4	1.09	0.17	92	91	14.7	67	16.2
	t = 4 hrs ^b	8.76 ± 0.01	14.3 ± 0.2	1.30 ± 0.07	0.20 ± 0.00	711 ± 29	108 ± 1	11 ± 1	401 ± 20	18 ± 3
7.00	t = 0 ^a	7.00	17.1	1.3	0.17	193	93	36.7	90	17
	t = 4 hrs ^b	8.40 ± 0.02	16.5 ± 0.27	1.8 ± 0.0	0.20 ± 0.0	1086 ± 8	110 ± 1	36 ± 1	598 ± 6	16 ± 0
6.00	t = 0 ^a	6.00	16.4	1.0	0.17	252	90	17.2	86	16.3
	t = 4 hrs ^b	8.06 ± 0.00	16.1 ± 0.1	1.3 ± 0.0	0.20 ± 0.0	871 ± 22	101 ± 1	17.6 ± 0.2	438 ± 12	16 ± 0

Note: ^a corresponds to the primary wastewater after the addition of ALE (including the dilution factor), except for the pH that corresponds to the valued measured for the primary wastewater. ^b corresponds to the wastewater in the end of the experiments (triplicate)

Table 15 - PO₄³⁻-P removal and practical loading from wastewater by ALE hydrogels at different initial pH. Conditions: dosage 3.75 g TS_{ALE}/L, temperature 21±1 °C, duration 240 minutes, triplicates.

pH	PO ₄ ³⁻ -P removal (%)	Practical loading (mg PO ₄ ³⁻ -P/g TS _{ALE})
8.57*	90.8 ± 0.0	0.40 ± 0.00
7.00	56.1 ± 2.0	0.28 ± 0.01
6.00	28.9 ± 0.8	0.16 ± 0.00

* Raw wastewater (without amendments).

4.3.3.4 Phosphorus content determination by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES)

The sorption of phosphorus by ALE hydrogels was confirmed by the mass balance derived from ICP-OES measurements of hydrogels before and after sorption. The sorption was especially demonstrated for the experiment at initial high PO₄³⁻ concentration. ALE hydrogels before sorption had up to 8120 mg P/kg ALE and the content of phosphorus clearly increased more than three times to 26102 mg P/kg ALE after the sorption experiment with PO₄³⁻ amendment (experiment #3).

4.3.4 Discussion

4.3.4.1 ALE hydrogels help recovering phosphates

A main result is that ALE hydrogels were able to efficiently remove only phosphate from domestic wastewater: removals of up to 90.8 ± 0.0% and practical loadings to 1.22 ± 0.07 mg PO₄³⁻-P/g TS_{ALE} were measured (Tables 10 and 12). Those values need to be carefully evaluated considering the context of the experimental conditions. In a study using ALE hydrogels beads and synthetic phosphate solutions, Dall'Agnol et al. (2020) obtained a higher practical loading of 57.25 ± 12.18 mg P/g TS_{ALE}, but at temperature of 45 °C, pH = 8, initial phosphate concentration of 100 mg P/L and ALE dosage of 0.39 g TS_{ALE}/L. By only changing the dosage to 3.95 g TS_{ALE}/L, the same authors obtained a phosphorus removal efficiency of 72.50 ± 2.79%. Although the removals obtained herein are similar to the one obtained by Dall'Agnol et al. (2020), the lower practical loading may be attributed to the different experimental conditions as type of absorbate (synthetic vs. real wastewater), temperature, shape

of adsorbent (beads vs. stripes), adsorbent dosage and initial phosphorus concentration. Adsorption capacity of phosphate ions by several different adsorbents ranges widely from 0.13 to 133.3 mg/g (DALL'AGNOL *et al.*, 2020; JANG; LEE, 2019). Hence, the comparison of values of sorption capacity/removals must be carefully done due to the high variability of results according to the experimental conditions.

Phosphate adsorption experiments using adsorbents with calcium show that precipitation in the bulk may contribute to phosphorus removal (SIWEK *et al.*, 2016; XU *et al.*, 2019). At high calcium concentration and pH values, phosphate ions may react with calcium ions to precipitate as calcium phosphates (JOKO, 1985). In this study, although ALE hydrogels went through several washing steps to remove the excess of Ca^{2+} , an increase of calcium ions in solution may have occurred slowly along the adsorption tests due to hydrolysis and deterioration of the crosslinked network (Tables 10, 12 and 14). However, at Ca^{2+} concentrations of 50-350 mg/L and pH values of 8.00-9.00 at the beginning of the adsorption experiments, phosphate might not have initially been precipitated (JOKO, 1985). A study which assessed phosphate adsorption by calcite have reported that at pH 8.3 there is no phosphate precipitation when the initial phosphate concentration is below 7.5 mg/L (LI *et al.*, 2017b), i.e., conditions similar to ours. In addition to that, at pH 9.1-10.1 and PO_4^{3-} concentrations below 10 mg/L, phosphate adsorption by calcite was the main mechanism for phosphate removal for until 24 hours reaction time (LI *et al.*, 2017b). Furthermore, ICP-OES measurements indicated an enrichment of phosphorus in ALE after the sorption experiments especially for the initial high phosphate concentrations, what demonstrates the entrapment of phosphate into ALE hydrogels.

Cross-linkage of calcium ions with ALE may play a key role in bridging phosphate ions through surface complexation providing positive sites (ANTELO; ARCE; FIOL, 2015; JANG; LEE, 2019). Cross-linking via the diffusion method as the one herein used produces hydrogels with a higher Ca^{2+} content on the surface (PAWAR; EDGAR, 2012), which in turn would favor surface complexation with phosphate. Previous studies using synthetic phosphate solutions have reported phosphate removal by Ca^{2+} -ALE hydrogel beads via the formation of calcium phosphates crystals on the surface of ALE (DALL'AGNOL *et al.*, 2020). Besides surface complexation, ligand exchange has effect on phosphate adsorption simultaneously (XUE; HOU; ZHU, 2009). Thus, possibly either phosphate was directly bound to a counter ion as Ca^{2+} on ALE surface, or an ion/compound was released to enable phosphate bind.

4.3.4.2 Influence of pH

Results showed that pH has a great impact on phosphate removal. Both removal efficiency and practical loading of phosphate decreased substantially when pH was reduced from 8.57 to 6.00. At the initial pH 7.00 and mainly at pH 6.00, an initial increase of PO_4^{3-} concentration followed by a further decrease occurred along sorption, while the pH rose to final values of 8.40 ± 0.02 and 8.06 ± 0.00 respectively (Figure 33E and Table 14). The increase of pH is an evidence of the release of OH^- from ALE and/or consumption of H^+ cations in solution. Initially, the lower pH values may have weakened the ALE hydrogels matrix which in turn increases the release of ions such as OH^- and phosphates, since ALE has phosphates in its composition (KIM *et al.*, 2020). Besides, the pH dependence for phosphate adsorption has been associated to the ligand exchange between phosphate and OH^- (XUE; HOU; ZHU, 2009; YANG *et al.*, 2006b). At low pH, OH^- has been showed to be released from alum sludge to supply active sites for phosphorus adsorption which in turn increases the pH (LIU *et al.*, 2016). The same mechanism may have occurred with ALE along the experiment by PO_4^{3-} replacing OH^- on the surface of the hydrogel and thus increasing the pH.

When the solution pH reached values over 7, ALE structure may be more stable and PO_4^{3-} sorption exceeds the rate of PO_4^{3-} release. At these conditions, a lower rate of OH^- release may have enabled a ligand exchange reaction with organic matter from ALE which favoured PO_4^{3-} sorption. During phosphate adsorption by alum sludge, the alkaline media (pH~9) induces more organic matter and humic substances release which accelerate the ligand exchange process (LIU *et al.*, 2016; YANG *et al.*, 2006a). Higher phosphate adsorption has been related to organic matter release (LIU *et al.*, 2016). Considering the high increase of COD, TP and TN along our adsorption tests, part of ALE organic matter components may have been displaced by phosphate ions in solution (i.e., ligand exchange), which in turn favored phosphate sorption.

Adsorption experiments have showed that the highest phosphate adsorption from synthetic solutions frequently occurs at acidic pH (KUMAR *et al.*, 2019). However, our results showed an opposite scenario. ALE has a point of zero charge at $\text{pH } 7.02 \pm 0.45$ (DALL'AGNOL *et al.*, 2020). At solution pH higher than the point of zero charge, the adsorbent surface is negatively charged which would result in decreasing phosphate adsorption due to electrostatic repulsion. Nevertheless, in these cases, ligand exchange may play a major role in sorption process (HU *et al.*, 2015) as explained before. Also, the high calcium content on ALE may decrease the repulsive electrostatic interactions (ANTELO; ARCE; FIOL, 2015). Besides,

adsorption can occur due to physical forces even when the solution pH is higher than the point of zero charge as previously reported for nitrate adsorption on chitosan hydrogels (CHATTERJEE; WOO, 2009). Interestingly, phosphate adsorption studies using chitosan/Ca-organically modified montmorillonite beads has showed a low pH dependence with a high phosphate removal up to 90% at pH 3 to 11 (JANG; LEE, 2019). Therefore, depending on the environmental conditions and the composition of the adsorbent employed, different patterns for pH dependence on phosphate removal can be obtained.

Results also showed that ALE hydrogels behaved as a buffer agent by heading pH to over 8 along most of the sorption tests performed at different initial pH (Table 14). Other hydrogels adsorbents has also been reported to act as a buffer agent at wide ranges of pH along ions adsorption, such as mixtures of drinking water treatment residuals and alginate (SHEN *et al.*, 2018), chitosan-Fe³⁺ complex (HU *et al.*, 2015) and alum sludge (LIU *et al.*, 2016). At alkaline conditions, the existence of OH⁻ in the bulk liquid can reduce the release rate of OH⁻ from ALE. Furthermore, in the presence of phosphate, organic matter is released for PO₄³⁻ and OH⁻ exchange (LIU *et al.*, 2016). In this case, phosphate adsorption can be also followed by H⁺ release which ultimately controls the pH variation (YANG *et al.*, 2006b). Those mechanisms may act as stabilizers to keep the pH balanced at the values between 8-9 as herein observed.

4.3.4.3 Influence of other ions

High PO₄³⁻ removals were obtained even in the presence of high concentrations of ions such as NH₄⁺ and NO₃⁻ (Figures 33C and D and Table 133). Usually, bivalent anions have higher adsorption tendency than monovalent ones (MEHDINEJADIANI; AMININASAB; MANHOOEI, 2019). At pH higher than 8, as of the wastewater herein used, most of phosphates species in domestic wastewater are in the form of HPO₄²⁻ (JENKINS; FERGUSON; MENAR, 1971). Therefore, the predominance of bivalent forms of phosphates would in turn favor the adsorption over other ions.

Nitrate has also been demonstrated to present negligible effects on phosphate adsorption by hydrogels in synthetic solutions (ZHENG; WANG, 2010). In our study, for high NO₃⁻ concentrations there was a lower removal efficiency and sorption probably because of the lower initial PO₄³⁻ concentration (1.7 mg PO₄³⁻-P/L), but with no clear influence of the high anion concentration. Initial concentrations act as a driving-force that can result in higher or lower sorption capacities (HU *et al.*, 2015; JANG; LEE, 2019; YANG *et al.*, 2006b). No NH₄⁺

sorption was observed by ALE hydrogels either. AGS and soluble ALE (non-crosslinked) have been showed to adsorb NH_4^+ as an ionic exchange mechanism with K^+ and thus to precipitate struvites with synthetic wastewater (LIN; BASSIN; VAN LOOSDRECHT, 2012). Nevertheless, herein ALE hydrogels did not present an effective capacity to adsorb ammonium from the wastewater probably to the fact of the crosslinking with calcium that changed the negative charge of functional groups.

It is noteworthy the high Cl^- concentrations found probably come from the CaCl_2 crosslinking solution, but with no detrimental effects on phosphate sorption either. Cl^- has been reported to have low/insignificant effects on PO_4^{3-} removal (JANG; LEE, 2019; XUE; HOU; ZHU, 2009; YANG *et al.*, 2006b). Even so, a longer and better washing of hydrogels is recommended to avoid the generation of a highly concentrated anionic solution with Cl^- and Ca^{2+} ions that could hinder potential large-scale applications.

4.3.4.4 Practical implications

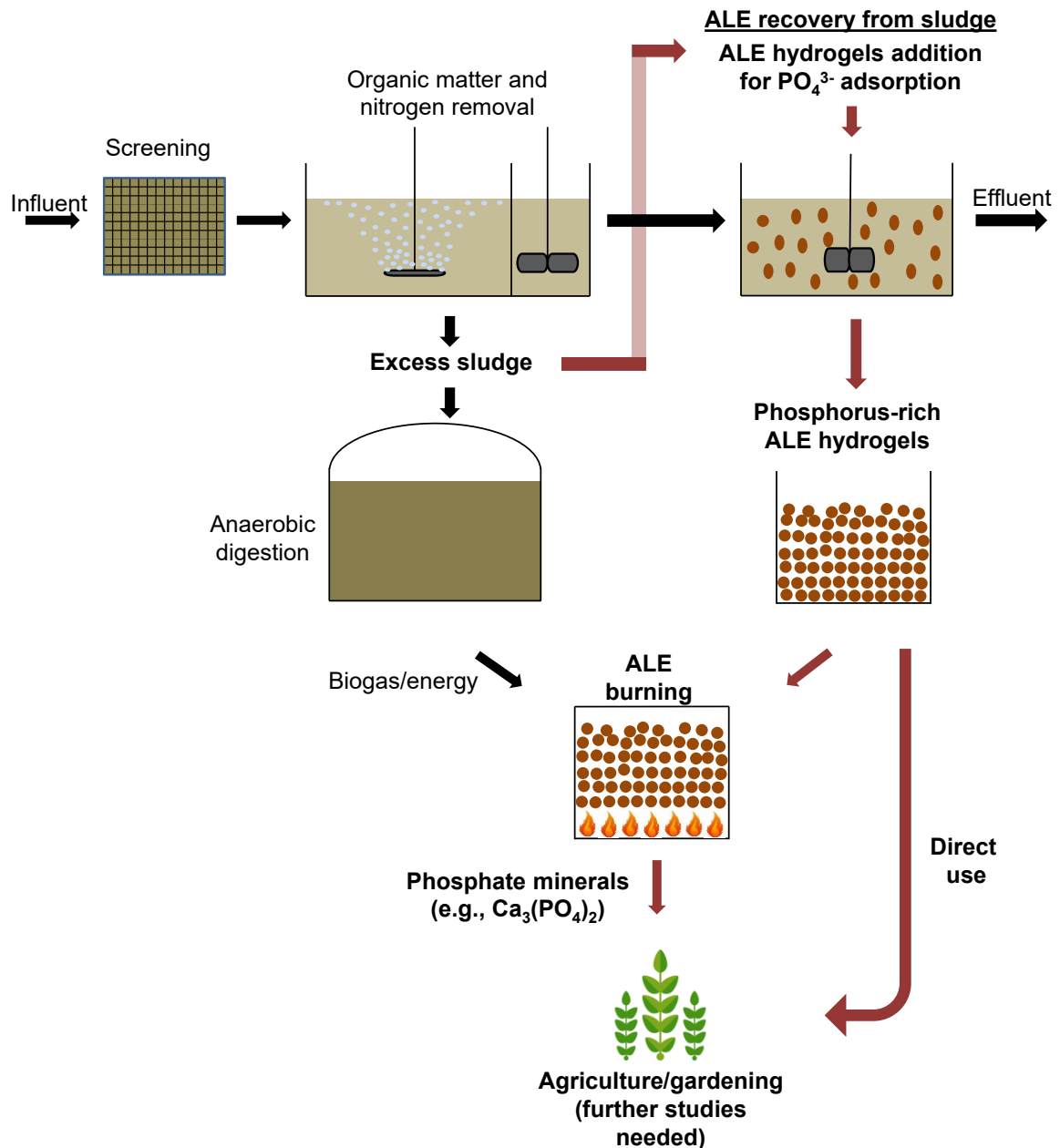
4.3.4.4.1 ALE as a potential agent for mainstream phosphorus recovery

The actual main products that can be recovered from WWTP are cellulose, bioplastics, phosphate and ALE (VAN LEEUWEN *et al.*, 2018). Our results showed that ALE hydrogels have high capacity for phosphate removal from basic wastewaters at the common phosphate range concentrations and even at higher concentrations. This feature makes ALE a potential biosorbent to recover phosphorus at WWTP. Biological nutrient removal efficiency is sometimes reported as an issue in some real wastewater treatment systems exhibiting poor stability (MILFERSTEDT *et al.*, 2017; NANCHARAI AH; KIRAN KUMAR REDDY, 2018; XU *et al.*, 2020). Furthermore, biological phosphorus removal requires different redox conditions to favor storage of polyphosphates, but its removal is eventually governed by the extraction of the sludge (excess sludge) (SARMA; TAY, 2018b). Thus, sorption has recently been regarded as a promising technology to simultaneously remove and recover nutrients from domestic wastewater, as ammonium, for example (CRUZ *et al.*, 2019). Domestic sewage is a promising source of phosphorus since the direct application of the sludge in the agriculture has laws restrictions in addition to the sanitation risks related to organic micropollutants and pathogens (EGLE *et al.*, 2016).

The choice of an adsorbent should be based not only on the satisfactory adsorption capacity, but also on an evaluation of several benefits and characteristics (KUMAR *et al.*, 2019). In this context, ALE hydrogels show advantages and potential as a biosorbent such as (i) fast settling ability facilitates rapid post separation, (ii) more diverse functions and characteristics which can be tailored/improved by surface modification and/or mixture with other polymers (iii) suitable for operation in stirred tank reactors (iv) recovery from a cheap and easy available raw material (i.e., waste biological sludge) (v) ALE beads enriched with phosphorus can be used as post-sorption biomaterial. The last two features dispense the need for reusability of ALE hydrogels, which would save the cost for regeneration processes.

ALE hydrogels have a high content of organic matter, nitrogen from proteins (SCHAMBECK *et al.*, 2020b) and especially phosphorus after adsorption. Besides, ALE are recovered under harsh conditions (i.e., high pH and temperature) which have the potential to eliminate/decrease pathogens. Those features make post-sorption ALE hydrogels potential agents for a direct application in the agronomic field as fertilizers/soil enhancers (SIWEK; BARTKOWIAK; WŁODARCZYK, 2019). Or else, the thermal degradation of ALE hydrogels after adsorption is an alternative. The heat generated by the energy use of biogas at WWTP that implement anaerobic digestion can be directed to thermally degrade ALE to obtain by-products such as calcium phosphates ashes which can be further used as fertilizers. In Figure 36 a new conceptual pathway is proposed that allows for continuous mainstream phosphate recovery from municipal wastewater based on ALE hydrogels adsorption coupled with further side-stream application. Nevertheless, further studies are needed to assess the potential agronomic use (e.g., P release in soil), economic impacts of this application as well as to perform a life cycle assessment to balance the positive and negative aspects.

Figure 36 – Schematic conceptual diagram for mainstream continuous phosphate sorption by ALE hydrogels followed by side-stream ALE use as input in the agronomic field. This scheme depicts an implementation example of the concept of wastewater resource recovery facility in a wastewater treatment plant.



4.3.4.4.2 ALE hydrogels structure along sorption

ALE hydrogels greatly contributed for the increment of COD and TN concentrations along the sorption tests. In addition to that, TP concentrations increased even with a significant

decay of PO_4^{3-} concentrations in most of the sets of experiments. This behaviour is also attributed to the fragile constitution of ALE hydrogels that detached debris to the media, consequently increasing carbon and organic nitrogen/phosphorus. There may be the release of components from ALE in the range of pH 6.5-8.5 (FELZ *et al.*, 2020b), as the ones tested herein. ALE is mainly composed by proteins, polysaccharides, humic acids and others phosphated compounds (FELZ *et al.*, 2019; KIM *et al.*, 2020; SCHAMBECK *et al.*, 2020b), which consequently acted as a source of carbon, nitrogen and phosphorus to the media. Besides, ligand exchange reactions during the sorption process can destabilize cross-linked hydrogels backbone which in turn results in the release of components (LEE; MOONEY, 2012). To tackle those drawbacks, ALE structure can be improved by different ways to tailor its mechanical properties and in turn to limit the debris detachment and release of ions/soluble organic constituents to the liquid. Sodium alginate alone or in mixture with other adsorbents can hinder or decrease the release of compounds to the bulk liquid (ROCHER *et al.*, 2008; SIWEK *et al.*, 2016). Thus, ALE can be mixed with other polymers (such as commercial alginate) before cross-linkage or else can be crosslinked with other cations as copper or zinc which have been showed to form stronger ALE hydrogels (FELZ *et al.*, 2020b). Manipulation and modification of biopolymers structure can enhance some properties as stiffness, solubility, hydrophobicity and affinity besides introducing new properties (LEE; MOONEY, 2012; PAWAR; EDGAR, 2012).

4.3.4.4.3 Influence of ALE hydrogels dosage on phosphate removal

Interestingly, no clear influence of ALE dosage on PO_4^{3-} sorption was ascertained (Figure 33A and Tables 10 and 11). The lowest dosage of 1.88 g $\text{TS}_{\text{ALE}}/\text{L}$ used in the experiments led to both a high removal efficiency and practical loading when compared to the other ALE dosages tested. This can be attributed to the higher initial PO_4^{3-} concentration in the wastewater used for the 1.88 g $\text{TS}_{\text{ALE}}/\text{L}$ sorption tests (2.8 mg $\text{PO}_4^{3-}\text{-P/L}$ vs. 2.2 mg $\text{PO}_4^{3-}\text{-P/L}$). Sorption is a concentration-dependent mechanism, in which higher initial concentrations acts as a gradient driving-force to overcome the mass transfer resistance between the aqueous and solid phases, resulting in higher sorption/removal capacities (HU *et al.*, 2015; JANG; LEE, 2019; YANG *et al.*, 2006b). This concentration dependency phenomenon is demonstrated by the test where KH_2PO_4 was added to increase PO_4^{3-} concentration to 7.3 mg $\text{PO}_4^{3-}\text{-P/L}$, which in turn markedly improved the practical loading (Table 13). Furthermore, the dosages of ALE

used in the experiments may have been larger than the optimum point to enable the highest sorption/removal. In this case, the number of active and available sites for interactions between the adsorbent and ions also goes up along with the adsorbent concentration, successively reducing the adsorptive capacity of the adsorbent (POORMAND; LEILI; KHAZAEI, 2017). Also, higher dosages of ALE hydrogels can increase de repulsion forces between phosphate and negatively charged functional groups what which would be negative for adsorption (BANERJEE *et al.*, 2019). As the overall final PO_4^{3-} concentrations were relatively low in the end of the sorption tests (1.1-0.2 mg PO_4^{3-} -P /L), lower dosages of ALE hydrogels could be employed in a real scenario which would be also helpful to decrease the release of COD, TN and TP.

It was not overlooked that the batch sorption tests had duration of 4 hours and concentration equilibrium was not reached. According to the kinetics, especially for the higher initial PO_4^{3-} concentration (Figure 33B), there is a tendency of even lower concentrations to be achieved in longer tests duration. Hence, ALE hydrogels most likely have a higher phosphorus sorption capacity and removal than the ones showed by our results. However, considering the use of adsorbent for municipal wastewater polishing, the focus should be on the adsorption capacity and affinity at the realistic phosphate concentrations (i.e., the practical loading) rather than the maximum adsorption capacity (KUMAR *et al.*, 2019). This work was a proof-of-concept approach to test the adsorption capacity of wastewater compounds by ALE hydrogels. Hence, adsorption equilibrium isotherms are encouraged to be performed in future experiments to acquire more information about ALE hydrogels phosphorus sorption dynamics and influencing factors.

4.3.5 Conclusions

Phosphate removal from municipal wastewater was successfully achieved using ALE hydrogels at alkaline pH (≈ 8.5) and even in the presence of high concentrations of ions such as ammonium and nitrate. Phosphate may have mainly been removed by surface complexation and ligand exchange. pH strongly affected phosphate removal. Neutral to acidic pH values (6.0 – 7.0) were detrimental for PO_4^{3-} removal, what suggests that the use of ALE hydrogels is more suitable for basic wastewaters. Besides, ALE hydrogels acted as a buffer agent leading pH to over 8 along adsorption. Our study demonstrates that a recovered bioresource (ALE) has a promising potential to be used for bioresource recovery (i.e., phosphorus). Nevertheless, further

studies are needed mainly to improve ALE hydrogels structure along adsorption and to decrease/eliminate COD, TN and TP release. Therefore, further applications of the phosphorus-rich ALE hydrogels can bring forward an effective resource-oriented approach to upgrade the fate of phosphorus in urban water management.

5 DISCUSSÃO INTEGRADA

A recuperação de recursos a partir do tratamento de esgoto doméstico é uma interessante ferramenta que pode trazer tanto benefícios econômicos quanto ambientais, contribuindo, assim, para uma economia circular. Dentro desse contexto, essa tese objetivou compreender melhor o biopolímero ALE recuperado a partir de lodo biológico de esgoto, considerando características quantitativas e qualitativas assim como testar uma aplicação desse biomaterial. Muitos desses aspectos eram na sua maior partes ainda não explorados, contudo os resultados aqui apresentados ajudam para um melhor entendimento do ALE.

Ressalta-se que a maior parte dos resultados foram obtidos com esgoto doméstico, ou seja, sob condições que se aproximam de um cenário real. Nos três artigos, o ALE foi recuperado a partir de biomassa que tratava esgoto doméstico tanto em reatores em escala piloto (Artigo 1) quanto em escala real (Artigos 2 e 3). Além disso, ALE com características semelhantes foi recuperado a partir de esgoto doméstico de países com características distintas (Brasil e Suíça).

O ALE recuperado se mostrou como importante constituinte da biomassa tanto na forma de grânulos quanto de flocos que tratava esgoto de diferentes países. Isso foi especialmente demonstrado nos Artigos 1 e 2. Contudo, o Artigo 1 demonstrou com base em uma longa série de dados e em condições reais que a quantidade de ALE extraída a partir de grânulos é consideravelmente maior que de flocos. Isso é um resultado interessante, já que a maior parte dos trabalhos na literatura fazem referência majoritariamente à recuperação de ALE a partir de lodo granular. Além disso, a maior parte das características químicas do ALE recuperado de flocos e grânulos que tratavam esgoto doméstico foram muito semelhantes. Somente a elasticidade dos hidrogéis de ALE de grânulos foi maior. Assim, a recuperação de ALE de flocos de lodo ativado, com características adequadas para aplicações industriais, possibilitaria a expansão da recuperação desse recurso para ETE com diferentes tecnologias biológicas de tratamento.

Os resultados também mostraram que a composição do esgoto impacta qualitativamente e quantitativamente o ALE recuperado. O Artigo 1 mostrou com base em análises estatísticas que a remoção de matéria orgânica e nutrientes esteve relacionada à dinâmica de produção de ALE pela biomassa durante o tratamento de esgoto doméstico. Isso vai ao encontro dos resultados do Artigo 2, o qual mostrou que esgoto rico em ácidos graxos voláteis impacta tanto a quantidade total de ALE quanto a concentração de ácidos urônicos do

ALE recuperado. Assim, esgotos domésticos com uma alta carga orgânica composta majoritariamente de ácidos graxos voláteis (ou seja, já submetidos a processos fermentativos) impactam as características do ALE que vier a ser extraído da biomassa.

A composição complexa do ALE indicada pelo Artigo 1 e principalmente pelo Artigo 2 deu subsídios para testar a capacidade de adsorção dos hidrogéis de ALE recuperado de grânulos. Conforme exposto no Artigo 3, os hidrogéis de ALE foram capazes de remover fósforo do esgoto doméstico, possivelmente devido à diversidade de grupos funcionais e polímeros constituintes (por exemplo, proteínas, ácidos húmicos e polissacarídeos). Essa capacidade de remoção de fósforo abre uma possibilidade de uso do ALE para o tratamento de esgoto. Considerando as características semelhantes entre o ALE recuperado de grânulos e flocos indicadas nos Artigos 1 e 2, hidrogéis de ALE recuperados de flocos possivelmente também manteriam a capacidade de remoção de fósforo.

De modo geral, os resultados advindos dessa pesquisa, principalmente do Artigo 1, dão suporte para futura implementação e aperfeiçoamento da recuperação de ALE em território brasileiro considerando as peculiaridades predominantes, como clima e características do esgoto. Além disso, há uma contribuição para uma perspectiva de melhora das condições sanitárias e ambientais nacionais, com a valorização do lodo biológico excedente e consequente diminuição da sua disposição final em aterros. Assim, espera-se que esse trabalho auxilie na mudança do conceito das estações de tratamento de esgoto no país como unidades não somente de tratamento, mas também como promissoras fontes de recuperação de recursos, o que contribui para uma economia mais circular.

6 CONCLUSÕES

Com base nas hipóteses, perguntas e objetivos que nortearam este trabalho, tem-se as seguintes conclusões:

1. Ao longo do processo de granulação, a quantidade média de ALE recuperada de grânulos é maior que de flocos: 236 ± 27 mg SV_{ALE}/g $SV_{grânulos}$ contra 187 ± 94 mg SV_{ALE}/g SV_{flocos} . Além disso, a quantidade de ALE é mais estável e continuamente aumenta nos grânulos uma vez que a granulação é atingida durante o tratamento de esgoto doméstico. Já a quantidade de EPS no lodo não é relacionada nem com o processo de granulação nem com a quantidade de ALE no lodo.
2. Uma maior quantidade de ALE no lodo se relaciona com o alcance da granulação, com uma composição estável da comunidade microbiana e com a conversão da matéria orgânica, nitrogênio e fósforo. Já a propriedade de formação de hidrogel, composição elementar e grupos funcionais do ALE não variam ao longo do processo de granulação durante o tratamento de esgoto doméstico.
3. A quantidade e composição do ALE extraído de diferentes tipos de lodo é de modo geral semelhante, sendo o ALE composto por polissacarídeos, proteínas e ácidos húmicos. Contudo, uma maior concentração de ácidos graxos voláteis no efluente leva a uma maior quantidade de ALE recuperada do lodo, assim como a uma maior concentração de ácidos urônicos no ALE. Além disso, a elasticidade dos hidrogéis de ALE varia conforme o tipo de lodo de origem. Hidrogéis de ALE extraídos de grânulos são mais elásticos que os extraídos de flocos quando ambos são provenientes de lodo que trata esgoto doméstico. Os ácidos manurônicos estão envolvidos na manutenção da estrutura de hidrogel do ALE.
4. O termo “polímeros semelhantes ao alginato” (*alginate-like polymers* - ALP) descreveria melhor esse biomaterial recuperado de lodo biológico já que os polímeros constituintes possivelmente não vêm somente do meio extracelular, como as proteínas provenientes da lise celular.
5. Os resultados abrem a possibilidade de expansão da recuperação de ALE não só de grânulos, como frequentemente reportado na literatura, mas também de flocos, como os gerados em sistema de lodos ativados.
6. Hidrogéis de ALE recuperados de lodo biológico podem ser empregados para remoção de fosfato de esgoto doméstico (até 90,8% de remoção). Contudo, há um grande

aumento da concentração de DQO, nitrogênio total e fósforo total para o meio líquido a partir dos hidrogéis de ALE ao longo do processo de sorção (até 117,6%, 118,0% e 42,5%, respectivamente). O pH impacta fortemente a remoção de fosfato. pH alcalino ($\approx 8,5$) é o mais favorável para remoção de fosfato, enquanto pH neutro a ácido (7,0-6,0) é o mais prejudicial. Assim, um recurso recuperado do tratamento de esgoto (ALE) tem potencial de uso para recuperação de outro recurso presente no esgoto (fósforo).

7. Por fim, esse trabalho obteve sucesso em recuperar ALE durante o tratamento de esgoto doméstico em diferentes condições. Espera-se que isso contribua para a ampliação das possibilidades de recuperação de recursos a partir do tratamento de esgoto além dos comumente estudados, como biogás e nutrientes. Assim, pode-se vislumbrar o alcance de uma economia mais circular a partir de mais alternativas para a valorização do lodo biológico de esgoto.

7 RECOMENDAÇÕES

Considerando os resultados obtidos com a presente pesquisa, sugere-se para trabalhos futuros:

- Realizar testes em bancada/sistemas piloto expostos a alta cargas orgânicas e de nutrientes (nitrogênio e fósforo), a fim de comprovar o aumento da produção de ALE nessas condições.
- Realizar mais caracterizações químicas do ALE recuperado de lodo granular e flocular, a fim de evidenciar a existência de possíveis diferenças que possam impactar futuras aplicações industriais. Por exemplo, sugere-se estudar como a proporção de ácidos manurônicos e gulurônicos influencia nas propriedades elásticas do ALE e sua relação com o lodo de origem (flocos e grânulos).
- Realizar estudos econômicos e de análise de ciclo de vida, a fim de verificar a viabilidade financeira e ambiental de recuperação de ALE de lodo biológico excedente, sobretudo considerando a realidade brasileira.
- Realizar caracterizações químicas mais detalhadas e complementares do ALE, a fim de conhecer mais a fundo a sua complexa composição e, assim, possibilitar novos usos desse biomaterial.
- Realizar estudos de adsorção de fosfato com esgoto real, misturando o ALE com alginato e/ou o reticulando com cobre e zinco a fim de diminuir a emissão de DQO, nitrogênio e fósforo para o meio líquido. Nessas condições, verificar se a capacidade de remoção de fósforo é mantida. Além disso, realizar os ensaios até que o equilíbrio seja atingido para obtenção de isotermas e outros parâmetros cinéticos que são úteis para elucidar mecanismos envolvidos na remoção de fósforo.
- Realizar estudos agronômicos para avaliar o potencial de uso dos hidrogéis de ALE enriquecidos em fósforo (pós-sorção).

REFERÊNCIAS

ADAV, S. S. *et al.* Aerobic granular sludge: Recent advances. **Biotechnology Advances**, [S. l.], v. 26, n. 5, p. 411–423, 2008. Disponível em: <https://doi.org/10.1016/j.biotechadv.2008.05.002>

ADAV, S. S.; LEE, D. J. Extraction of extracellular polymeric substances from aerobic granule with compact interior structure. **Journal of Hazardous Materials**, [S. l.], v. 154, n. 1–3, p. 1120–1126, 2008. Disponível em: <https://doi.org/10.1016/j.jhazmat.2007.11.058>

ADAV, S. S.; LEE, D. J.; LAI, J. Y. Functional consortium from aerobic granules under high organic loading rates. **Bioresource Technology**, [S. l.], v. 100, n. 14, p. 3465–3470, 2009. Disponível em: <https://doi.org/10.1016/j.biortech.2009.03.015>

ADAV, S. S.; LEE, D. J.; TAY, J. H. Extracellular polymeric substances and structural stability of aerobic granule. **Water Research**, [S. l.], v. 42, n. 6–7, p. 1644–1650, 2008. Disponível em: <https://doi.org/10.1016/j.watres.2007.10.013>

AMIR, S. *et al.* Structural study of humic acids during composting of activated sludge-green waste: Elemental analysis, FTIR and ¹³C NMR. **Journal of Hazardous Materials**, [S. l.], v. 177, n. 1–3, p. 524–529, 2010. Disponível em: <https://doi.org/10.1016/j.jhazmat.2009.12.064>

ANDERSON, L. A.; ISLAM, M. A.; PRATHER, K. L. J. Synthetic biology strategies for improving microbial synthesis of “green” biopolymers. **Journal of Biological Chemistry**, [S. l.], v. 293, n. 14, p. 5053–5061, 2018. Disponível em: <https://doi.org/10.1074/jbc.TM117.000368>

ANTELO, J.; ARCE, F.; FIOL, S. Arsenate and phosphate adsorption on ferrihydrite nanoparticles. Synergetic interaction with calcium ions. **Chemical Geology**, [S. l.], v. 410, p. 53–62, 2015. Disponível em: <https://doi.org/10.1016/j.chemgeo.2015.06.011>

APHA. Standard Methods for the Examination of Water and Wastewater Standard Methods for the Examination of Water and Wastewater. **Public Health**, [S. l.], n. 1, 2005.

BANERJEE, S. *et al.* Effect of alginate concentration in wastewater nutrient removal using alginate-immobilized microalgae beads: Uptake kinetics and adsorption studies. **Biochemical Engineering Journal**, [S. l.], v. 149, n. January, p. 107241, 2019. Disponível em: <https://doi.org/10.1016/j.bej.2019.107241>

BASSIN, J. P. *et al.* Ammonium adsorption in aerobic granular sludge, activated sludge and anammox granules. **Water Research**, [S. l.], v. 45, n. 16, p. 5257–5265, 2011.

Disponível em: <https://doi.org/10.1016/j.watres.2011.07.034>

BOLEIJ, M. *et al.* Identification of Glycoproteins Isolated from Extracellular Polymeric Substances of Full-Scale Anammox Granular Sludge. research-article. **Environmental Science and Technology**, [S. l.], v. 52, n. 22, p. 13127–13135, 2018. Disponível em: <https://doi.org/10.1021/acs.est.8b03180>

BOLTZ, J. P. *et al.* From biofilm ecology to reactors: A focused review. **Water Science and Technology**, [S. l.], v. 75, n. 8, p. 1753–1760, 2017. Disponível em: <https://doi.org/10.2166/wst.2017.061>

BORTOLIN, A. *et al.* Investigação do Processo de Absorção de Água de Hidrogéis de Polissacarídeo: Efeito da Carga Iônica, Presença de Sais, Concentrações de Monômero e Polissacarídeo. **Polimeros**, [S. l.], v. 22, n. 4, p. 311–317, 2012. Disponível em: <https://doi.org/10.1590/S0104-14282012005000046>

CAPORASO, J. G. *et al.* Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. **ISME Journal**, [S. l.], v. 6, n. 8, p. 1621–1624, 2012. Disponível em: <https://doi.org/10.1038/ismej.2012.8>

CAUDAN, C. *et al.* Extracellular polymeric substances (EPS) from aerobic granular sludges: Extraction, fractionation, and anionic properties. **Applied Biochemistry and Biotechnology**, [S. l.], v. 166, n. 7, p. 1685–1702, 2012. Disponível em: <https://doi.org/10.1007/s12010-012-9569-z>

CAUDAN, C. *et al.* Multiple EPS interactions involved in the cohesion and structure of aerobic granules. **Chemosphere**, [S. l.], v. 117, n. 1, p. 262–270, 2014. Disponível em: <https://doi.org/10.1016/j.chemosphere.2014.07.020>

CHAO, Y.; ZHANG, T. Surface-enhanced Raman scattering (SERS) revealing chemical variation during biofilm formation: From initial attachment to mature biofilm. **Analytical and Bioanalytical Chemistry**, [S. l.], v. 404, n. 5, p. 1465–1475, 2012. Disponível em: <https://doi.org/10.1007/s00216-012-6225-y>

CHATTERJEE, S.; WOO, S. H. The removal of nitrate from aqueous solutions by chitosan hydrogel beads. **Journal of Hazardous Materials**, [S. l.], v. 164, n. 2–3, p. 1012–1018, 2009. Disponível em: <https://doi.org/10.1016/j.jhazmat.2008.09.001>

COMA, M. *et al.* Enhancing aerobic granulation for biological nutrient removal from domestic wastewater. **Bioresource Technology**, [S. l.], v. 103, n. 1, p. 101–108, 2012. Disponível em: <https://doi.org/10.1016/j.biortech.2011.10.014>

CORSINO, S. F. *et al.* Fate of aerobic granular sludge in the long-term: The role of

EPSs on the clogging of granular sludge porosity. **Journal of Environmental Management**, [S. l.], v. 183, p. 541–550, 2016. Disponível em: <https://doi.org/10.1016/j.jenvman.2016.09.004>

CRUZ, H. *et al.* Rapid removal of ammonium from domestic wastewater using polymer hydrogels. **Scientific Reports**, [S. l.], v. 8, n. 1, p. 1–6, 2018. Disponível em: <https://doi.org/10.1038/s41598-018-21204-4>

CRUZ, H. *et al.* Mainstream ammonium recovery to advance sustainable urban wastewater management. **Environmental Science and Technology**, [S. l.], v. 53, n. 19, 2019. Disponível em: <https://doi.org/10.1021/acs.est.9b00603>

DALL'AGNOL, P. *et al.* A comparative study of phosphorus removal using biopolymer from aerobic granular sludge: A factorial experimental evaluation. **Journal of Environmental Chemical Engineering**, [S. l.], v. 8, n. 2, p. 103541, 2020. Disponível em: <https://doi.org/10.1016/j.jece.2019.103541>

DE KREUK, M. K. *et al.* Behavior of polymeric substrates in an aerobic granular sludge system. **Water Research**, [S. l.], v. 44, n. 20, p. 5929–5938, 2010. Disponível em: <https://doi.org/10.1016/j.watres.2010.07.033>

DE MELO, B. A. G.; MOTTA, F. L.; SANTANA, M. H. A. Humic acids: Structural properties and multiple functionalities for novel technological developments. **Materials Science and Engineering C**, [S. l.], v. 62, p. 967–974, 2016. Disponível em: <https://doi.org/10.1016/j.msec.2015.12.001>

DERLON, N. *et al.* Formation of aerobic granules for the treatment of real and low-strength municipal wastewater using a sequencing batch reactor operated at constant volume. **Water Research**, [S. l.], v. 105, p. 341–350, 2016. Disponível em: <https://doi.org/10.1016/j.watres.2016.09.007>

DING, Z. *et al.* Role of extracellular polymeric substances (EPS) production in bioaggregation: application to wastewater treatment. **Applied Microbiology and Biotechnology**, [S. l.], v. 99, n. 23, p. 9883–9905, 2015. Disponível em: <https://doi.org/10.1007/s00253-015-6964-8>

DREYWOOD, R. Qualitative Test for Carbohydrate Material. **Industrial and Engineering Chemistry - Analytical Edition**, [S. l.], v. 18, n. 8, p. 499, 1946. Disponível em: <https://doi.org/10.1021/i560156a015>

DUBOIS, M. *et al.* Colorimetric Method for Determination of Sugars and Related Substances. **Analytical Chemistry**, [S. l.], v. 28, n. 3, p. 350–356, 1956. Disponível em: <https://doi.org/https://doi.org/10.1021/ac60111a017>

EGLE, L. *et al.* Phosphorus recovery from municipal wastewater: An integrated comparative technological, environmental and economic assessment of P recovery technologies. **Science of the Total Environment**, [S. l.], v. 571, p. 522–542, 2016. Disponível em: <https://doi.org/10.1016/j.scitotenv.2016.07.019>

FELZ, S. *et al.* Extraction of Structural Extracellular Polymeric Substances from Aerobic Granular Sludge. **Journal of Visualized Experiments**, [S. l.], n. 115, p. 1–8, 2016. Disponível em: <https://doi.org/10.3791/54534>

FELZ, S. *et al.* Chemical characterization methods for the analysis of structural extracellular polymeric substances (EPS). **Water Research**, [S. l.], v. 157, p. 201–208, 2019. Disponível em: <https://doi.org/10.1016/j.watres.2019.03.068>

FELZ, S. *et al.* Aerobic granular sludge contains Hyaluronic acid-like and sulfated glycosaminoglycans-like polymers. **Water Research**, [S. l.], v. 169, p. 115291, 2020 a. Disponível em: <https://doi.org/10.1016/j.watres.2019.115291>

FELZ, S. *et al.* Impact of metal ions on structural EPS hydrogels from aerobic granular sludge. **Biofilm**, [S. l.], v. 2, n. November 2019, p. 100011, 2020 b. Disponível em: <https://doi.org/10.1016/j.bioflm.2019.100011>

FLEMMING, H. C.; WINGENDER, J. The biofilm matrix. **Nature Reviews Microbiology**, [S. l.], v. 8, n. 9, p. 623–633, 2010. Disponível em: <https://doi.org/10.1038/nrmicro2415>

FREITAS, F.; TORRES, C. A. V.; REIS, M. A. M. Engineering aspects of microbial exopolysaccharide production. **Bioresource Technology**, [S. l.], v. 245, p. 1674–1683, 2017. Disponível em: <https://doi.org/10.1016/j.biortech.2017.05.092>

FROLUND, H.; T. GRIEBE, P.; NIELSEN, B. Enzymatic activity in the activated-sludge floc matrix. **Applied Microbiology and Biotechnology**, [S. l.], v. 43, n. November 1994, p. 755–761, 1995.

GÓMEZ, A. *et al.* Potential and cost of electricity generation from human and animal waste in Spain. **Renewable Energy**, [S. l.], v. 35, n. 2, p. 498–505, 2010. Disponível em: <https://doi.org/10.1016/j.renene.2009.07.027>

GUO, X.; WANG, X.; LIU, J. Composition analysis of fractions of extracellular polymeric substances from an activated sludge culture and identification of dominant forces affecting microbial aggregation. **Scientific Reports**, [S. l.], v. 6, n. June, p. 1–9, 2016. Disponível em: <https://doi.org/10.1038/srep28391>

HAY, I. D. *et al.* Microbial alginate production, modification and its applications.

Microbial Biotechnology, [S. l.], v. 6, n. 6, p. 637–650, 2013. Disponível em: <https://doi.org/10.1111/1751-7915.12076>

HEALY, M. G. *et al.* Resource recovery from sewage sludge. In: **Sewage treatment plants: economic evaluation of innovative technologies for energy efficiency**. London: International Water Association, 2015. v. 32p. 139–162. *E-book*. Disponível em: <https://doi.org/http://dx.doi.org/10.1680/geot.2008.T.003>

HONG, P. N. *et al.* Optimum selection of extraction methods of extracellular polymeric substances in activated sludge for effective extraction of the target components. **Biochemical Engineering Journal**, [S. l.], v. 127, p. 136–146, 2017. Disponível em: <https://doi.org/10.1016/j.bej.2017.08.002>

HU, Q. *et al.* Nitrate adsorption from aqueous solution using granular chitosan-Fe 3+ complex. **Applied Surface Science**, [S. l.], v. 347, p. 1–9, 2015. Disponível em: <https://doi.org/10.1016/j.apsusc.2015.04.049>

IVLEVA, N. P. *et al.* In situ surface-enhanced raman scattering analysis of biofilm. **Analytical Chemistry**, [S. l.], v. 80, n. 22, p. 8538–8544, 2008. Disponível em: <https://doi.org/10.1021/ac801426m>

IVLEVA, N. P. *et al.* Towards a nondestructive chemical characterization of biofilm matrix by Raman microscopy. **Analytical and Bioanalytical Chemistry**, [S. l.], v. 393, n. 1, p. 197–206, 2009. Disponível em: <https://doi.org/10.1007/s00216-008-2470-5>

JACKSON, D. A. Stopping Rules in Principal Components Analysis : A Comparison of Heuristical and Statistical Approaches Stable. **Ecology**, [S. l.], v. 74, n. 8, p. 2204–2214, 1993.

JANG, J.; LEE, D. S. Effective phosphorus removal using chitosan/Ca-organically modified montmorillonite beads in batch and fixed-bed column studies. **Journal of Hazardous Materials**, [S. l.], v. 375, n. October 2018, p. 9–18, 2019. Disponível em: <https://doi.org/10.1016/j.jhazmat.2019.04.070>

JENKINS, D.; FERGUSON, J. F.; MENAR, A. B. Chemical processes for phosphate removal. **Water Research**, [S. l.], v. 5, n. 7, p. 369–389, 1971. Disponível em: [https://doi.org/10.1016/0043-1354\(71\)90001-7](https://doi.org/10.1016/0043-1354(71)90001-7)

JENSEN, J.; JEPSEN, S.-E. The production, use and quality of sewage sludge in Denmark. **Waste Management**, [S. l.], v. 25, n. 3, p. 239–247, 2005. Disponível em: <https://doi.org/10.1016/j.wasman.2004.08.011>

JOKO, I. Phosphorus removal from wastewater by the crystallization method. **Water**

Science and Technology, [S. l.], v. 17, n. 2-3-3 pt 1, p. 121–132, 1985. Disponível em: <https://doi.org/10.2166/wst.1985.0124>

KARAKAS, I. *et al.* Resource recovery from an aerobic granular sludge process treating domestic wastewater. **Journal of Water Process Engineering**, [S. l.], v. 34, n. July 2019, p. 101148, 2020. Disponível em: <https://doi.org/10.1016/j.jwpe.2020.101148>

KAZY, S. K. *et al.* Extracellular polysaccharides of a copper-sensitive and a copper-resistant *Pseudomonas aeruginosa* strain: Synthesis, chemical nature and copper binding. **World Journal of Microbiology and Biotechnology**, [S. l.], v. 18, n. 6, p. 583–588, 2002. Disponível em: <https://doi.org/10.1023/A:1016354713289>

KIM, N. K. *et al.* Flame retardant property of flax fabrics coated by extracellular polymeric substances recovered from both activated sludge and aerobic granular sludge. **Water Research**, [S. l.], v. 170, p. 115344, 2020. Disponível em: <https://doi.org/10.1016/j.watres.2019.115344>

KUMAR, P. S. *et al.* Understanding and improving the reusability of phosphate adsorbents for wastewater effluent polishing. **Water Research**, [S. l.], v. 145, p. 365–374, 2018. Disponível em: <https://doi.org/10.1016/j.watres.2018.08.040>

KUMAR, P. S. *et al.* Adsorption as a technology to achieve ultra-low concentrations of phosphate: Research gaps and economic analysis. **Water Research X**, [S. l.], v. 4, p. 100029, 2019. Disponível em: <https://doi.org/10.1016/j.wroa.2019.100029>

LADNORG, S. *et al.* Alginate-like exopolysaccharide extracted from aerobic granular sludge as biosorbent for methylene blue: Thermodynamic, kinetic and isotherm studies. **Journal of Environmental Chemical Engineering**, [S. l.], v. 7, n. 3, p. 103081, 2019. Disponível em: <https://doi.org/10.1016/j.jece.2019.103081>

LAGUNA, A. *et al.* A simple and low cost technique for determining the granulometry of upflow anaerobic sludge blanket reactor sludge. **Water Science and Technology**, [S. l.], v. 40, n. 8, p. 1–8, 1999.

LAYER, M. *et al.* Organic substrate diffusibility governs microbial community composition, nutrient removal performance and kinetics of granulation of aerobic granular sludge. **Water Research X**, [S. l.], v. 4, p. 100033, 2019. Disponível em: <https://doi.org/10.1016/j.wroa.2019.100033>

LAYER, M. *et al.* Limited simultaneous nitrification-denitrification (SND) in aerobic granular sludge systems treating municipal wastewater: Mechanisms and practical implications. **Water Research X**, [S. l.], v. 7, p. 100048, 2020. Disponível em:

<https://doi.org/10.1016/j.wroa.2020.100048>

LE, C.; KUNACHEVA, C.; STUCKEY, D. C. «protein» Measurement in Biological Wastewater Treatment Systems: A Critical Evaluation. **Environmental Science and Technology**, [S. l.], v. 50, n. 6, p. 3074–3081, 2016. Disponível em: <https://doi.org/10.1021/acs.est.5b05261>

LE, C.; STUCKEY, D. C. Colorimetric measurement of carbohydrates in biological wastewater treatment systems: A critical evaluation. **Water Research**, [S. l.], v. 94, p. 280–287, 2016. Disponível em: <https://doi.org/10.1016/j.watres.2016.03.008>

LEE, K. Y.; MOONEY, D. J. Alginate: Properties and biomedical applications. **Progress in Polymer Science (Oxford)**, [S. l.], v. 37, n. 1, p. 106–126, 2012. Disponível em: <https://doi.org/10.1016/j.progpolymsci.2011.06.003>

LI, X. *et al.* Seawater-based wastewater accelerates development of aerobic granular sludge: A laboratory proof-of-concept. **Water Research**, [S. l.], v. 115, p. 210–219, 2017 a. Disponível em: <https://doi.org/10.1016/j.watres.2017.03.002>

LI, Y. *et al.* Formation of artificial granules for proving gelation as the main mechanism of aerobic granulation in biological wastewater treatment. **Water Science and Technology**, [S. l.], v. 70, n. 3, p. 548–554, 2014. Disponível em: <https://doi.org/10.2166/wst.2014.260>

LI, Z. *et al.* Phosphate adsorption and precipitation on calcite under calco-carbonic equilibrium condition. **Chemosphere**, [S. l.], v. 183, p. 419–428, 2017 b. Disponível em: <https://doi.org/10.1016/j.chemosphere.2017.05.139>

LIN, Y. *et al.* Characterization of alginate-like exopolysaccharides isolated from aerobic granular sludge in pilot-plant. **Water Research**, [S. l.], v. 44, n. 11, p. 3355–3364, 2010. Disponível em: <https://doi.org/10.1016/j.watres.2010.03.019>

LIN, Y. *et al.* Glycosylated amyloid-like proteins in the structural extracellular polymers of aerobic granular sludge enriched with ammonium-oxidizing bacteria. **MicrobiologyOpen**, [S. l.], v. 7, n. 6, p. 1–13, 2018. Disponível em: <https://doi.org/10.1002/mbo3.616>

LIN, Y. M. *et al.* Sustainable polysaccharide-based biomaterial recovered from waste aerobic granular sludge as a surface coating material. **Sustainable Materials and Technologies**, [S. l.], v. 4, p. 24–29, 2015. Disponível em: <https://doi.org/10.1016/j.susmat.2015.06.002>

LIN, Y. M.; BASSIN, J. P.; VAN LOOSDRECHT, M. C. M. The contribution of

exopolysaccharides induced struvites accumulation to ammonium adsorption in aerobic granular sludge. **Water Research**, [S. l.], v. 46, n. 4, p. 986–992, 2012. Disponível em: <https://doi.org/10.1016/j.watres.2011.11.072>

LIN, Y. M.; SHARMA, P. K.; VAN LOOSDRECHT, M. C. M. The chemical and mechanical differences between alginate-like exopolysaccharides isolated from aerobic flocculent sludge and aerobic granular sludge. **Water Research**, [S. l.], v. 47, n. 1, p. 57–65, 2013. Disponível em: <https://doi.org/10.1016/j.watres.2012.09.017>

LIU, R. *et al.* Evaluation of natural organic matter release from alum sludge reuse in wastewater treatment and its role in P adsorption. **Chemical Engineering Journal**, [S. l.], v. 302, p. 120–127, 2016. Disponível em: <https://doi.org/10.1016/j.cej.2016.05.019>

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**, [S. l.], v. 65, n. 2, p. 143–148, 2004. Disponível em: <https://doi.org/10.1007/s00253-004-1657-8>

LIU, Y.; YANG, S.; TAY, J. Improved stability of aerobic granules by selecting slow-growing nitrifying bacteria. **Journal of biotechnology**, [S. l.], v. 108, p. 161–169, 2004. Disponível em: <https://doi.org/10.1016/j.jbiotec.2003.11.008>

LOTTI, T. *et al.* Hydrogels formed by anammox extracellular polymeric substances: Structural and mechanical insights. **Scientific Reports**, [S. l.], v. 9, n. 1, p. 1–9, 2019. Disponível em: <https://doi.org/10.1038/s41598-019-47987-8>

LV, J. *et al.* The microbial attachment potential and quorum sensing measurement of aerobic granular activated sludge and flocculent activated sludge. **Bioresource Technology**, [S. l.], v. 151, p. 291–296, 2014. Disponível em: <https://doi.org/10.1016/j.biortech.2013.10.013>

MCSWAIN, B. S. *et al.* Composition and distribution of extracellular polymeric substances in aerobic flocs and granular sludge. **Applied and environmental microbiology**, [S. l.], v. 71, n. 2, p. 1051–1057, 2005. Disponível em: <https://doi.org/10.1128/AEM.71.2.1051-1057.2005>

MEHDINEJADIANI, B.; AMININASAB, S. M.; MANHOOEI, L. Enhanced adsorption of nitrate from water by modified wheat straw: Equilibrium, kinetic and thermodynamic studies. **Water Science and Technology**, [S. l.], v. 79, n. 2, p. 302–313, 2019. Disponível em: <https://doi.org/10.2166/wst.2019.047>

MENG, F. *et al.* Enhanced amount and quality of alginate-like exopolysaccharides in aerobic granular sludge for the treatment of salty wastewater. **BioResources**, [S. l.], v. 14, n. 1, p. 139–165, 2019. Disponível em: <https://doi.org/10.15376/biores.14.1.139-165>

MIAO, L. *et al.* Advanced nitrogen removal from landfill leachate via Anammox system based on Sequencing Biofilm Batch Reactor (SBBR): Effective protection of biofilm. **Bioresource Technology**, [S. l.], v. 220, p. 8–16, 2016. Disponível em: <https://doi.org/10.1016/j.biortech.2016.06.131>

MILFERSTEDT, K. *et al.* Biogranules applied in environmental engineering. **International Journal of Hydrogen Energy**, [S. l.], v. 42, n. 45, p. 27801–27811, 2017. Disponível em: <https://doi.org/10.1016/j.ijhydene.2017.07.176>

MOHAN, S. V. *et al.* Waste biorefinery models towards sustainable circular bioeconomy: Critical review and future perspectives. **Bioresource Technology**, [S. l.], v. 215, p. 2–12, 2016. Disponível em: <https://doi.org/10.1016/j.biortech.2016.03.130>

MÜLLER, J. M.; DOS SANTOS, R. L.; BRIGIDO, R. V. Produção de alginato por microrganismos. **Polímeros**, [S. l.], v. 21, n. 4, p. 305–310, 2011.

NANCHARAI, Y. V.; KIRAN KUMAR REDDY, G. Aerobic granular sludge technology: Mechanisms of granulation and biotechnological applications. **Bioresource Technology**, [S. l.], v. 247, n. September 2017, p. 1128–1143, 2018. Disponível em: <https://doi.org/10.1016/j.biortech.2017.09.131>

NI, B. J. *et al.* Granulation of activated sludge in a pilot-scale sequencing batch reactor for the treatment of low-strength municipal wastewater. **Water Research**, [S. l.], v. 43, n. 3, p. 751–761, 2009. Disponível em: <https://doi.org/10.1016/j.watres.2008.11.009>

NI, S. Q. *et al.* Distribution of extracellular polymeric substances in anammox granules and their important roles during anammox granulation. **Biochemical Engineering Journal**, [S. l.], v. 101, p. 126–133, 2015. Disponível em: <https://doi.org/10.1016/j.bej.2015.05.014>

NIZAMI, A. S. *et al.* Waste biorefineries: Enabling circular economies in developing countries. **Bioresource Technology**, [S. l.], v. 241, p. 1101–1117, 2017. Disponível em: <https://doi.org/10.1016/j.biortech.2017.05.097>

PAWAR, S. N.; EDGAR, K. J. Alginate derivatization: A review of chemistry, properties and applications. **Biomaterials**, [S. l.], v. 33, n. 11, p. 3279–3305, 2012. Disponível em: <https://doi.org/10.1016/j.biomaterials.2012.01.007>

PEÑA-MÉNDEZ, E. M.; HAVEL, J.; PATOČKA, J. Humic substances - compounds of still unknown structure: applications in agriculture, industry, environment, and biomedicine. **Journal of Applied Biomedicine**, [S. l.], v. 3, n. 1, p. 13–24, 2005. Disponível em: <https://doi.org/10.32725/jab.2005.002>

POORMAND, H.; LEILI, M.; KHAZAEI, M. Adsorption of methylene blue from

aqueous solutions using water treatment sludge modified with sodium alginate as a low cost adsorbent. **Water Science and Technology**, [S. l.], v. 75, n. 2, p. 281–295, 2017. Disponível em: <https://doi.org/10.2166/wst.2016.510>

PRONK, M. *et al.* Effect and behaviour of different substrates in relation to the formation of aerobic granular sludge. **Applied Microbiology and Biotechnology**, [S. l.], v. 99, n. 12, p. 5257–5268, 2015 a. Disponível em: <https://doi.org/10.1007/s00253-014-6358-3>

PRONK, M. *et al.* Full scale performance of the aerobic granular sludge process for sewage treatment. **Water Research**, [S. l.], v. 84, p. 207–217, 2015 b. Disponível em: <https://doi.org/10.1016/j.watres.2015.07.011>

PRONK, M. *et al.* The acid soluble extracellular polymeric substance of aerobic granular sludge dominated by *Deffluviicoccus* sp. **Water Research**, [S. l.], v. 122, p. 148–158, 2017. Disponível em: <https://doi.org/10.1016/j.watres.2017.05.068>

QIN, L.; LIU, Y.; TAY, J. H. Effect of settling time on aerobic granulation in sequencing batch reactor. **Biochemical Engineering Journal**, [S. l.], v. 21, n. 1, p. 47–52, 2004. Disponível em: <https://doi.org/10.1016/j.bej.2004.03.005>

RAMOS, P. E. *et al.* Effect of alginate molecular weight and M/G ratio in beads properties foreseeing the protection of probiotics. **Food Hydrocolloids**, [S. l.], v. 77, p. 8–16, 2018.

REMMINGHORST, U.; REHM, B. H. A. Bacterial alginates: From biosynthesis to applications. **Biotechnology Letters**, [S. l.], v. 28, n. 21, p. 1701–1712, 2006. Disponível em: <https://doi.org/10.1007/s10529-006-9156-x>

ROCHER, V. *et al.* Removal of organic dyes by magnetic alginate beads. **Water Research**, [S. l.], v. 42, n. 4–5, p. 1290–1298, 2008. Disponível em: <https://doi.org/10.1016/j.watres.2007.09.024>

ROCKTÄSCHEL, T. *et al.* Influence of the granulation grade on the concentration of suspended solids in the effluent of a pilot scale sequencing batch reactor operated with aerobic granular sludge. **Separation and Purification Technology**, [S. l.], v. 142, p. 234–241, 2015. Disponível em: <https://doi.org/10.1016/j.seppur.2015.01.013>

RONDEL, C.; MARCATO-ROMAIN, C.; GIRBAL-NEUHAUSER, E. Development and validation of a colorimetric assay for simultaneous quantification of neutral and uronic sugars. **Water Research**, [S. l.], v. 47, n. 8, p. 2901–2908, 2013. Disponível em: <https://doi.org/10.1016/j.watres.2013.03.010>

RUSANOWSKA, P. *et al.* Changes in extracellular polymeric substances (EPS)

content and composition in aerobic granule size-fractions during reactor cycles at different organic loads. **Bioresource Technology**, [S. l.], v. 272, n. October 2018, p. 188–193, 2019. Disponível em: <https://doi.org/10.1016/j.biortech.2018.10.022>

SALEHIZADEH, H.; YAN, N.; FARNOOD, R. Recent advances in polysaccharide bio-based flocculants. **Biotechnology Advances journal**, [S. l.], v. 36, n. May 2017, p. 92–119, 2018. Disponível em: <https://doi.org/10.1016/j.biotechadv.2017.10.002>

SAM, S. B.; DULEKGURGEN, E. Characterization of exopolysaccharides from floccular and aerobic granular activated sludge as alginate-like-exoPS. **Desalination and Water Treatment**, [S. l.], v. 7, n. 3, 2015. Disponível em: <https://doi.org/10.1080/19443994.2015.1052567>

SARMA, S. J.; TAY, J. H. Aerobic granulation for future wastewater treatment technology: Challenges ahead. **Environmental Science: Water Research and Technology**, [S. l.], v. 4, n. 1, p. 9–15, 2018 a. Disponível em: <https://doi.org/10.1039/c7ew00148g>

SARMA, S. J.; TAY, J. H. Carbon, nitrogen and phosphorus removal mechanisms of aerobic granules. **Critical Reviews in Biotechnology**, [S. l.], v. 38, n. 7, p. 1077–1088, 2018 b. Disponível em: <https://doi.org/10.1080/07388551.2018.1451481>

SARMA, S. J.; TAY, J. H.; CHU, A. Finding Knowledge Gaps in Aerobic Granulation Technology. **Trends in Biotechnology**, [S. l.], v. 35, n. 1, p. 66–78, 2017. Disponível em: <https://doi.org/10.1016/j.tibtech.2016.07.003>

SARTORI, C.; FINCH, D. S.; RALPH, B. Determination of the cation content of alginate thin films by FTi.r. spectroscopy. **Polymer**, [S. l.], v. 38, n. 1, p. 43–51, 1997.

SCHAMBECK, C. M. *et al.* Biopolymers recovery: dynamics and characterization of alginate-like exopolymers in an aerobic granular sludge system treating municipal wastewater without sludge inoculum. **Journal of Environmental Management**, [S. l.], v. 263, n. February, 2020 a. Disponível em: <https://doi.org/10.1016/j.jenvman.2020.110394>

SCHAMBECK, C. M. *et al.* Chemical and physical properties of alginate-like exopolymers of aerobic granules and flocs produced from different wastewaters. **Bioresource Technology**, [S. l.], p. 123632, 2020 b. Disponível em: <https://doi.org/10.1016/j.biortech.2020.123632>

SEVIOUR, T. *et al.* Understanding the Properties of Aerobic Sludge Granules as Hydrogels. **Biotechnology and Bioengineering**, [S. l.], v. 102, n. 5, p. 1483–1493, 2009. Disponível em: <https://doi.org/10.1002/bit.22164>

SEVIOUR, T. *et al.* Aerobic sludge granulation : A tale of two polysaccharides?

Water Research, [S. l.], v. 46, p. 4803–4813, 2012. Disponível em: <https://doi.org/10.1016/j.watres.2012.06.018>

SEVIOUR, T. *et al.* Extracellular polymeric substances of biofilms: Suffering from an identity crisis. **Water Research**, [S. l.], v. 151, p. 1–7, 2019. Disponível em: <https://doi.org/10.1016/j.watres.2018.11.020>

SEVIOUR, T.; DONOSE, B. C.; PIJUAN, M. Purification and Conformational Analysis of a Key Exopolysaccharide Component of Mixed Culture Aerobic Sludge Granules. **Environmental Science and Technology**, [S. l.], v. 44, n. 12, p. 4729–4734, 2010.

SHEN, C. *et al.* Adsorption of phosphorus with calcium alginate beads containing drinking water treatment residual. **Water Science and Technology**, [S. l.], v. 78, n. 9, p. 1980–1989, 2018. Disponível em: <https://doi.org/10.2166/wst.2018.473>

SHI, S. *et al.* Strategies of valorization of sludge from wastewater treatment. **Journal of Chemical Technology & Biotechnology**, [S. l.], v. 93, n. December, p. 936–944, 2018. Disponível em: <https://doi.org/10.1002/jctb.5548>

SILVERSTEIN, R. M.; WEBSTER, F. X.; DAVID, J. K. **Spectrometric identification of organic compounds**. 7th. arg. [S. l.]: John Wiley & Sons, 2005. *E-book*.

SIWEK, H. *et al.* Removal of Phosphate from Aqueous Solution Using Alginate/Iron (III) Chloride Capsules: a Laboratory Study. **Water, Air, and Soil Pollution**, [S. l.], v. 227, n. 11, 2016. Disponível em: <https://doi.org/10.1007/s11270-016-3128-0>

SIWEK, H.; BARTKOWIAK, A.; WŁODARCZYK, M. Adsorption of phosphates from aqueous solutions on alginate/goethite hydrogel composite. **Water (Switzerland)**, [S. l.], v. 11, n. 4, p. 1–13, 2019. Disponível em: <https://doi.org/10.3390/w11040633>

SOLON, K. *et al.* Resource recovery and wastewater treatment modelling. **Environmental Science: Water Research and Technology**, [S. l.], v. 5, n. 4, p. 631–642, 2019. Disponível em: <https://doi.org/10.1039/c8ew00765a>

STOKHOLM-BJERREGAARD, M. *et al.* A critical assessment of the microorganisms proposed to be important to enhanced biological phosphorus removal in full-scale wastewater treatment systems. **Frontiers in Microbiology**, [S. l.], v. 8, n. APR, p. 1–18, 2017. Disponível em: <https://doi.org/10.3389/fmicb.2017.00718>

SUN, C.; ZHAN, H.; WANG, L. Enhanced Aerobic Sludge Granulation by Seeding Concentrated Activated Sludge with Ca-Alginate Gel. **Water Environment Research**, [S. l.], v. 89, n. 12, p. 2078–2087, 2017. Disponível em: <https://doi.org/10.2175/106143017x14839994522786>

TAY, J. H.; LIU, Q. S.; LIU, Y. The effect of upflow air velocity on the structure of aerobic granules cultivated in a sequencing batch reactor. **Water Science and Technology**, [S. l.], v. 49, n. 1, p. 35–40, 2004.

TU, X. *et al.* Fractionation and characterization of dissolved extracellular and intracellular products derived from floccular sludge and aerobic granules. **Bioresource Technology**, [S. l.], v. 123, n. July, p. 55–61, 2012. Disponível em: <https://doi.org/10.1016/j.biortech.2012.07.075>

VAN DER HOEK, J. P.; DE FOUIJ, H.; STRUKER, A. Wastewater as a resource: Strategies to recover resources from Amsterdam's wastewater. **Resources, Conservation and Recycling**, [S. l.], v. 113, p. 53–64, 2016. Disponível em: <https://doi.org/10.1016/j.resconrec.2016.05.012>

VAN DER HOEK, J. P.; FOUIJ, H. De; STRUKER, A. Resources , Conservation and Recycling Wastewater as a resource : Strategies to recover resources from Amsterdam ' s wastewater. **Resources, Conservation & Recycling**, [S. l.], v. 113, p. 53–64, 2016. Disponível em: <https://doi.org/10.1016/j.resconrec.2016.05.012>

VAN LEEUWEN, K. *et al.* The Energy & Raw Materials Factory : Role and Potential Contribution to the Circular Economy of the Netherlands. **Environmental Management**, [S. l.], v. 61, p. 786–795, 2018. Disponível em: <https://doi.org/10.1007/s00267-018-0995-8>

VAN LOOSDRECHT, M. C. M.; BRDJANOVIC, D. Anticipating the next century of wastewater treatment. **Science**, [S. l.], v. 344, n. 6191, p. 1452–1453, 2014. Disponível em: <https://doi.org/10.1126/science.1255183>

VASILAKI, V. *et al.* Relating N₂O emissions during biological nitrogen removal with operating conditions using multivariate statistical techniques. **Water Research**, [S. l.], v. 140, p. 387–402, 2018. Disponível em: <https://doi.org/10.1016/j.watres.2018.04.052>

WAGNER, J. *et al.* Effect of particulate organic substrate on aerobic granulation and operating conditions of sequencing batch reactors. **Water Research**, [S. l.], v. 85, p. 158–166, 2015. Disponível em: <https://doi.org/10.1016/j.watres.2015.08.030>

WAN, C. *et al.* Disintegration of aerobic granules: Role of second messenger cyclic di-GMP. **Bioresource Technology**, [S. l.], v. 146, p. 330–335, 2013. Disponível em: <https://doi.org/10.1016/j.biortech.2013.07.073>

WAN, J. *et al.* Evolution of bioaggregate strength during aerobic granular sludge formation. **Biochemical Engineering Journal**, [S. l.], v. 58–59, n. 1, p. 69–78, 2011. Disponível em: <https://doi.org/10.1016/j.bej.2011.08.015>

WAN, J.; BESSIÈRE, Y.; SPÉRANDIO, M. Alternating anoxic feast/aerobic famine condition for improving granular sludge formation in sequencing batch airlift reactor at reduced aeration rate. **Water Research**, [S. l.], v. 43, n. 20, p. 5097–5108, 2009. Disponível em: <https://doi.org/10.1016/j.watres.2009.08.045>

WANG, F. *et al.* Characteristics of aerobic granule and nitrogen and phosphorus removal in a SBR. **Journal of Hazardous Materials**, [S. l.], v. 164, p. 1223–1227, 2009. Disponível em: <https://doi.org/10.1016/j.jhazmat.2008.09.034>

WANG, L. *et al.* Recent advances on biosorption by aerobic granular sludge. **Journal of Hazardous Materials**, [S. l.], v. 357, n. June, p. 253–270, 2018. Disponível em: <https://doi.org/10.1016/j.jhazmat.2018.06.010>

WANG, R. *et al.* Understanding the role of extracellular polymeric substances in an enhanced biological phosphorus removal granular sludge system. **BIORESOURCE TECHNOLOGY**, [S. l.], v. 169, p. 307–312, 2014. Disponível em: <https://doi.org/10.1016/j.biortech.2014.06.040>

WANG, X. *et al.* Probabilistic evaluation of integrating resource recovery into wastewater treatment to improve environmental sustainability. **Proceeding of the National Academy of Sciences of the United States of America**, [S. l.], v. 112, n. 5, p. 1630–1635, 2015. Disponível em: <https://doi.org/10.1073/pnas.1410715112>

WANG, Y.; QIAN, P. Y. Conservative fragments in bacterial 16S rRNA genes and primer design for 16S ribosomal DNA amplicons in metagenomic studies. **PLoS ONE**, [S. l.], v. 4, n. 10, 2009. Disponível em: <https://doi.org/10.1371/journal.pone.0007401>

WEI, D. *et al.* Role of extracellular polymeric substances in biosorption of dye wastewater using aerobic granular sludge. **Bioresource Technology**, [S. l.], v. 185, p. 14–20, 2015. Disponível em: <https://doi.org/10.1016/j.biortech.2015.02.084>

WEISSBRODT, D. G.; SHANI, N.; HOLLIGER, C. Linking bacterial population dynamics and nutrient removal in the granular sludge biofilm ecosystem engineered for wastewater treatment. **FEMS Microbiology Ecology**, [S. l.], v. 88, n. 3, p. 579–595, 2014. Disponível em: <https://doi.org/10.1111/1574-6941.12326>

WHITELEY, C. G.; LEE, D. J. Bacterial diguanylate cyclases: Structure, function and mechanism in exopolysaccharide biofilm development. **Biotechnology Advances**, [S. l.], v. 33, n. 1, p. 124–141, 2015. Disponível em: <https://doi.org/10.1016/j.biotechadv.2014.11.010>

WINKLER, M. K. H. *et al.* Evaluating the solid retention time of bacteria in flocculent and granular sludge. **Water Research**, [S. l.], v. 46, n. 16, p. 4973–4980, 2012. Disponível em:

<https://doi.org/10.1016/j.watres.2012.06.027>

WINTER, H. H.; CHAMBON, F. Analysis of Linear Viscoelasticity of a Crosslinking Polymer at the Gel Point. **Journal of Rheology**, [S. l.], v. 30, n. 2, p. 367–382, 1986. Disponível em: <https://doi.org/10.1122/1.549853>

XIA, J. *et al.* Microbial community structure and function in aerobic granular sludge. **Applied Microbiology and Biotechnology**, [S. l.], v. 102, n. 9, p. 3967–3979, 2018. Disponível em: <https://doi.org/10.1007/s00253-018-8905-9>

XU, Y. *et al.* Removal behaviors and mechanisms of orthophosphate and pyrophosphate by calcined dolomite with ferric chloride assistance. **Chemosphere**, [S. l.], v. 235, p. 1015–1021, 2019. Disponível em: <https://doi.org/10.1016/j.chemosphere.2019.07.018>

XU, Y. *et al.* Using Microbial Aggregates to Entrap Aqueous Phosphorus. **Trends in Biotechnology**, [S. l.], p. 1–12, 2020. Disponível em: <https://doi.org/10.1016/j.tibtech.2020.03.012>

XUE, Y.; HOU, H.; ZHU, S. Characteristics and mechanisms of phosphate adsorption onto basic oxygen furnace slag. **Journal of Hazardous Materials**, [S. l.], v. 162, n. 2–3, p. 973–980, 2009. Disponível em: <https://doi.org/10.1016/j.jhazmat.2008.05.131>

YANG, G. *et al.* Extraction and characterization of stratified extracellular polymeric substances in *Geobacter* biofilms. **Bioresource Technology**, [S. l.], v. 276, p. 119–126, 2019. Disponível em: <https://doi.org/10.1016/j.biortech.2018.12.100>

YANG, Y. *et al.* Dewatered alum sludge: A potential adsorbent for phosphorus removal. **Water Science and Technology**, [S. l.], v. 54, n. 5, p. 207–213, 2006 a. Disponível em: <https://doi.org/10.2166/wst.2006.564>

YANG, Y. *et al.* Characteristics and mechanisms of phosphate adsorption on dewatered alum sludge. **Separation and Purification Technology**, [S. l.], v. 51, n. 2, p. 193–200, 2006 b. Disponível em: <https://doi.org/10.1016/j.seppur.2006.01.013>

YANG, Y. *et al.* Accelerated aerobic granulation using alternating feed loadings: Alginate-like exopolysaccharides. **BIORESOURCE TECHNOLOGY**, [S. l.], v. 171, p. 360–366, 2014. Disponível em: <https://doi.org/10.1016/j.biortech.2014.08.092>

ZHANG, Q.; HU, J.; LEE, D. J. Aerobic granular processes: Current research trends. **Bioresource Technology**, [S. l.], v. 210, p. 74–80, 2016. Disponível em: <https://doi.org/10.1016/j.biortech.2016.01.098>

ZHANG, Z. *et al.* Understanding of aerobic sludge granulation enhanced by sludge retention time in the aspect of quorum sensing. **Bioresource Technology**, [S. l.], v. 272, n. 866,

p. 226–234, 2019 a. Disponível em: <https://doi.org/10.1016/j.biortech.2018.10.027>

ZHANG, Z. *et al.* A novel mode of air recycling favored stable operation of the aerobic granular sludge process via calcium accumulation. **Chemical Engineering Journal**, [S. l.], v. 371, n. 866, p. 600–608, 2019 b. Disponível em: <https://doi.org/10.1016/j.cej.2019.04.083>

ZHAO, L. *et al.* Characteristics of extracellular polymeric substances from sludge and biofilm in a simultaneous nitrification and denitrification system under high salinity stress. **Bioprocess and Biosystems Engineering**, [S. l.], v. 39, p. 1375–1389, 2016. Disponível em: <https://doi.org/10.1007/s00449-016-1613-x>

ZHENG, Y.; WANG, A. Nitrate adsorption using Poly(dimethyl diallyl ammonium chloride)/polyacrylamide hydrogel. **Journal of Chemical and Engineering Data**, [S. l.], v. 55, n. 9, p. 3494–3500, 2010. Disponível em: <https://doi.org/10.1021/je100169r>

ZHENG, Y.; XIE, Y.; WANG, A. Rapid and wide pH-independent ammonium-nitrogen removal using a composite hydrogel with three-dimensional networks. **Chemical Engineering Journal**, [S. l.], v. 179, p. 90–98, 2012. Disponível em: <https://doi.org/10.1016/j.cej.2011.10.064>

ZHU, L. *et al.* Specific component comparison of extracellular polymeric substances (EPS) in flocs and granular sludge using EEM and SDS-PAGE. **Chemosphere**, [S. l.], v. 121, p. 26–32, 2015. Disponível em: <https://doi.org/10.1016/j.chemosphere.2014.10.053>

GLOSSÁRIO

Ácidos graxos voláteis - ácidos graxos de cadeia carbônica curta compostos principalmente de ácidos carboxílicos produzidos no processo de digestão anaeróbia como o acetato e propionato, por exemplo.

Ácidos húmicos - são macromoléculas que compreendem as substâncias húmicas que são a matéria orgânica distribuída no solo terrestre, água natural e sedimentos resultante de processos de degradação. Ácidos húmicos diferem das outras frações de substâncias húmicas porque são solúveis em meios alcalinos, parcialmente solúveis em água e insolúveis em meios ácidos.

Ácidos urônicos - classe de ácidos de açúcar com cargas negativas e com grupos funcionais de carbonila e ácido carboxílico. Eles são açúcares nos quais o grupo hidroxila do carbono terminal foi oxidado a um ácido carboxílico. Os nomes dos ácidos urônicos são geralmente baseados no açúcar de onde ele veio: ácido glucurônico (derivado da glicose), ácido manurônico (da manose) e ácido gulurônico (da gulose).

Activated sludge – ver lodos ativados.

Açúcar – termo genérico para carboidratos solúveis.

Açúcares neutros – polissacarídeos sem carga como a glicose, diferentemente dos ácidos urônicos que são carregados negativamente.

Adsorção – processo de aderência física ou ligação de íons e moléculas à uma superfície.

Adsorption – ver adsorção.

Agente tampão – um agente tampão ajusta e estabiliza o pH de uma solução. Ele mantém o pH de uma solução em um certo valor, prevenindo a sua mudança.

Amplitude sweep – é um teste oscilatório para avaliar a deformação de materiais em que ocorre a variação da amplitude enquanto a frequência é mantida constante. A amplitude é o máximo do movimento oscilatório.

Análise de ciclo de vida – análise dos efeitos ambientais associados às atividades produtivas ao longo de todas as etapas necessárias para que um produto cumpra sua função na cadeia de produtividade. Isso vai desde a extração e processamento da matéria-prima até o descarte final, passando pelas fases de transformação, produção, transporte, distribuição, uso, reuso, manutenção e reciclagem.

Anammox – sigla do inglês para *anaerobic ammonium oxidation*. É um processo onde a remoção do nitrogênio para gás nitrogênio (N_2) ocorre diretamente do nitrogênio amoniacal e nitrito (NO_2^-) sob condições anaeróbias com oxidação autotrófica.

Anfifílico – propriedade característica de molécula ou substância que possui em sua estrutura química uma parte polar e hidrofílica e outra apolar e hidrofóbica e que seja capaz de promover a interação entre meios que apresentam polaridades diferentes.

Anoxia – condição em que o oxigênio (O_2) para respiração microbiana provém do nitrato (NO_3^-).

Biofilme – bioestrutura complexa que aparece em superfícies que estão regularmente em contato com água. Um biofilme consiste de células procarióticas e outros microrganismos como leveduras, fungos e protozoários que secretam um revestimento gelatinoso protetor onde eles estão (ou seja, as substâncias poliméricas extracelulares - EPS).

Buffer agent – ver agente tampão.

Capacidade prática - termo usado aqui para descrever o valor da capacidade de sorção de uma substância por uma superfície sorvente (massa de substância por massa de sorvente) sob um determinado conjunto de condições. Visto que diferentes estudos de adsorção são conduzidos em condições variáveis, torna-se muito difícil comparar as capacidades máximas de adsorção entre diferentes sorventes. Assim, usar o termo “capacidade máxima” pode ser enganoso, sendo o termo “capacidade prática” mais adequado conforme proposto por Kumar et al. (2019).

Carbohydrates – ver polissacarídeos.

Carboidratos – biomoléculas compostas de carbono, oxigênio e hidrogênio. Em bioquímica é um sinônimo de sacarídeos, que compreende os monossacarídeos, dissacarídeos, oligossacarídeos e polissacarídeos.

Contaminantes emergentes - contaminantes ambientais, incluindo certos produtos farmacêuticos, produtos de higiene pessoal e produtos químicos industriais que podem ter efeitos negativos na saúde humana ou no ecossistema receptor, mas sem diretrizes rígidas sobre sua liberação e monitoramento ambiental.

Crosslinking – ver reticulação.

Desnitrificação – redução do nitrato (NO_3^-) a nitrito (NO_2^-) e depois a N_2 na ausência de O_2 com oxidação da matéria orgânica realizada por microrganismos heterotróficos.

Domestic wastewater – ver esgoto doméstico.

Elasticidade - propriedade que descreve os materiais que retornam à sua forma de repouso depois que as tensões aplicadas são removidas.

Elasticity – ver elasticidade.

Esgoto doméstico – Esgoto sanitário real ou águas residuais produzidas por uma comunidade de pessoas em suas atividades cotidianas domésticas. No Brasil, pode conter uma parcela de esgoto industrial dependendo das características do esgoto e da rede coletora.

Esgoto sanitário – ver esgoto doméstico.

Estado estacionário – condição de um sistema na qual um parâmetro ou parâmetros se estabilizaram ou não variam mais ao longo do tempo.

Flocos – denominação da biomassa similar à forma de flocos presente comumente em sistemas de lodos ativados.

Flocs – ver flocos.

Flocculent sludge – ver flocos.

Functional groups – ver grupos funcionais.

G' – módulo elástico (*storage modulus*). Porção elástica do comportamento viscoelástico. Parâmetro que descreve a comportamento quase sólido da amostra. Sólidos viscoelásticos possuem $G' > G''$.

G'' – módulo viscoso (*loss modulus*). Porção viscosa do comportamento viscoelástico. Parâmetro que representa o comportamento líquido da amostra. Líquidos viscoelásticos possuem $G'' > G'$.

Gelificação – processo para formação de géis como reticulação iônica, reticulação covalente, gelificação térmica e reticulação celular.

Glycoside hydrolase - enzima que catalisa a hidrólise da ligação glicosídica de carboidratos.

Grupos funcionais - em química orgânica, os grupos funcionais são átomos específicos dentro das moléculas com carbono que são responsáveis por reações químicas características. Mesmo que outras partes da molécula sejam bastante diferentes, certos grupos funcionais tendem a reagir maneiras semelhantes.

Hidrogel - material polimérico que exibe a capacidade de inchar e reter uma fração significativa de água dentro de sua estrutura, mas sem se dissolver nela.

Humic acids – ver ácidos húmicos.

Índice volumétrico de lodo (IVL) – parâmetro usado para descrever as características de sedimentação do lodo (biomassa). É definido como o volume (em mL) ocupado por 1 grama de lodo após sedimentação por determinado período (geralmente 5, 10 ou 30 minutos).

Isoterma – são equações matemáticas usadas para descrever, em termos quantitativos, a adsorção de substâncias em superfícies a temperaturas constantes. A isoterma de adsorção pode mostrar a quantidade máxima da substância adsorvida por uma superfície adsorvente, em função da concentração de equilíbrio da substância.

Isotherm – ver isoterma.

Licor misto - mistura de esgoto e biomassa em suspensão por meio da agitação promovida pela aeração.

Ligand exchange – ver troca de ligantes.

Loading – peso de cada variável normalizada dentro da componente principal.

Lodo seco – lodo resultante do processo de desidratação ou desaguamento a fim de reduzir sua quantidade de água.

Lodo úmido – lodo com 90-95% de teor de umidade que ainda não passou pelos processos de desidratação ou desaguamento.

Lodos ativados – sistema de tratamento de esgoto composto minimamente por um tanque de aeração ou reator, tanque de decantação e recirculação de lodo. Esse processo biológico envolve a massa ativa de microrganismos sedimentados (lodo) que são recirculados do decantador novamente para o reator aumentando a concentração de biomassa em suspensão no tanque de aeração.

Mixed liquor – ver licor misto.

Municipal wastewater – ver esgoto doméstico.

Neutral sugars – ver açúcares neutros.

Nitrificação – oxidação autotrófica do nitrogênio amoniacal a nitrito (NO_2^-) e posteriormente a nitrato (NO_3^-) sob condições aeróbias onde o gás carbônico (CO_2) é a principal fonte de carbono.

Operational taxonomic unit (OTU)– ver unidade taxonômica operacional.

PAO – sigla em inglês para *polyphosphate-accumulating organisms* (organismos acumuladores de fósforo) que são heterotróficos de crescimento lento e seu metabolismo envolve ciclos de formação e consumo de polímeros intracelulares (poli-fosfatos, glicogênio e PHA) sob condições de oxidação-redução alternadas (condições anaeróbias e aeróbias ou anóxicas).

PHA (polihidroxicanoatos) – São poliésteres acumulados intracelularmente por bactérias como reserva de carbono e/ou energia sob a limitação de um nutriente que vêm sendo empregados para produção de bioplásticos.

Practical loading – ver capacidade prática.

Pressão de seleção - grupo de fatores ambientais como temperatura, oxigênio dissolvido, DQO e tempo de sedimentação que favorecem certo grupo de microrganismos a dominarem um consórcio microbiano.

Primary effluent wastewater – esgoto que passou por processo de sedimentação para retirada dos sólidos suspensos afluentes da rede de coleta.

Principal Component Analysis (PCA) - Análise de Componentes Principais. É uma técnica de análise multivariada estatística que pode ser usada para analisar inter-relações entre um grande número de variáveis. Procura-se condensar a informação contida nas várias variáveis originais em um conjunto menor de variáveis estatísticas (componentes principais) com uma perda mínima de informação.

Principal components (PC) – componentes principais. São novas variáveis construídas como combinações lineares ou misturas das variáveis iniciais. Essas combinações são feitas de tal forma que as novas variáveis (isto é, componentes principais) não estão correlacionadas e a maioria das informações dentro das variáveis iniciais é comprimida nos primeiros componentes. Isso permite reduzir a dimensionalidade do grupo de variáveis sem perder muita informação, descartando os componentes com pouca informação.

Polissacarídeos - são grandes carboidratos formados por cadeias de monossacarídeos ligados entre si por ligações glicosídicas.

Polysaccharides – ver polissacarídeos.

Protease - enzima que quebra ligações peptídicas entre os aminoácidos das proteínas.

Proteínas - tipo de biomolécula formada a partir de um conjunto de aminoácidos ligados entre si por ligações denominadas de peptídicas.

Proteins – ver proteínas.

Quorum sensing – processo de coordenação da expressão gênica dependente da densidade populacional observada em comunidades microbianas. Moléculas autoindutoras são liberadas para o ambiente em correlação com a densidade microbiana dentro de uma comunidade. Em resposta à concentração dessas moléculas, a expressão gênica é coordenada para um propósito específico, como a formação de biofilme.

Reator em bateladas – reator que trata um volume pré-definido de esgoto em determinado tempo sem haver vazões de entrada ou saída ao longo do processo.

Reator de fluxo de pistão – reator onde as reações que ocorrem no tratamento variam não somente ao longo do tempo, mas também ao longo da distância (comprimento) do reator, não havendo uma mistura completa.

Rejeitos - resíduos sólidos que, depois de esgotadas todas as possibilidades de tratamento e recuperação por processos tecnológicos disponíveis e economicamente viáveis, não apresentem outra possibilidade que não a disposição final ambientalmente adequada.

Reologia – ciência que estuda as propriedades viscosas, viscoelásticas e elásticas dos materiais.

Reticulação – reação onde cadeias poliméricas lineares ou ramificadas são interligadas por ligação covalente. Assim, as ligações cruzadas (ou *crosslinking*) entre moléculas lineares produzem polímeros tridimensionais com alta massa molar tornando a estrutura mais rígida, muitas vezes em forma de gel.

Rheology – ver reologia.

Richness – ver riqueza.

Riqueza - é o número de diferentes espécies representadas em uma comunidade. É simplesmente uma contagem de espécies e não leva em consideração as abundâncias das espécies ou suas distribuições de abundância relativa.

Sludge volumetric index (SVI) – ver Índice volumétrico de lodo.

Solid retention time – idade do lodo. É o tempo que a fração sólida (biomassa) fica em determinada unidade do sistema de tratamento de esgoto.

Sólidos suspensos voláteis – fração de sólidos que se perde após queima da amostra a 550 °C. Usado como indicador da fração orgânica (biomassa).

Sorption – ver sorção.

Sorção – processo físico e químico pelo qual uma substância se liga a outra. Geralmente engloba os processos de absorção, adsorção e troca iônica.

Start-up ou partida – período inicial após o começo de operação do reator caracterizado pelo ajuste dos parâmetros operacionais e desempenho de tratamento até alcançar a estabilidade.

Steady-state – ver estado estacionário.

Student t-test – teste t de Student. É um teste de hipótese que usa conceitos estatísticos para rejeitar ou não uma hipótese nula quando a estatística de teste segue uma distribuição t de Student. O teste t informa o quão significativas são as diferenças entre grupos de dados. Em outras palavras, permite saber se essas diferenças medidas em médias podem ter acontecido por acaso, ou seja, não seguem um padrão, ou então, se são estatisticamente significativas.

Sugar – ver açúcar.

Tratamento primário - nível de tratamento de esgoto onde ocorre a remoção de sólidos em suspensão sedimentáveis e de sólidos flutuantes no esgoto afluente.

Troca de ligantes – processo de troca de íons entre uma solução e uma superfície. Uma troca de ligante é um tipo de reação química na qual um ligante em um composto é substituído por outro.

UASB – sigla em inglês para *upflow anaerobic sludge blanket* o que equivaleria a um reator anaeróbio de fluxo ascendente e manta de lodo. Essa tecnologia de tratamento de esgoto é baseada na decomposição anaeróbia da matéria orgânica, consistindo em uma coluna de escoamento ascendente (composta de zonas de digestão e sedimentação) e de dispositivo separador de fases gás-sólido-líquido.

Unidade taxonômica operacional – definição usada para classificar grupos de indivíduos próximos/semelhantes. Simplificadamente, significa o grupo de indivíduos similares que está sendo estudado.

Uronic sugar/acids – ver ácidos urônicos.

Viscoelasticidade - é a propriedade dos materiais que apresentam características viscosas e elásticas ao sofrer deformação. Materiais viscosos, como água, resistem ao cisalhamento e à deformação linearmente com o tempo quando uma tensão é aplicada. Os materiais elásticos se tensionam ao serem esticados e retornam imediatamente ao seu estado original assim que a tensão é removida. Os materiais viscoelásticos têm elementos de ambas as propriedades.

Viscoelasticity – ver viscoelasticidade.

Volatile suspended solids (VSS) – ver sólidos suspensos voláteis.

Volatile fatty acids (VFA) – ácidos graxos voláteis.