Density and sludge volume index estimation in mature aerobic granular sludge by quantitative image analysis and chemometric tools

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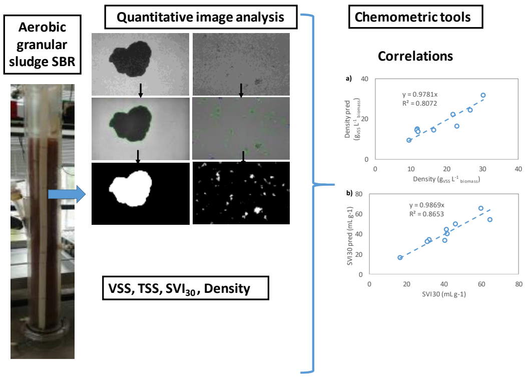

Quantitative image analysis (QIA) has already been applied for activated sludge dysfunctions and anaerobic granular sludge processes elucidation and its application to aerobic granular sludge characterization is considered of major interest. In this work, a sequencing batch reactor (SBR) was operated using mature aerobic granular sludge for pharmacologically active compounds (PAC) removal. The physicochemical parameters (total and volatile suspended solids, sludge volume index and density), prior to the introduction of PAC, were determined by analytical methods. The mature aerobic granular and suspended sludge morphological and structural parameters were established by QIA. The objective of this study is to characterize this mature granular sludge by QIA, and establish relationships for physicochemical parameters (aggregates density and SVI30) with QIA data using chemometric tools. Indeed, relevant relationships could be found for both density (R^2 of 0.81) and SVI_{30} (R^2 of 0.87).

Introduction

Conventional activated sludge (CAS) systems are prone to be affected by dysfunctions (pinpoint flocs formation, filamentous and viscous bulking, foaming, etc.) leading to a decrease in treatment efficiency. However, the traditional methods to monitor these phenomena are usually time-consuming. In this sense, quantitative image analysis (QIA) has already been applied to monitor and predict these abnormalities in CAS [1].
On the other hand, the aerobic granular sludge (AGS) systems are a promising technology to replace CAS due to the smaller footprint and the possibility to remove organic matter and nutrients (N and P) simultaneously. Furthermore, its use for pharmacologically active compounds (PAC) removal, within a sequencing batch reactor (SBR), is gaining attention. However, the granular aggregates can suffer from instability phenomena, which affects the performance of the biological process. Recently, it has been recognized that the stability of the AGS process is dependent on a good balance between the suspended and granular sludge [2]. Again, QIA techniques have been successfully applied to monitor the anaerobic granulation process performance [3]. Therefore, the application of QIA can be a useful tool to characterize the biomass structure and even to predict and diagnose changes in AGS by the presence of certain compounds like PAC. This research presents the first study, to the authors' knowledge, on the application of QIA to the suspended and granular fractions of a mature AGS system directed to estimate its density and sludge volume index (SVI). Due to the large amount of data obtained by QIA, chemometric tools were applied to pursue and validate the obtained correlations.

Objectives

The main objective of this study is to characterize mature aerobic granular sludge in an SBR, prior to the introduction of PAC, by QIA and establish relevant relationships for aggregates density and SVI_{30} using chemometric tools.

Methods

A 5 L SBR was fed with a synthetic medium [4] for 6 hours cycles encompassing 120 min of feeding in plug-flow, 232 min of aeration, 3 min of settling and 5 min of effluent withdrawal, presenting a hydraulic retention time of 12h. The SBR was inoculated with AGS from a municipal WWTP (Portugal). A stabilization period of 66 days was allowed before monitoring took place. Then, the characterization of the mature and stable AGS was performed once per week for a period of two months. Total and volatile suspended solids (TSS and VSS) and SVI at 30 minutes (SVI_{30}) were determined according to standard methods [5], whereas the density was determined with Blue dextran [4]. For the biomass morphological and structural characterization, the suspended and granular sludge fractions were separated with a 500 µm mesh sieve. The suspended biomass images were further acquired with an Olympus BX51 microscope (Olympus, Shinjuku, Japan) with a total magnification of 40x, and for the granular biomass an Olympus SZ 40 stereomicroscope (Olympus, Shinjuku, Japan) was used with a total magnification of 15x. The image processing and analysis programs, for the characterization of the suspended (flocs and filaments) and granular sludge were developed in-house in Matlab 7.3 (The Mathworks, Inc., Natick, USA), adapting a previous version developed by Amaral [6]. The total filaments length (TL) and the flocs size values were obtained by QIA. The flocs were then divided into three size classes in equivalent diameter (Deq): F1 (<25 µm in Deq), F2 (25-250 µm), and F3 (>250 µm). Regarding the granular biomass also three classes were used: G1 (<0.25 mm in Deq); G2 (0.25-2.5 mm); and G3 (>2.5 mm). The performed overlapping allowed accounting for flocculent biomass that could be trapped in the sieve, though presenting a diameter smaller than 500 µm. Finally, a multiple linear regression (MLR) was performed in order to establish relevant relationships concerning the studied parameters: aggregates density and SVI_{30}.

Results

Table 1 presents the main physicochemical parameters determined in the monitored operational period.
During the monitoring period TSS were always above 4.6 g L^{-1}, presenting the largest value in the last monitoring sample. Throughout this entire period, the VSS/TSS ratio presented a value around 0.9. The SVI_{30} values were always below 65 mL g-

[1], indicating a good sludge settleability, with particular emphasis towards the monitoring period end (16.3 mL g^{-1}). Taking into account the granules volume, the obtained density, presented an average value of 18.1 g $_{VSS}$ L^{-1} $_{biomass}$, which can be considered relatively low according to [4] (below 50 g $_{VSS}$ L^{-1} $_{biomass}$), though it raised at the end of period. In addition, the main mature biomass structural parameters were determined by QIA in the monitored operational period (Table 2).

Table 1. Main physicochemical parameters values

Parameter	Min	Avg	Max	STD	Final
TSS (g L^{-1})	4.6	6.3	13.4	2.7	13.4
VSS/TSS	0.87	0.90	0.91	0.01	0.90
SVI_{30} (mL g^{-1})	16.3	41.4	64.6	14.7	16.3
Density (g_{VSS} L^{-1} $_{biomass}$)	9.4	18.1	30.2	7.5	30.2
Gran. vol. %	22.6	41.7	70.5	16.1	40.1

Avg: Average; Final: Value at the end of the experimental period; Max: Maximum; Min: Minimum; STD: standard deviation.

Table 2. Main mature biomass structural parameters values

Parameter	Min	Avg.	Max	STD	Final
F1 area %	0.07	0.81	2.41	0.66	0.07
F2 area %	1.4	8.0	11.3	3.1	1.4
F3 area %	0.71	1.88	3.31	1.06	0.71
G1 area %	0.05	0.11	0.29	0.08	0.29
G2 area %	17.2	25.8	40.3	8.1	24.3
G3 area %	45.7	63.5	75.1	10.4	73.2
Compactness (gran.)	0.74	0.77	0.80	0.02	0.75
Eccentricity (gran.)	0.69	0.77	0.74	0.02	0.74
TL/TSS (m mg^{-1})	0.21	3.21	6.79	2.29	0.21

The overall granules area percentage was, throughout the monitoring period, always above 85% and reaching 97.8% in the end. All, but one, of the samples presented G3 (larger granules) area % above 50%, reaching 73.2% at the end. This configures a highly granular biomass stratification. Such granules were also found to possess good morphological qualities. Given the TL/TSS values always under 7 m mg^{-1} [7], and reaching 0.2 m mg^{-1} at the end, no filamentous bulking occurred. Furthermore, the SVI_{30} values under 65 mL g^{-1} indicate good granules settling properties, whereas the F1 area percentage below 2.5% excluded the existence of a pinpoint flocs problem [1].
Next, a multiple linear regression (MLR) was performed in order to establish the relationships concerning the studied parameters: aggregates density and SVI_{30}, presented in Figure 1. Regarding the density correlation, a 0.81 R^2 value (with root mean square of error, RMSE, value of 2.87 g $_{VSS}$ L^{-1} $_{biomass}$) was obtained for the following relationship with the aggregates area percentages: Density = – 9.77F1 + 3.93F2 + 127.53G1 – 5.55F3 – 0.88G2 + 0.22G3
With respect to the SVI_{30} correlation, a 0.87 R^2 value (with a RMSE value of 4.99 mL g^{-1}) was obtained for the following relationship: SVI_{30} = 0.61Density – 1.62TSS + 6.66(TL/TSS) + 0.05Gran. vol. %
Furthermore, analyzing each parameter weight (factor x absolute value), it could also be determined that the density was more heavily dependent on the F2 and G2 aggregates classes, and the SVI_{30} on the TL/TSS and Gran. vol. %.

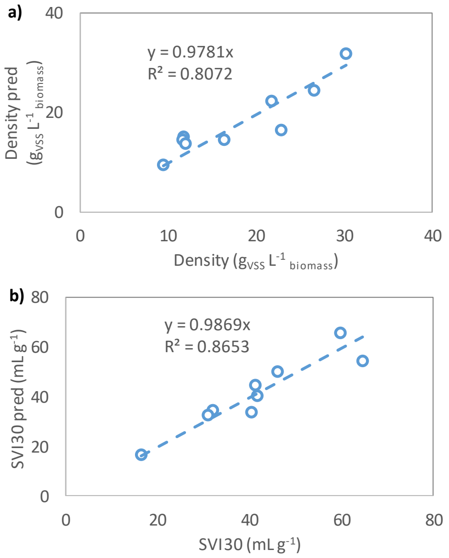

Figure 1. Relationship between the parameters predicted by MLR (pred) and the experimental measurements for: (a) Density and (b) SVI_{30}.

Conclusion

The use of QIA, coupled with chemometric analysis allowed to characterize mature AGS and establish relevant relationships for the aggregates density and SVI_{30} with QIA data using chemometric tools. Thus, it is expected that the use of such techniques and correlations can be useful in further experiments regarding the AGS use for PAC removal, to predict possible changes in the biomass morphology due to the presence of those compounds.

Acknowledgements

The authors thank the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UID/BIO/04469 unit, COMPETE 2020 (POCI-01-0145-FEDER-006684) and BioTecNorte operation (NORTE-01-0145-FEDER-000004) funded by the European Regional Development Fund under the scope of Norte2020 - Programa Operacional Regional do Norte. Cristiano Leal is recipient of a fellowship supported by a doctoral advanced training (call NORTE-69-2015-15) funded by the European Social Fund under the scope of Norte2020 - Programa Operacional Regional do Norte. A. Val del Rio is supported by Xunta de Galicia (ED418B 2017/075) and program Iacobus (2017/2018).

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