Journal of Environmental Chemical Engineering 8 (2020) 104156

Contents lists available at ScienceDirect

Journal of Environmental Chemical Engineering

journal homepage: www.elsevier.com/locate/jece

Variability in the composition of extracellular polymeric substances from a full-scale aerobic granular sludge reactor treating urban wastewater

Ana S. Oliveira[a], Catarina L. Amorim[a,*], Miguel A. Ramos[a], Daniela P. Mesquita[b], Paulo Inocêncio[c], Eugénio C. Ferreira[b], Mark van Loosdrecht[d], Paula M.L. Castro[a]

[a] Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho 1327, 4169-005, Porto, Portugal
[b] CEB - Centre of Biological Engineering, Universidade do Minho, Campus de Gualtar, 4710-057, Braga, Portugal
[c] Águas do Tejo Atlântico, S.A. Avenida de Ceuta, 1300-254, Lisboa, Portugal
[d] Department of Biotechnology, Delft University of Technology, van der Maasweg 9, 2629 HZ, Delft, the Netherlands

ARTICLE INFO

Editor: Teik Thye Lim

Keywords:
Aerobic granular sludge
Extracellular polymeric substances
Resource recovery
Waste valorization
EPS characterization.

ABSTRACT

Within the framework of the circular economy, there is a need for waste management alternatives that promote the reuse of materials produced in wastewater treatment plants (WWTP). An interesting option is the recovery of extracellular substances from sludge. The variability of characteristics of potential recovered bioproducts has to be assessed in full scale operational settings. In this study, aerobic granular sludge (AGS) from a full-scale WWTP treating urban wastewater was regularly collected for 4 months to assess variability in extracellular polymeric substances (EPS) composition and in granular morphology. Variations in the EPS composition occurred with time. Proteins and humic substances were the main EPS components (329–494 and 259 – 316 mg/g VSS of AGS, respectively), with polysaccharides and DNA representing minor components. The application of an extra purification step after extraction to obtain a purer EPS led to a decrease in the yield of each EPS component, particularly pronounced for the polysaccharides. The final product had a rather constant composition for the monthly samples. The granules showed morphological stability throughout the sampling period and the yield of EPS was correlated to the size of the granules, higher when there was a higher content of small granules (Deq < 150 μm) comparing to intermediate (150 ≤ Deq < 1500 μm) or large granules (Deq ≥ 1500 μm). This is the first time that a potential valorization strategy for surplus AGS biomass is studied in a full-scale environment. Knowledge on yield and product homogeneity is important as these features are essential for downstream application of the recovered EPS.

1. Introduction

Water is one of the most valuable resources. Water scarcity and excessive use are increasing the need to reuse wastewater and to develop wastewater treatment systems environmental friendly, energy and cost efficient, potentially combined with resource recovery. Currently, activated sludge systems are still the most commonly used systems for biological wastewater treatment. These systems require large surface areas and nowadays land is a limited resource, especially in densely populated regions. Activated sludge systems are not very flexible regarding sewage characteristics, as changes in sewage composition often lead to adverse effects on the system and hence on the effluent quality [1]. Aerobic granular sludge (AGS) technology is an innovative wastewater treatment system, economically outcompeting the conventional activated sludge systems. This is related to lower investment costs (10–30 %), around 30 % savings in energy consumption and ca. 70 % less space needed [2]. AGS is considered a special case of suspended biofilms in which self-immobilized microorganisms form spherical sludge aggregates. Microorganisms are embedded in a self-produced extracellular polymeric substances (EPS) matrix thus avoiding the need for any carrier [3–5]. The formation of AGS can be accomplished using sequencing batch reactors (SBR), alternating between aerobic and anaerobic periods [6]. Interesting properties, such as high biomass retention, settling properties (increasing the amount of water that can be treated in a certain period), tolerance to chemical toxicity, high biosorption capacity, ability to remove organic carbon, nitrogen and phosphorus simultaneously, make this technology increasingly attractive over the conventional activated sludge systems

* Corresponding author.
E-mail address: acatarinamorim@hotmail.com (C.L. Amorim).

https://doi.org/10.1016/j.jece.2020.104156
Received 20 April 2020; Received in revised form 5 June 2020; Accepted 6 June 2020
Available online 09 June 2020

and over other biofilm technologies [1,7–9]. EPS is composed of proteins, polysaccharides, humic substances and nucleic acids, produced and excreted by the composing bacteria. EPS play an important role in the resistance of the granules as EPS accumulated on the cells surface form a protective barrier preventing microbial cells from direct contact with the external environment [10,11].

In line with a circular economy approach, the demand for biotechnological processes that could offer an economic and versatile way to transform waste products into valuable products is growing. Water utilities are becoming increasingly aware of the need to implement resource recovery across wastewater treatment cycle [12]. Wastewater treatment plants (WWTPs) are facing a paradigm shift in which these plants are not only considered for their wastewater treatment functions, but also as a biorefinery, the basis for the exploitation of potential resources [13]. The identification of new strategies to valorize wastewater derived biomass is a crucial issue. There is a strong need of technical and economical feasible strategies for WWTP companies to valorize waste, which could also contribute for a positive environmental balance. EPS extraction plants are starting to be constructed, and a first extraction plant in the Netherlands, is already operating [14]. The EPS is marketed under the brand name Kaumera (https://kaumera.com/english/).

The implementation of AGS technology in full-scale WWTPs is rapidly increasing and the recovery of biomaterials such as EPS, could contribute to meet the strategies and recommendations outlined by the European Commission and the EU-28 member states to develop a knowledge based on bioeconomy in the coming years and reducing the waste disposal in 2050 [15,16]. Indeed, several efforts have been made to find new applications for surplus biomass from WWTPs in the construction and energy sector [12,17–19]. Surface coating material, composite plastics and binder material are bio-based products that can potentially be obtained using granular sludge as the primary source [19,20]. AGS-derived biopolymers have also been commercialized as a product for the construction engineering market, offering improvement for curing of cement (http://www.ngcm.nl/curing-compound.html). Further, EPS recovered from WWTPs processes have been reported to have potential in the chemical sector, as a soil enhancer in agriculture, brick addictive [21], flocculation, dewatering chemicals, biosorption agents for wastewater treatment [22,23], coating materials or the development of flame-retardant materials and coatings [14,19,24,25]. EPS recovered from aerobic granular sludge has also been referred as having potential in the food sector [26]. However, there is a strong stigma regarding resources recovered from sewage for food application and quality products.

Up to date, extraction and characterization of EPS was mainly evaluated from granular sludges collected from lab-scale reactors [27–29] and pilot plants [30–32]. EPS extraction from lab-scale reactors has consistently shown proteins as the major EPS component [27–29], whereas pilot plant studies have presented different conclusions regarding the main compositional component being proteins or polysaccharides [30–32].

To the best of our knowledge, this study presents for the first-time characterization of EPS from granular biomass collected from a full-scale WWTP, correlating it with WWTP loading dynamics. Knowledge on the variability of produced EPS, especially in full-scale WWTPs, deserves more attention from the point of view of product stability towards different conditions and of potential resource recovery approaches. In this work, EPS was extracted from granular sludge collected at a full-scale treatment plant to assess EPS composition and variability and to analyze how the extraction procedure would affect the product characteristics, determinant for downstream applications.

2. Materials and methods

2.1. Granular biomass

AGS was provided by an urban WWTP located in Frielas, Portugal. Frielas WWTP (GPS coordinates 38°49′08.65″N, 9°08′56.52″W) is the world's first retrofit conventional continuous activated sludge plant to Nereda® technology. The process configuration is composed by grit removal, followed by a primary settler and lastly by a secondary treatment. At the moment, the secondary treatment is provided by Nereda® technology (20 % daily volume) and conventional activated sludge (80 % of daily volume). The flow rate fed to the Nereda section was constant. This WWTP which receives domestic, pluvial and industrial (15–17 %) wastewater, is treating approximately $55000-60000\ m^3/$ day. The AGS was collected from the top of the aeration tank during the aeration period of the treatment cycle. Eight sampling campaigns were performed throughout approximately 4 months. The samples were numbered from 1 to 8 according to their sequential collection: first sampling campaign occurred in October of 2016; second, third and fourth occurred in the beginning, middle and end of December of 2016, respectively; fifth occurred in January of 2017; sixth and seventh occurred at the beginning and end of February of 2017, respectively; and the eighth occurred in March of 2017. Full-scale WWTPs analytical influent characterization was performed and provided by the Frielas Laboratory Unit, accredited under EN ISO 17025. The applied methodologies are expressed in the IPAC technical annex L-0287 − 2, which are based in Standard Methods for Examination of Water and Wastewater 22nd Edition, or ISO references.

2.2. Image analysis

The morphology of the granules collected from the full-scale WWTP was assessed by image analysis. Three samples (n = 3) with a volume of ca. 2 mL of granules were prepared for image acquisition. The granules were washed with phosphate-buffered saline (PBS) and after the addition of 4% formaldehyde and PBS were then incubated for 2 h at 4 °C. After incubation, the granules were washed again with PBS and subsequently, a mixture of 1:1 of PBS and ethanol 96 % was added to the granules. The granules were stored at -20 °C until analysis.

The granules were transferred to a Petri dish for image acquisition using the method described elsewhere [33]. The granules were divided into three classes based on the equivalent diameter (Deq): small granules (Deq below 150 μm), intermediate granules (Deq between 150 and 1500 μm), and large granules (Deq above 1500 μm). A specially developed program in Matlab (The Mathworks, Inc., Natick) allowed the treatment of the collected images in order to characterize AGS samples, in terms of some relevant morphological parameters: size, roundness, area, robustness, and compactness [33–35].

2.3. Extraction of Na-EPS and H-EPS from aerobic granules

The granules were sieved in the laboratory prior to EPS extraction. Sodium-EPS (Na-EPS) and acidic-EPS (H-EPS) were extracted from AGS. The extraction was performed using sodium carbonate (Na_2CO_3), with heat and constant mixing, following a procedure described by Felz et al. (2016) [31]. After the alkaline extraction, the supernatant with Na-EPS samples was divided into two fractions: one underwent the acidic precipitation resulting in H-EPS, and the other fraction was used directly as Na-EPS for biochemical characterization. Four successive extractions were performed using the pellet obtained in each extraction to recover more EPS.

2.4. EPS biochemical characterization

Colorimetric methods were used to determine the proteins [36], polysaccharides [37] and humic acids like substances contents [38],

and a fluorometric method was used to determine nucleic acids, specifically DNA content, using a Qubit fluorometer (Thermo Fisher Scientific).

2.5. Fourier-transform infrared spectroscopy (FTIR) of EPS

The spectra of Na-EPS and H-EPS were obtained with a Fourier transform infrared spectrometer (FTIR) (PerkinElmer Spectrum-100), using a horizontal attenuated total reflectance (ATR) accessory, with a diamond/ZnSe crystal. All spectra were acquired with 16 scans and a resolution of 4 cm^{-1}, in the region between 4500 and 450 cm^{-1}. Air was used for the background spectrum. The extracted EPS samples were lyophilized prior to analysis.

2.6. Statistical analysis

Statistical analysis was performed using the SPSS program (SPSS Inc., Chicago, IL Version 24.0). Each EPS extraction comprised six replicates (n = 6). Data are presented as mean ± standard deviation.

Normal distribution was verified with the Shapiro-Wilk test with a level of $p > 0.05$ set for significance. Homogeneity of variance was tested with the Levene's test and the assumption of homogeneity of variance was violated. According to Everitt (1996) [39] the ANOVA is robust to homogeneity of variance assumption violations as long as group sizes are equal (the ratio of the largest to smallest group being less than 1.5).

The statistical analysis was carried out by one-way ANOVA and subsequent post-hoc Tukey comparison to investigate differences in the concentration of each EPS component. A *t*-test was also used to evaluate differences between total Na-EPS and H-EPS. A $p < 0.05$ was considered as significant.

Pearson's correlation coefficient (r) was used to evaluate the linear correlation between EPS content, influent composition, and morphology of the granules. A $p < 0.05$ was considered as statistically significant for correlations.

Correlation matrices were performed using the software R 3.5.1, in association with the R-Studio interface 1.1.463 (www.rstudio.com 2018).

3. Results and discussion

3.1. Granules morphology and characteristics

Eight sampling campaigns were performed throughout approximately 4 months. The characteristics of the full-scale WWTP influent over that period are presented in Table 1. Considering this WWTP receives urban effluents, the weather conditions could affect the chemical composition of the wastewater. Temperature on sampling days ranged from 7 °C to 17.5 °C. Despite the large temperature range observed on sampling days, no correlation was found between ambient temperature and EPS composition or morphology characteristics (data not shown). No rainfall was observed in the days preceding the sampling campaigns.

Table 1
Full-scale WWTPs influent characterization over the four-months collection period. Means and standard deviation values of BOD_5 (mg/L), COD (mg/L), COD/BOD_5 ratio and TSS (mg/L) for one week before sampling campaigns (information provided by WWTP at Frielas, Portugal).

Sampling campaign	Biochemical oxygen demand (BOD_5, mg/L)	Chemical oxygen demand (COD, mg/L)	COD/BOD_5	Total suspended solids (TSS, mg/L)
1	225.0 ± 59.2	462.5 ± 103.1	2.1 ± 0.1	195.0 ± 34.2
2	175.0 ± 31.1	357.5 ± 70.9	2.0 ± 0.2	182.5 ± 17.1
3	192.5 ± 26.3	427.5 ± 75.4	2.2 ± 0.1	202.5 ± 40.3
4	282.5 ± 98.8	562.5 ± 133.8	2.1 ± 0.3	290.0 ± 74.8
5	170.0 ± 62.4	390.0 ± 131.1	2.4 ± 0.2	153.3 ± 23.1
6	136.7 ± 15.3	310.0 ± 40.0	2.3 ± 0.2	166.7 ± 25.2
7	213.3 ± 30.6	426.7 ± 15.3	2.0 ± 0.3	193.3 ± 47.3
8	213.3 ± 25.2	413.3 ± 32.1	2.0 ± 0.3	170.0 ± 10.0

In addition to the influent characteristics found in Table 1, other chemical parameters were evaluated less frequently, and consequently, it was not adequate to include them in the statistical analysis. During the 4 months of sampling campaigns variations were observed in NH_4^+ (31 − 61 mg/L NH_4^+), Kjeldahl nitrogen (36 − 62 mg/ N), phosphorous (4.7 − 10 mg/L P), and NO_3^- (1 mg/L N) contents of the wastewater. After wastewater treatment, the effluent composition was also analyzed and showed small variations for BOD_5 (6 − 10 mg/L), COD (31 − 64 mg/L), COD/BOD_5 ratio (4.3–9), TSS (6 − 21 mg/L). Less frequently analyzed parameters showed variations in NH_4^+ (3.9 − 26 mg/L NH_4^+), Kjeldahl nitrogen (5 − 25 mg/L N), phosphorous (2.2–2.4 mg/L P), and NO_3^- (2.4 − 16 mg/L N).

Morphology features and characteristics of the AGS collected are shown in Fig. 1. The biomass was divided into three groups according to its size (equivalent diameter, Deq): small granules with a Deq value below 150 µm, intermediate granules with a Deq value between 150 and 1500 µm, and large granules with a Deq value above 1500 µm (Fig. 2). During the first two sampling campaigns, biomass had an evenly distributed number of granules from the three groups. From the 3rd sampling campaign onwards, biomass in reactor was mainly constituted by small granules (in average 60 %). Intermediate and large granules were less abundant (around 20 % each). Small granules are often originated from the breakage of large and intermediate granules [33]. A negative correlation between the number of large granules and the number of small granules was found in the present study ($r = -0.805$, $p < 0.01$). Interestingly, a negative correlation between the number of large granules and the area of intermediate granules was also found ($r = -0.607$, $p < 0.01$), indicating that possibly large granules break into small and intermediate granules and not only into small granules.

It can be hypothesized that before the 4th sampling campaign operational parameters caused the breakage of the large and/or intermediate granules. Looking at Table 1, indeed levels of organic matter in the influent showed the highest values in the week before this sampling campaign. These observations are consistent with a study conducted by Costa et al. (2009), where loading disturbances caused granules fragmentation [40]. In the subsequent sampling campaigns (5–8), large granules area increased, but they are still outnumbered by the small granules, maybe due to a slow recovery from the condition that caused the granules breakage. The roundness, compactness and robustness of the biomass are indicative factors of the granule's morphological stability. For each size group, these three parameters were maintained almost constant over the 4-months collection period. Overall, image analysis allowed to infer that during the operation of the reactor, the granular biomass stability was consistent overtime despite variations observed in the area (%) and number (%) that were probably caused by fluctuations on incoming wastewater. However, this did not compromise the performance of granular biomass and effluent quality.

3.2. EPS extraction and characterization

Na-EPS and H-EPS were recovered from the granules, the first corresponding to the EPS extracted from the heat and alkaline extraction and the latter to the EPS obtained after the acidic precipitation step. Na-EPS and H-EPS protein, polysaccharides, humic substances and DNA contents were assessed. The sum of proteins, polysaccharides, humic substances and DNA content was considered to be the total Na-EPS and total H-EPS for each EPS form. Fig. 3 shows the concentration of each of the components (mg/g VSS of AGS) and the protein-polysaccharide ratio (PN/PS) for the AGS collected over the 4-months period.

Total Na-EPS concentration recovered from AGS over the 4 months differed significantly between sampling campaigns ($p < 0.05$), ranging

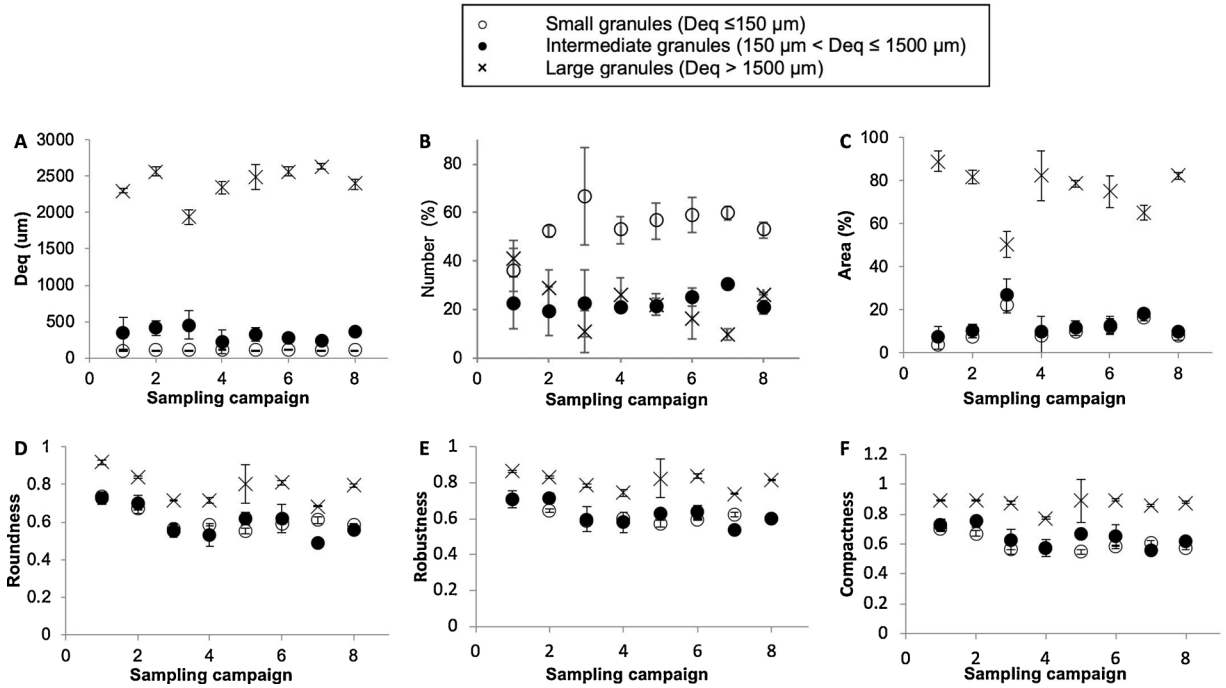

Fig. 1. Equivalent diameter (A), percentage of granules number of each size (B), percentage of area (C), roundness (D) robustness (E), and compactness (F) of the aerobic granules from the samples collected during approximately 4 months. Three samples (n = 3) of granules were used for image analysis. Marks and error bars represent the average and standard deviation of the evaluated parameters. The granular biomass was divided into three groups according to its Deq: small (○), intermediate (●) and large (×).

from 672 to 896 mg/g VSS of AGS. In addition, a *t*-test showed there were statistically significant differences ($p < 0.05$) in the total EPS of each sampling campaign before (Na-EPS) and after acidic precipitation (H-EPS). After acidic precipitation, although total EPS concentration was reduced, its concentration showed stable values ranging from 122 to 149 mg/g VSS of AGS. Pilot-scale studies have reported total EPS (sum of proteins and polysaccharides) yields of 235.2–262.8 mg/g VSS [30] and 370 mg/g VSS [32], lower than the total Na-EPS concentration obtained in the present study. The differences in EPS yield could be related to different extraction procedures used. Also, operational conditions differed in pilot and full-scale reactors, which may affect EPS production.

Proteins and humic substances were the main components of both Na-EPS and H-EPS, showing concentrations ranging from 298–485 mg/g VSS and 239–317 mg/g VSS, respectively. In contrast, Adav et al. (2008) obtained 537 mg/g VSS of proteins and 85 mg/g VSS of humic substances when extracting EPS (with a procedure that includes ultrasounds, formamide and NaOH) from a lab-scale AGS-SBR system [28]. The content of proteins obtained in the present study was approximately in the same order of magnitude, but the ones of humic substances were considerably different from the study of Adav et al. (2008) [28], which corroborates the previous observation that EPS extraction procedure and operational conditions can lead to different EPS characterization results. DNA showed a significant decrease in concentration from the first to the last sampling. For all other components, their concentration in EPS varied over the sampling period, presenting a cyclic pattern of increase and decrease of concentration. The PN/PS ratio varied between 5.7 and 8.7, in Na-EPS. Chen et al. (2010) [41] and Adav et al. (2008) [42] reported that narrow PN/PS ratio ranges are indicative of strong granular structure and stability. Hence, in the present study, the PN/PS ratio range corroborate the fact that granular structure and stability were not affected by loading variations over time.

Several positive and negative correlations were found between the evaluated Na-EPS biochemical parameters (Fig. 4 **A**). A positive correlation between total Na-EPS and proteins, humic substances ($r = 0.854$ and $r = 0.73$, respectively and both $p < 0.001$) and polysaccharides ($r = 0.436$, $p < 0.01$), also indicating that proteins and humic substances were the main components of the EPS, followed by polysaccharides. The acidic precipitation step forms EPS whose composition is more homogeneous when compared to Na-EPS samples regarding the concentration of each component. Specially proteins, the main EPS

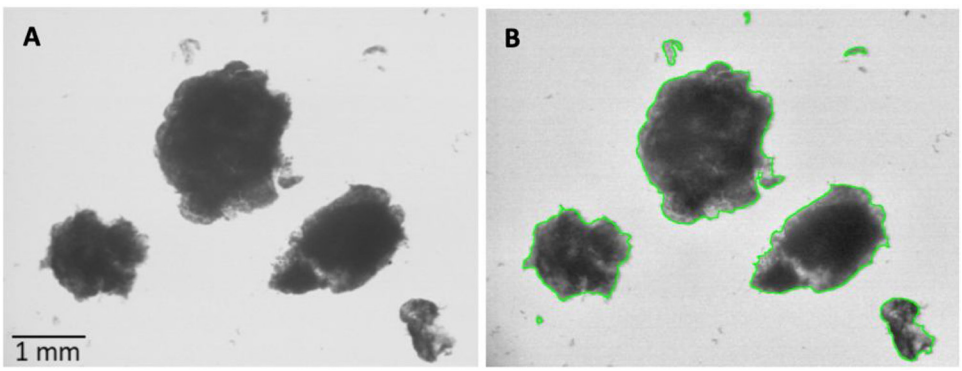

Fig. 2. Image acquisition of granules depicting the three groups according to equivalent diameter (Deq). (A) Large granule at the top center (Deq > 1500 µm), two intermediate granules at the center left and right (150 µm < Deq ≤ 1500 µm) and small granules at the bottom right (Deq ≤150 µm). (B) Granules as they are recognized by the software.

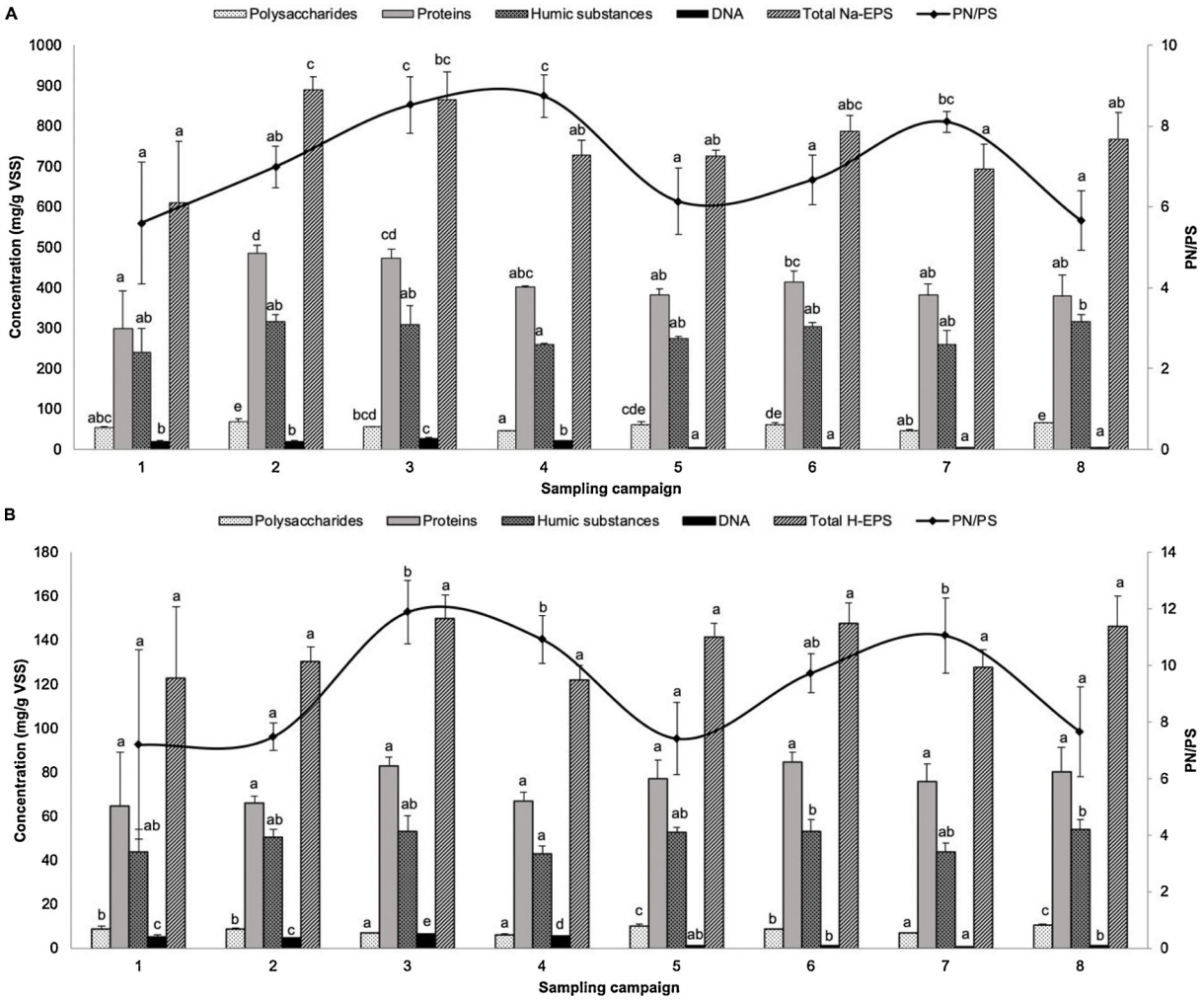

Fig. 3. EPS characterization based on the polysaccharides, proteins, humic substances, and DNA content, total EPS, and PN/PS ratioof (A) Na-EPS and (B) H-EPS. Means that do not share a letter in columns of the same group differed significantly according to Tukey's test at $p < 0.05$.

components, showed no significant differences between sampling campaigns in H-EPS.

Previous studies reported that the biochemical composition of EPS is affected by environmental conditions of microbial growth. The carbohydrate content is significantly affected by factors such as microbial species, carbon source, nutrient supplementation (N, P) whilst the protein content of EPS could be affected by the nitrogen concentration present in media [43]. Fig. 4B indicates that a negative correlation was found between the COD and the TSS of the influent with the polysaccharide's concentration of the granules EPS ($r = -0.883$ and $r = -0.907$, respectively and both $p < 0.05$). Influents with high COD content are usually accompanied by high TSS content which, in turn, decrease the polysaccharide production in granules EPS.

After the acidic precipitation, the concentration of each EPS component decreased: polysaccharides content decreased 6 to 8-fold; protein content decreased 5 to 7-fold; humic substances content decreased 5 to 6-fold; DNA content decreased 3 to 5-fold; total EPS content decreased 5 to 7-fold. The decrease in protein concentration was less pronounced than in polysaccharides concentration, leading to an increase of the PN/PS ratio. Moreover, the protein content in H-EPS was stable ($p > 005$) whilst in Na-EPS varied significantly throughout the different sampling campaigns ($p < 0.05$). No biochemical mechanism, regarding the decrease of EPS components after the acidic precipitation, has been proposed in the literature.

Although H-EPS appears to have a more homogeneous composition throughout sampling campaigns, the extraction yield decreased after acidic precipitation. Extraction yields for H-EPS showed a narrow range of 11–14 %, while extraction yields of Na-EPS ranged from 65 to 89%, after the quadruple extraction. Such broad range of extraction yields for Na-EPS reinforce the heterogeneous nature of these samples. Considering the H-EPS homogeneity over time, this is likely more stable also when different WWTPs would be investigated. Therefore, EPS recovery from WWTP surplus biomass can be considered a feasible process and two main approaches can be explored depending on the desired application of the extracted EPS. We envisage that Na-EPS extraction could be considered if high amounts of EPS are required regardless of the composition variability (e.g. construction or agricultural sector), whereas H-EPS extraction should be used when applications require a more homogenous EPS (e.g. flocculation agent in water treatment or polymer for composite materials production, coating materials or the development of flame-retardant materials and coatings). However, further studies are needed to assess the adequacy of Na-EPS and H-EPS for the suggested applications as well as its economic viability.

The relation between morphology features, EPS components and influent characteristics were also analyzed. A positive correlation was found between total Na-EPS and the number of small granules ($r = 0.71$, $p < 0.001$) (Fig. 4 **C**). Large granules, despite the size, may have a higher percentage of hollow space and/or water content. Furthermore, the number of small granules was also positively correlated to proteins concentrations in EPS ($r = 0.753$, $p < 0.01$). Thus, small granules, which contain higher EPS concentration than other size

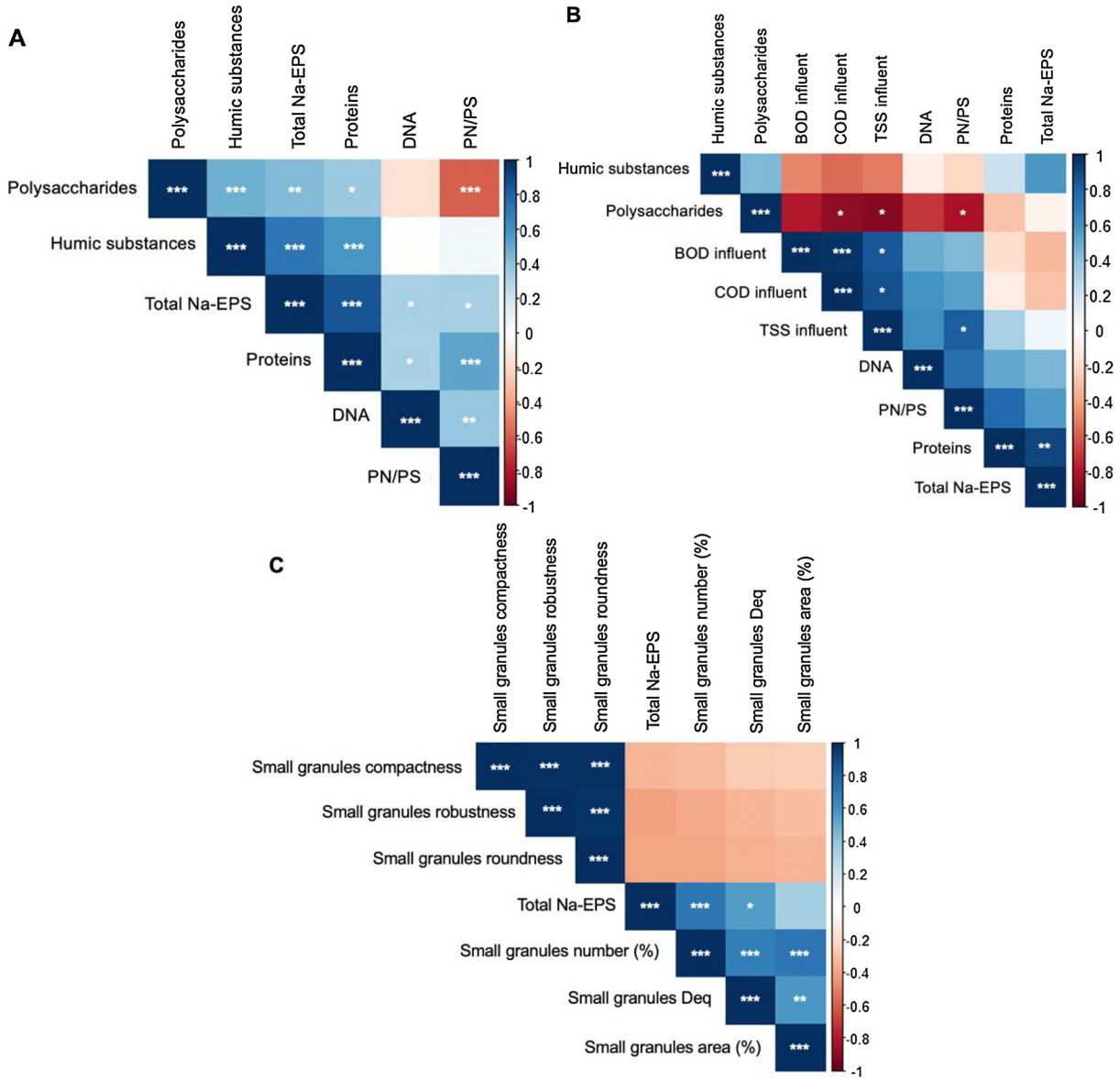

Fig. 4. Heat map of Pearson's correlation coefficients computed (A) between EPS composition parameters (n = 48), (B) between EPS compositions parameters and WWTP influent parameters (n = 8), and (C) between total Na-EPS and morphological parameters of small granules (n = 20). The values and directions of the correlation coefficients are displayed according to the colour key: positive correlations as blue gradients from 0 to 1 and inverse correlations as red gradients from 0 to -1. Significance of p-values are as followed: $p < 0.001$ represented as ***; $p < 0.01$ represented as **; $p < 0.05$ represented as * (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

granules, have higher concentration of proteins.

The extraction technique used in the present study showed higher yields than other techniques described in a study developed by Felz et al. (2016) [31]. Essentially other extraction techniques do not dissolve the granular sludge matrix. For that reason, this extraction technique was chosen. However, there are no guaranties that the extraction method used, or any other extraction method known so far, is able to extract EPS components in the same proportion as they are present in granules. Extraction techniques should be further optimized and studied in order to understand which one is the most appropriate and to increase yield of resource recovery. Characterization methods should also be improved in order to decrease biases and knowledge constraints. All those efforts should contribute to the standardization of an EPS extraction and characterization methods, allowing the comparison of EPS samples used in different studies [44,45]. Comparisons between studies is only viable if the same extraction and characterization methods are used.

The principle that underlies the EPS recovery for future applications, as a valuable resource, is based on the use of waste granules. The yield of biomass in AGS systems can range from 0.3 to 0.6 MLVSS/ g COD [46,47]. Considering an AGS biomass yield of 0.4 g MLVSS/ g COD, for an WWTP receiving ca. 16 ton COD/day, the biomass produced would allow for 7 – 10 ton of Na-EPS per day or 1 – 2 ton of H-EPS per day, based on the extraction yield obtained in the present study. However, in a large-scale setting, quadruple extraction may not be feasible, and a lower extraction yield would be obtained from a single extraction. According to Felz et al. (2016), the quadruple extraction increases the total yield by 46 % when compared to a single extraction [31]. Consequently, it could be expected that the same WWTP would allow for the recovery of 4 – 5 ton of Na-EPS per day or 0.6 – 1 ton of H-EPS per day, if considering a single extraction. The application of this knowledge could lead to WWTPs becoming closer to biorefineries, aligned with the circular economy concept, by exploiting potential resources. This study could be of interest for extraction plants

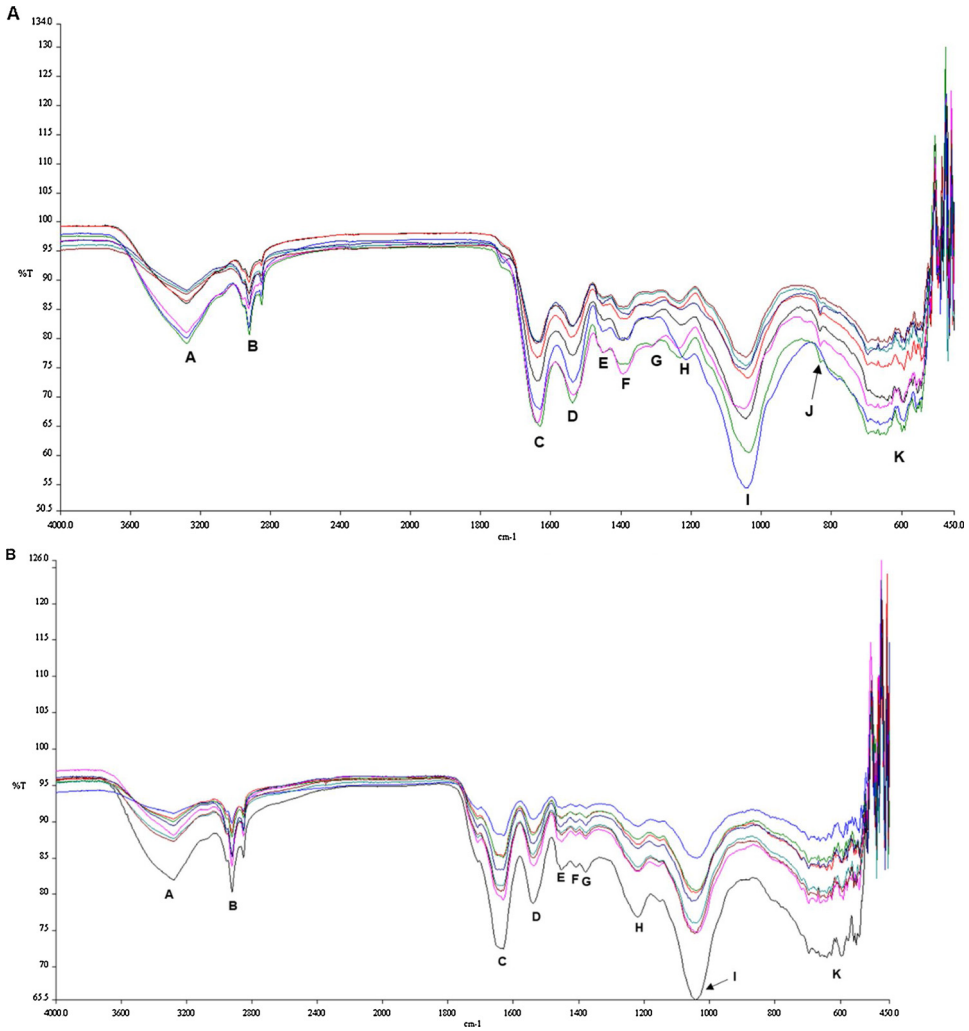

Fig. 5. FTIR spectra of the extracted Na-EPS (a) and H-EPS (b) from the AGS collected during the eight sampling campaigns. Bands marked with letters in the figure are referenced in Table 2.

that are starting to be constructed and operating, such as the Kaumera factory, a raw material factory in Zutphen, Netherlands, as information on recovered EPS compositional stability over time is provided.

3.3. FTIR analysis of EPS

The functional groups of Na-EPS and H-EPS were identified by FTIR analysis. Fig. 5 shows FTIR spectra obtained for the lyophilized Na-EPS and H-EPS extracted from the eight sampling campaigns and the respective band assignments are given in Table 2 [48–51]. FTIR spectra revealed high homology between samples from each type of EPS (Na-EPS and H-EPS) collected on different days, indicating that no major changes in the functional groups of EPS were observed after the acidic precipitation. Remarkably, a band around 833 nm (assigned as band J in Fig. 2) was only present in Na-EPS spectra. Thus, it can be hypothesized that the acidic precipitation, as an extra purification step, was responsible for the elimination of a certain protein or lipid as indicated by the assignment of this band. Studies performed by Liang et al. (2010) [52] and D'Abzac et al. (2010) [53] also showed EPS spectra with a band having similar shape and wavelength as the one

Table 2
Assignment of the bands found in FTIR spectra of extracted Na-EPS and H-EPS.

Band	Frequency (cm^{-1})	Assignment
A	3285	O—H stretching of hydroxyl groups
B	2930 – 2910	C—H stretching asymmetric (fatty acids)
C	1633	Amide I (C═O and C═C stretching in proteins)
D	1538	Amide II (N—H deformation, C—N stretching in proteins)
E	1450	C—H deformation of $-CH_2$
F	1400	C═O stretching symmetric of COO^-
G	1300	Amide III (N—H deformation, C—N stretching in proteins)
H	1233	N—H deformation, C—N stretching
I	1075 – 1048	C—O—C and C—H stretching (polysaccharides and/or nuclei acids)
J	833	Tri-substituted alkene sp^2 C—H deformation (in lipids) or para di-substituted aromatic sp^2 C—H deformation (in proteins)
K	900 – 600	"fingerprint region"

assigned as band J in the present study. The band observed in such studies showed high intensity, but no assignment was made.

Most of the bands were concentrated in the region among 1800–900 cm^{-1} that correspond to bands of amide, carboxylic and carbohydrate functional groups. These bands were also found in previous studies from Lin et al. (2010) [54], Wang et al. (2018) [55], and Zhu et al. (2012) [56]. Similar assignments were attributed to those bands. Moreover, comparing the FTIR spectra of both types of EPS samples, differences were noted in intensity of the bands indicating that there was variation in the quantity of each individual component, corroborating the biochemical EPS characterization presented in section 3.2.

4. Conclusions

The granular sludge showed morphological stability despite the variability of the EPS composition and granules size variations caused by influent composition changes, which did not lead to loss of WWTP efficiency.

Variations in the EPS production and composition could be due to chemical differences in the influent stream, with EPS polysaccharides concentration lower at higher COD and TSS in the influent. The yield of the extraction was higher for Na-EPS, but the extra acidic precipitation step allowed for a more homogeneous EPS, H-EPS, which could be important for a range of downstream applications of the recovered EPS.

To our knowledge, the work presents the first insight on the dynamics of the recovery of EPS from granular sludge from an urban WWTP, creating the base for obtaining high value bio-based products from the surplus biomass.

CRediT authorship contribution statement

Ana S. Oliveira: Conceptualisation, Formal analysis, Investigation, Writing - original draft. **Catarina L. Amorim:** Conceptualisation, Formal analysis, Supervision, Writing - review & editing. **Miguel A. Ramos:** Formal analysis. **Daniela P. Mesquita:** Formal analysis, Writing - review & editing. **Paulo Inocêncio:** Resources. **Eugénio C. Ferreira:** Formal analysis, Supervision. **Mark van Loosdrecht:** Conceptualisation, Supervision, Writing - review & editing. **Paula M.L. Castro:** Conceptualisation, Formal analysis, Funding acquisition, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors wish to thank the company SIMTEJO for supplying the granules and influent and effluent characterization data. This work was financed by FCT under the project AGeNT - PTDC/BTA-BTA/31264/2017 (POCI-01-0145-FEDER-031264). We would like to thank the scientific collaboration of CBQF under the FCT project UID/Multi/50016/2019 and NORTE-08-5369-FSE-000007 and CEB under the FCT project UID/BIO/044697/2019 and BioTecNorte operation (NORTE-01-0145-FEDER-000004).

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