EPS and aggregates changes on activated sludge under atrazine exposure

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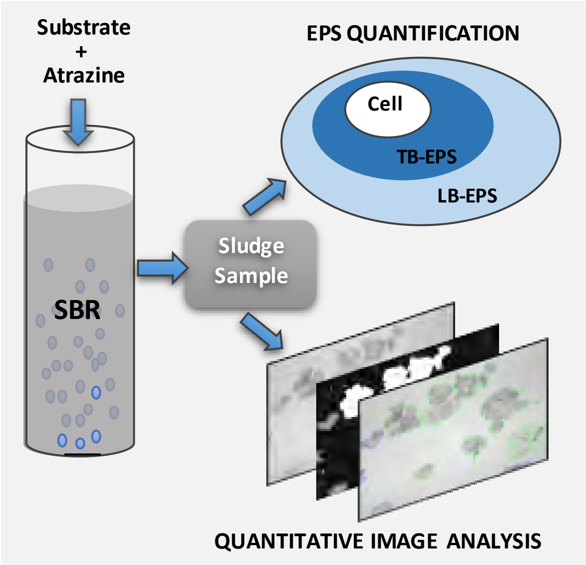

Extracellular polymeric substances (EPS) play a vital role in biological wastewater treatment systems, affecting their performance in aggregates settling, structure and arrangement, and interacting with micropollutants present in wastewater. In this study, the effects of herbicide atrazine (ATZ) on the EPS yield and composition and aggregates structure were investigated on activated sludge (AS) in a sequencing batch reactor (SBR). The results demonstrated that TB-EPS and LB-EPS increased under ATZ exposure, indicating that microorganisms release EPS as a self-mechanism of defense against environmental changes. Above 5.5 mg L^{-1} of ATZ aggregates become larger. Principal component analysis (PCA) was useful in highlighting biomass changes during the experimental phases, and Pearson correlation revealed that TB-EPS content correlate well with large aggregates (0.996).

Introduction

Among pesticides, atrazine (ATZ) is commonly used in agricultural activity worldwide. As a consequence, ATZ can be found in wastewater treatment (WWT) systems and natural aquatic environments. In biological WWT, microorganisms live and grow held together by a slime matrix comprised of extracellular polymeric substances (EPS), forming a three-dimensional microbial structure of aggregates and by chemical binding forces. The EPS are typically composed of organic substances such as polysaccharides (PS), proteins (PN), humic acid substances (HAS), nucleic acids, and lipids. In biological WWT, EPS is important because they play an essential role in aggregates flocculation, settling, and dewatering [1]. Moreover, in the presence of toxic substances, such as pesticides, EPS forms a protective layer for the aggregated biomass against environmental disturbances that might play an important role in the transport and transformation of micropollutants [2]. Also, the increase of EPS concentration under toxic conditions have been reported [2]. Besides that, the content of EPS and its components (PS, PN, HAS), and variation in the PN/PS ratio have been associated with the stability and size of aggregates in WWT. Quantitative Image Analysis (QIA) has proven to be a suitable tool for monitoring biological WWT systems, and for the assessment of aggregates in AS as well [3]. Furthermore, multivariate statistics, such as principal component analysis (PCA), have become important in organizing and extracting relevant information from such comprehensive datasets [4]. In this context, this work presents the effect on EPS production and on aggregates structure of different concentrations of atrazine (ATR).

Methods

A sequencing batch reactor (SBR) consisting of a 2 L working volume was operated at room temperature. SBR was fed with synthetic medium containing ATZ at 2.0 (phase II for 32 days), 5.5 (phase III for 32 days), and 12.0 mg L^{-1} (phase IV for 33 days), and without ATZ (phase I for 34 days). EPS was determined as loosely bound (LB-EPS), tightly bound (TB-EPS), and total EPS according to [5]. Aggregates were evaluated by QIA where an Olympus BX51 microscope (Olympus, Shinjuku, Japan) at 40 × coupled to an Olympus DP72 camera (Olympus, Tokyo, Japan) was used. Images were then processed, and aggregates size was classified considering their equivalent diameter (Deq) as: small (Deq < 25 µm), intermediate (25 < Deq < 250 µm), and large (Deq > 250 µm). The aggregate area percentage (%Area) was also determined for the small ($\%Area_{small}$), intermediate ($\%Area_{intermediate}$), and large aggregates ($\%Area_{large}$). PCA was performed using EPS and QIA aggregates data. More detail about the techniques employed can be found elsewhere [5]. QIA procedures and PCA were conducted using Matlab™ 8.5 (The MathWorks Inc, USA). Pearson correlation was used to relate EPS production and QIA data.

Results

Different concentrations of ATZ had some impact on biomass activity by increasing LB-EPS from 18 to 45, TB-EPS from 34 to 66, and from 53 to 111 mg EPS g $MLVSS^{-1}$ (Table 1). When the aggregates were exposed to the highest ATZ concentrations (5.5. and 12 mg L^{-1}) the aggregates became larger (Figure 1a). Principal components analysis (PCA) was performed and is present in Figure 1b. The explained variance was 41, 15 and 12% for PC1, PC2, and PC3, respectively, performing 68% of the total explained variance. Two well-defined clusters were found corresponding to phase I and phase II. Considering PC1 axis, phase I was placed in the right side and phase II was placed from the middle to the left side. In contrast, for phases III and IV, samples were combined in two close clusters located in the bottom and upper of the left side, demonstrating that ATZ above 5.5 mg L^{-1} did not cause significant changes on biomass properties. These results showed the usefulness of this technique identifying changes in AS properties, showing the transition from predominantly small and intermediate aggregates to large aggregates as well as the increase in EPS content under ATZ exposure. From Pearson correlation (Table 2), it was verified the strong relationship between EPS content (mainly TB-EPS) and the $\%Area_{large}$. Therefore, the present results showed a close relationship between large aggregates and high EPS content. Additionally, the increase of EPS content can be also considered a self-defense mechanism from microorganisms when exposed to toxic compounds.

Table 1. Average values followed by the standard deviation of EPS (mg EPS g $MLVSS^{-1}$).

EPS	Phase	Mean	Standard Deviation
LB-EPS	I	17.71	0.98
	II	49.26	1.10
	III	37.12	1.20
	IV	44.74	1.23
TB-EPS	I	34.12	1.71
	II	46.51	1.13
	III	57.27	1.73
	IV	66.40	1.14
Total EPS	I	51.82	1.97
	II	95.77	1.57
	III	94.39	2.10
	IV	111.14	1.68

Conclusions

Changes of aggregates size and EPS content were studied to assess the impact of ATZ on AS system. The effect was more evident above 5.5 mg L^{-1}. TB-EPS had values greater than LB-EPS during all experimental phases, indicating their interaction with ATZ as a response mechanism of the biomass. PCA accomplished with QIA and EPS data provided insights into the EPS role in the biomass structure changes along operation and allowed to distinguish the experimental phases as well. Pearson correlation revealed that total EPS and TB-EPS were strongly correlated to %Area of aggregates, rather than LB-EPS forms.

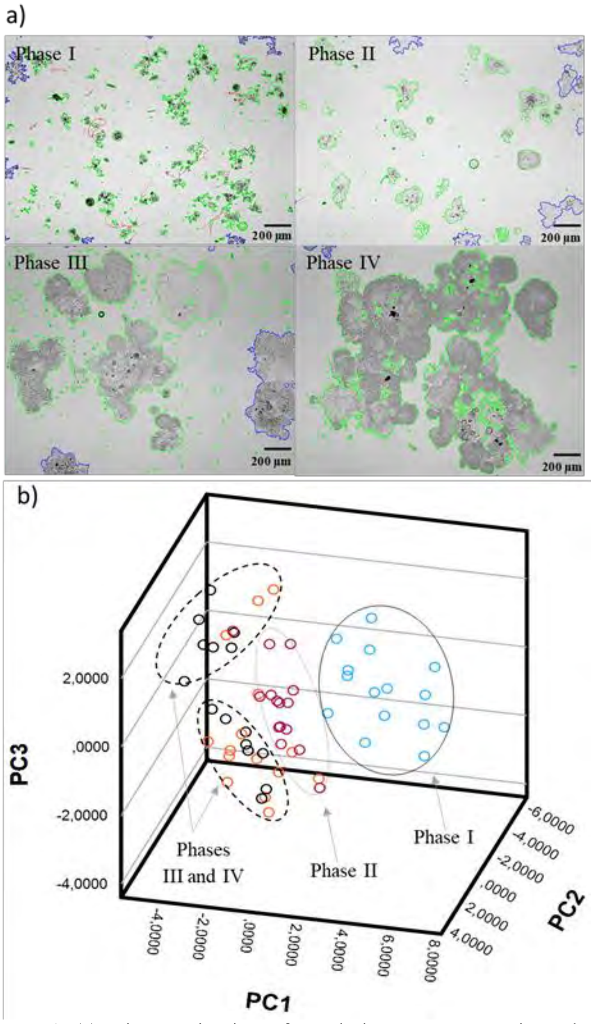

Figure 1. (a) Microscopic view of AS during reactor operation. The image corresponds to the beginning of each phase. (b) PCA scores plot of the SBR operational parameters, EPS and QIA dataset.

Table 2. Pearson correlation coefficients computed between EPS components and %Area of aggregates.

	LB-EPS	TB-EPS	Total EPS	$\%Area_{small}$	$\%Area_{intermediate}$	$\%Area_{large}$
LB-EPS	1.000					
TB-EPS	0.658	1.000				
Total EPS	0.913	0.908	1.000			
$\%Area_{small}$	0.239	-0.494	-0.135	1.000		
$\%Area_{intermediate}$	-0.672	-0.992	-0.912	0.418	1.000	
$\%Area_{large}$	0.630	0.996	0.891	-0.481	-0.998	1.000

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