ANALYSIS OF THE CHARACTERISTICS OF EXTRACELLULAR POLYMERIC SUBSTANCES (EPS) OF ACTIVATED SLUDGE MIXED LIQUOR AND RECIRCULATION STREAM OF DOMESTIC WASTEWATER TREATMENT PLANT OF THESSALONIKI.

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ABSTRACT

Extracellular polymeric substances (EPS) represent a complex mixture of biopolymers with high molecular mass, consisted of proteins, carbohydrates, humic substances, DNA and RNA. Nevertheless, their major constituents in biofilms and activated sludge are proteins and polysaccharides. They are produced by a number of pathways including excretion by microorganisms, lysis, hydrolysis and adsorption of organic matter from wastewater. They are important in biomass aggregation, in settleability in wastewater treatment systems and they are highly responsible for membrane fouling in membrane bioreactors (MBR). Therefore, EPS play a particularly important role in various wastewater treatment systems. Hence, the main objective of this work is the study of the behavior and composition of EPS in a typical activated sludge process. Samples were collected from the aeration tank and the recirculation channel of a municipal wastewater treatment and were analyzed for the determination of proteins' and polysaccharides' concentration using the modified Lowry method and Dubois method, respectively. Specifically, the analysis aimed to the identification of soluble EPS (sEPS) (or soluble microbial products (SMP)) and of extractable EPS with the form of loosely bound EPS (LBEPS) and tightly bound EPS (TBEPS). According to the results, PS are much greater than PN, the tendency of PN/PS ratio is almost the same in both cases (aerated tank and recirculation stream) and the total EPS concentration increased significantly in TBEPS comparing with sEPS.

Keywords: *activated sludge; extracellular polymeric substances (EPS); EPS extraction; proteins; polyssacharides*

1. INTRODUCTION

Microorganisms develop and live in aggregated forms, the so-called biofIlms. The common characteristic of these forms is that microorganisms are embedded in a matrix of extracellular polymeric substances (EPS). In natural environments the production of EPS is a typical property of microorganisms. BiofIlms can be detected everywhere, such as in natural soil, in aquatic environments and in technical systems like filters and other porous materials, as well [1]. BiofIlms usually adhere at interfaces like solid-water, water-oil, water-air and solid-air interfaces. Biofilms are consisted in microorganisms, EPS, multivalent cations, biogenic and inorganic particles, as well as colloidal and dissolved compounds [2]. Between all these compounds, EPS are mainly responsible for the structural and functional unity of biofilms and they determine the physicochemical and biological properties of biofilms. In particular, EPS form a three-dimensional, gel-like, highly hydrated and often charged biofilm

matrix, in which the microorganisms are embedded and immobilized [2]. The ratio of EPS in biofilms varies between 50 and 90% of the total organic matter [3]. According to Nielsen and Jahn [4], EPS are composed of proteins, carbohydrates, humic substances, DNA and RNA. Nevertheless, the major component of EPS in biofilms and activated sludge are proteins [3].

EPS are a matrix of large polymeric molecules. EPS can be classified into soluble EPS and bound EPS. Bound EPS consist of a dynamic double-layered structure, which is divided into loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS) [5]. This classification depends on the bounding strength between EPS and cells. According to Laspidou and Rittmann [6], soluble EPS and soluble microbial products (SMP) are the same. SMP are soluble organic compounds which are released into solution during cell lysis (biomass decay), are excreted during substrate metabolism (biomass growth) and are lost during synthesis (SMP degradation) [7]. SMP which are connected by metal ions or other inorganic/organic substances could form colloidal microbial products (CMP) while CMP can also be hydrolyzed to SMP [7]. According to a recent study of Wang and Waite [8], the loosely term of “soluble” microbial products could also include colloidal microbial products. Therefore they proposed the united term of soluble and colloidal microbial products (SCMP). The relationships between CMP, SMP (or SCMP), LB-EPS and TB-EPS are illustrated in Fig. 1.

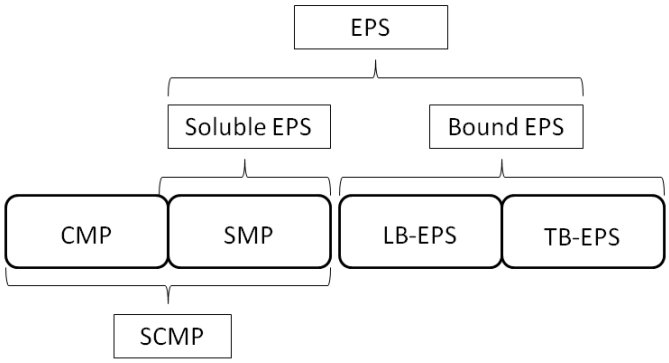

Figure 1. The relationships between CMP, SMP (or SCMP), LB-EPS and TB-EPS.

2. IMPORTANCE OF EPS IN WASTEWATER TREATMENT

Activated sludge (AS) process is the most common biological process in wastewater treatment. During sludge flocculation, microbial cells are transformed into aggregates and therefore biomass and water are separated efficiently. This process is critical to the overall treatment efficiency performance. The major components of the AS matrix are microbial extracellular polymeric substances (EPS) [9]. EPS create links between the cells and they form sludge flocs. Therefore, the role of EPS in flocculation and sludge settleability is crucial and lots of research has been carried out on this topic until today [10, 11]. Nevertheless, many of the results are conflicting. For instance, Urbain et al. [12] showed that EPS content is correlated positively with bioflocculation. On the other hand, Liao et al. [13] showed that they are negatively correlated. Furthermore, some researchers have pointed that the quantity of EPS is not of such importance in sludge flocculation as are their composition and properties [13]. Consequently, more research has to be carried out about the role of EPS in AS process.

According to Poxon and Darby [14], bacteria in the AS suspension and floc matrix have a dynamic double-layered EPS structure consisted of loosely bound EPS (LB-EPS) diffused from the tightly bound EPS (TB-EPS), which surrounds the cells. The LB-EPS in the flocs may operate as the primary surface for cell attachment and flocculation [5]. Most of the research has been conducted for the total EPS and not for LB-EPS and TB-EPS separately. This is due to the applied EPS extraction method. This method consisted of thorough washing followed by harsh extraction [15] and the EPS that were measured by this method was actually the total EPS. According to Li and Yang [5], EPS are necessary in sludge floc

formation, but excessive EPS in the form of LB-EPS may deteriorate cell attachment and weaken the floc structure, resulting in poor sludge–water separation.

3. EVALUATION AND SELECTION OF EPS EXTRACTION METHOD

Today, a large number of EPS extraction methods has been presented in the literature; however it should be pointed out that different methods have different extraction efficiencies, resulting in various compositions in the EPS [16, 17, 18]. Therefore, the evaluation and selection of the appropriate extraction method of EPS according to the sample or the modification of an existing method is a crucial step for the further analysis of EPS. The optimal EPS extraction method should be effective, causing minimal cell lysis and not disintegrating the EPS structure [9]. According to Nielsen and Jahn [4], the extraction efficiency for a given sample can be defined as the total amount of EPS extracted from the total organic matter or the total amount of EPS extracted from the total EPS pool. As mentioned, cell lysis may occur during EPS extraction but the extent of cell lysis is difficult to be evaluated. Furthermore, in certain EPS extraction methods, such as boiling and alkaline treatments, the disruption of macromolecules may take place [19].

For the extraction of soluble EPS, centrifugation is always used [4]. For the extraction of bound EPS, the methods which are used are classified into physical methods, chemical methods and a combination of physical and chemical methods [4]. The physical extraction methods utilize the external forces, which can be caused by ultrasonic, centrifugation or heating, to detach the EPS from cells and dissolve them in solution. In the chemical extraction methods, chemical compounds, such as cation exchange resin (CER) [9], EDTA [17] or HCHO/NaOH [16], are added in order to disrupt the bonds between the EPS and the cells and dissolve the EPS in solution. Chemical extraction methods are more efficient in comparison with the physical extraction methods; however they cause specific problems in the extraction process itself or in the subsequent EPS analysis, such as cell lysis, disruption of macromolecules, contamination of the EPS extraction or others.

In order to study the composition of the LB-EPS and TB-EPS, the bound EPS can be extracted in two phases [19]. A mild method can be used for the LB-EPS extraction, like high-rate shear, heating at low temperatures or high speed centrifugation. Thereafter, a harsh method should be applied for the TB-EPS extraction, such as heating at high temperatures, sonication or chemical extraction methods.

4. ANALYTICAL METHODS FOR QUANTIFICATION OF PROTEINS AND POLYSACCHARIDES

According to Raunkjær et al. [20], for the quantification of the various components of EPS, conventional chemical colorimetric analyses should be used.

The proteins are analysed by the Lowry method, the Bradford method or the total N-content method. The Lowry method has a higher recovery of proteins than the Bradford method [9] and it is frequently applied for protein analyses in EPS characterization. As the phenolic functional groups of humic acids react with the Lowry reagent, the appropriate correction is always needed [21]. Bovine serum albumin (BSA) can be used as standard.

The polysaccharides content, with the form of carbohydrates, should be analysed using the phenol–sulfuric acid method [21, 22] and the Anthrone method [9, 23]. The phenol–sulfuric acid method is sensitive and the color is stable. A comparison between the two methods for carbohydrates determination in EPS showed that the two methods yielded similar results [9]. Glucose can be used as standard.

Standard Addition Method is used in most cases to study eventual interferences between the sample and the analytical method [21].

The aim of this work is the selection of the appropriate method for EPS extraction and measurement of proteins and polysaccharides, in order to analyse the characteristics of EPS in a conventional activated sludge process.

5. MATERIALS AND METHODS

Samples were collected from the aeration tank and the recirculation stream of a full scale municipal wastewater treatment plant. The EPS were extracted via a thermal extraction method, according to Hwang et al. [24], with separation of LBEPS and TBEPS. Proteins were analysed with modified Lowry method [9, 21] and polysaccharides in the form of carbohydrates were analysed with phenol-sulfuric acid method [21, 22]. Standard addition method was used for both proteins and carbohydrates.

6. RESULTS AND DISCUSSION

Table 1 shows the average concentration of polysaccharides and proteins for the sEPS and the extracted samples from the aerated tank and the recirculation stream. Figures 2 and 3 illustrate the weight ratio (%) of polysaccharides and proteins (columns) for sEPS, LB-EPS and TB-EPS and the total concentration of EPS (line) of activated sludge mixed liquor of the aerated tank and of the recirculation stream, respectively.

Table 1. Average concentrations of polysaccharides and proteins for the sEPS and the extracted samples.

	Aerated tank		Recirculation	
Samples	Polysaccharides (mg gluc./g VSS)	Proteins (mg BSA/g VSS)	Polysaccharides (mg gluc./g VSS)	Proteins (mg BSA/g VSS)
sEPS	1.96±1.26	7.18±3.41	0.29±0.34	6.06±4.26
LBEPS	2.85±3.27	9.28±3.81	0.06±0.08	0.50±0.71
TBEPS	19.69±10.97	60.11±7.92	12.42±1.27	66.78±3.63

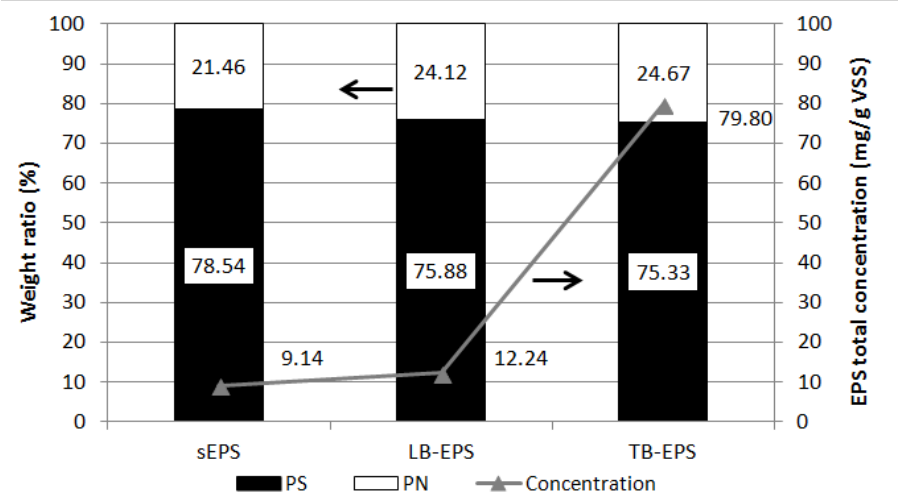

Figure 2. PS and PN of sEPS, LB-EPS and TB-EPS in activated sludge mixed liquor of aerated tank; the columns represent the weight ratio of PS and PN; the line represent the total concentration of PS and PN.

According to the results showed in Table 1 and Figures 2 and 3, it can be concluded that in all cases PS fraction is higher than PN fraction and this is especially evident in sEPS. Moreover, PN and PS concentrations increased after the extraction in a greater extent, i.e. in TB-EPS, comparing with SMP, and PN/PS ratios increased, as well. These facts prove the effectiveness of EPS extraction for the TB-EPS fraction. This is demonstrated, also, by the fact that TB-EPS concentration was higher than 20mg/g VSS, which is near to the lower boundary of the wide ranges that gives the literature for e-EPS of aerobic sludge. Furthermore, differences found between PN and PS concentrations from the aerated tank and the recirculation stream and it probably happens due to the greater value of VSS in the

recirculation stream (2.93g VSS/L for the aerated tank and 4.83g VSS/L for the recirculation stream). Similar results have been presented by other works [21, 24].

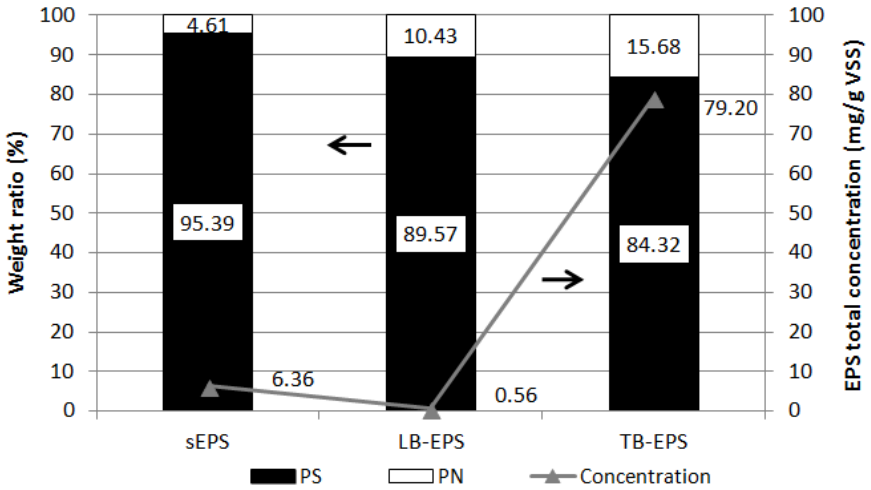

Figure 3. PS and PN of sEPS, LB-EPS and TB-EPS in liquor of recirculation; the columns represent the weight ratio of PS and PN; the line represent the total concentration of PS and PN.

7. CONCLUSIONS

In this project, the characteristics of EPS of activated sludge mixed liquor and liquor of the recirculation stream were analysed. For the needs of this work two samplings were carried out from domestic wastewater treatment plant of Thessaloniki. The EPS were extracted and proteins and polysaccharides were measured for the samples. Finally, it was resulted PN/PS ratios for aerated tank and recirculation stream, which appeared a similar decreasing tendency. The PN of the recirculation stream were lower than them of the aerated tank. Furthermore, the PS in all cases were greater than the PN, especially in sEPS and the total EPS concentration increased significantly after the extraction procedure.

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