Article

Effects of Waste Activated Sludge Extracellular Polymeric Substances (EPS) Composition on Biosorption for Primary Carbon Diversion

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Abstract: Extracellular polymeric substances make up a significant fraction of the organic matter in waste activated sludge, strongly influence the sludge's physicochemical properties, as well as serve biological functions. Biosorption is a physicochemical process in which biomass, such as waste activated sludge, acts as a sorbent which uptakes external nutrients and compounds from a sorbate such as raw wastewater into its cell matrix. This paper characterizes the composition of waste activated sludge extracellular polymeric substances from various treatment processes and analyzes their ability to conduct biosorption when mixed with raw wastewater. Biosorption effectiveness was characterized as the ability to remove the soluble fraction of the organic matter from the wastewater. Due to different waste activated sludge bioaggregate structures, the amount of total organics available in the sludge could not be used as an indicating factor for how effective the sludge was at conducting biosorption. However, a positive correlation was found between the amount of extracellular polymeric substances present in the organic fraction of the sludges and the normalized removal of soluble organic matter. The extracellular polymeric substances, regardless of its chemical composition, removed nearly the same amount of soluble and truly soluble organics removing 1.43±0.15 (n=16) mg of soluble chemical oxygen demand, and 1.20±0.18 (n=16) mg of truly soluble chemical oxygen demand, per mg of cation exchange resin extracted extracellular polymeric substances. Additionally, it was observed that utilizing multiple extraction methods in series could increase extracellular polymeric substances extraction yields and provide a more holistic insight into its composition as well as its biosorption properties.

Keywords: biosorption; extracellular polymeric substances; cation exchange resin; sulfide extraction; base extraction; high-rate biological contactor, primary wastewater treatment

1. Introduction

Waste activated sludges (WAS) are bioaggregates of cells as well as organic matter exteriors known as extracellular polymeric substances (EPS). EPS production is dependent on microbial growth phases and conditions though the mechanisms are not well understood. The composition of EPS consists of a combination of secretions, cell lysis products, hydrolyzed macromolecules, and sorbed organics and compounds. EPS plays a vital role in the physicochemical properties of the sludge including their structure, surface charge, flocculation, settling and dewatering properties, and adsorption ability [1]. EPS also serves as means for biological functions such as cell-to-cell recognition and communication, though the physicochemical properties are more pertinent for this study—particularly adsorption ability.

The literature suggests that a large fraction of the organic matter contained in WAS consists of EPS, but due to the dynamic nature of sludges and the variance between sludges from different treatment processes, there is no consensus about what exactly constitutes EPS. EPS are often categorized as loosely-bound EPS, tightly-bound EPS, and soluble EPS, also referred to as soluble microbial products (SMP). Physicochemical properties of the sludge are typically attributed to the bound portion of the EPS though some studies suggest that the SMP also may play a role [1]. The

SMP and bound EPS can be separated by centrifugation, but the extraction of bound EPS requires physical or chemical treatment in order to be released from the cell matrix before centrifugation [2].

Biosorption is a physicochemical process where WAS acts as a sorbent which uptakes external nutrients and compounds from a sorbate—such as raw, untreated wastewater (WW)—into its cell matrix. In this process, particulate and soluble organic matter contained in the WW adsorbs onto the WAS flocs. Conventional wastewater primary treatments such as screens and gravity sedimentation are effective at removing the particulate organic fraction from wastewater however, they do not effectively remove the soluble fraction. The high-rate biological contactor (HRBC) is a primary treatment process that removes particulate organic matter and a portion of the soluble organic matter by means of biosorption. This process operates in short hydraulic retention time (HRT) and low dissolved oxygen (DO) concentration in promoting more carbon diversion to the anaerobic digesters for methane gas production as well as reducing aeration requirements in secondary treatment processes. High biomethane potential (BMP) values of the float in a pilot test using a trickling filter/solids contact (TF/SC)'s WAS as a biosorbent revealed that oxidation of the sorbed organic matter was at a minimum [3].

EPS contained in the WAS has been shown to have an influence on the biosorption process in past studies and in the crucial role that they play in the biosorption of heavy metals [4]. Additionally, while some biological activity occurs in the raw wastewater, it has been shown that properties of the sludge have a larger influence on the biosorption than the properties of the influent wastewater [3]. Though it is suspected that higher EPS concentrations within the WAS correlates to more biosorption, the study seeks to identify, if any, key compositional fractions of the EPS responsible for biosorption. To evaluate the objective of this study the EPS from different structural sources and treatment processes were extracted and related to the ability of the WAS to remove soluble organic matter from WW in a simulated HRBC process.

2. Materials and Methods

2.1 Procedure for Biosorption

WW and WAS were collected and transported at ambient conditions and were tested within two hours of collection. Samples were taken at three different wastewater treatment plants (WWTPs) on the island of O'ahu with varying treatment methods and design capacities. Honouliuli WWTP in Ewa Beach uses a trickling filter and solid contact (TF/SC) process with a design capacity of 38 million gallons per day (MGD). Wahiawa WWTP utilizes a membrane bioreactor (MBR) and is designed to treat up to 2.49 MGD. Waimanalo WWTP is a conventional activated sludge (CAS) plant with a design capacity 0.6 MGD.

A primary treatment HRBC biosorption contact tank was simulated by using a bench scale setup. WW and WAS were mixed at a 5% WAS by volume mixing ratio in a five-liter contact tank while being constantly stirred and aerated to 1 mg/L dissolved oxygen for a 30-minute contact time.

To simulate the solid-liquid separation of the treated effluent of the biosorption contactor, a bench scale dissolved air floatation (DAF) system was utilized. Tap water was pressurized to 60 psi in a vessel which was vigorously shaken. 150 ml of pressurized tap water was added to 850 ml of biosorption effluent in a one-liter graduated cylinder in which a float formed separately from the subnatant. After a 3-minute contact time, the subnatant was extracted. DAF processes use pressurized DAF subnatant instead of tap water, thus the DAF separation was conducted a second time using pressurized extracted subnatant instead of tap water. This secondary subnatant was used for analysis of biosorption.

WW, WAS, and DAF effluent were analyzed to determine the effects of biosorption. Total chemical oxygen demand (COD), soluble COD (sCOD), filtered and flocculated COD (ffCOD), colloidal COD (cCOD), total suspended solids (TSS), and volatile suspended solids (VSS) were measured based on Standard Methods [5] and according to previous studies [6]. Normalized removals of the various types of COD were determined by dividing the difference between WW COD and DAF effluent COD by the mass of VSS from the WAS added to the biosorption contactor such

that removal was expressed in mg COD removed per g of WAS VSS. The normalized values of interest for this study were the soluble and truly soluble COD fractions expressed as mg sCOD removed per g VSS, and mg ffCOD removed per g VSS, respectively.

2.2 Procedure for Extracellular Polymeric Substances Extraction and Characterization

The WAS used for biosorption was analyzed in parallel for its EPS properties so that the removals observed could be compared to the EPS in the WAS. In order to extract EPS from WAS, three extraction methods were employed. The primary extraction method used was the cation exchange resin (CER) method [7] which was run for 24 hours. CER was predominantly used due to its ubiquity in literature and its relatively higher yields. Since the CER method targets calcium and magnesium bound EPS, it does not extract all of the EPS bound in the cell matrix. The base extraction method and sulfide extraction method described by Park et. al. [8] were also utilized to gain further insight into WAS EPS as they target other cations within the sludge flocs. CER, base, and sulfide extractions were conducted in parallel and in series for analysis. Sludge pellets were rinsed and resuspended to original volumes with deionized water between extractions when conducted in series.

The extracted EPS were characterized and measured using colorimetric methods according to wastewater literature [9] and specific EPS literature [10] for concentrations of proteins [11], carbohydrates [12], humic acids [7], and uronic acids [13].

In order to produce EPS values that could be compared, measured weights of centrifuged WAS pellets from known volumes of WAS were added to the set volume of extraction solutions such that EPS could be expressed in mg EPS per L of WAS and even further as mg EPS per g WAS VSS. These normalized EPS values were used to compare with the normalized biosorption values in order to determine the relationship between EPS and soluble organic removal.

3. Results and Discussion

3.1. Extracellular Polymeric Substances Extraction and Characterization

The three WWTPs studied in this research use different types of biological processes for treatment and thus generate different types of sludge. The EPS compositions of the WAS reflected that. Table 1 shows the chemical composition of the WAS from the various plants using the CER extraction method. 25-30% of the organic fraction of the WAS was extracted as EPS from the TF/SC plant, 28-35% for the CAS plant, and 14-15% for the MBR. The MBR had a significantly smaller EPS fraction likely due to the nature of the sludge's growth conditions. TF/SC systems require the sludge to develop as a biofilm, and CAS systems require sludge that has good settling properties. Both biofilm development and settleability are processes that are heavily associated with EPS composition. In an MBR, there is no biological need for these properties and is likely why a lower EPS fraction is observed. The largest fraction of the TF/SC sludge was humic acids which could likely be characterized by the fact that the wasted sludge produced by biofilm systems are humus sludges. Proteins were the major fraction of the other two sludges though humic acids also had a strong presence. A large humic portion is not atypical. Studies done by Wilen et. al. [14] and Frolund et. al. [15] characterize large portions of the EPS as humic acids—both characterizing it as either the second largest, or sometimes the largest fraction, of EPS. It is important to recognize that when analyzing the properties of EPS, it is only in regard to the fraction that was extracted. It was likely that only a fraction of the total amount of EPS was extracted due to the nature of the CER extraction, thus other extraction methods in parallel and in series were looked into.

Table 1. EPS composition of WAS using CER extraction (Honouliuli; n=6, Waimanalo; n=5, Wahiawa; n=5)

			mg/gVSS			
WWTP	Process	EPS/VSS (%)	Carbohydrate	Protein	Humic Acid	Uronic Acid
Honouliuli	TF/SC	28±1.7	32±2.0	89±11.2	151±11.7	4±0.7
Waimanalo	CAS	30±2.9	46±5.6	124±12.3	128±27.2	5±0.8
Wahiawa	MBR	14±0.4	27±1.5	57±1.3	53±2.0	6±0.2

As standalone extraction methods, CER yielded the most amount of EPS, then the base, followed by the sulfide method. These findings align with the literature [8]. When comparing methods in series, CER values in the base-to-sulfide-to-CER and sulfide-to-base-to-CER extractions were significantly lower than standalone CER values whereas base and sulfide values were comparable to their standalone values when CER was conducted first. Two series, CER-to-base-to-sulfide and CER-to-sulfide-to-base were tested in duplicate to validate which series of methods would yield the most EPS. Figure 1 shows the results of maximizing the amount of EPS extracted from WAS using these methods. The CER extraction needed to be conducted first followed by the base method then the sulfide method. About 20% more EPS was extracted from the CER-to-base-to-sulfide series compared to the other one. It is not clear why this phenomenon was observed. It is a possibility that conducting certain extractions first could alter the structure of the flocs in a way that either increases or decreases sequential yields though more research is needed to verify this.

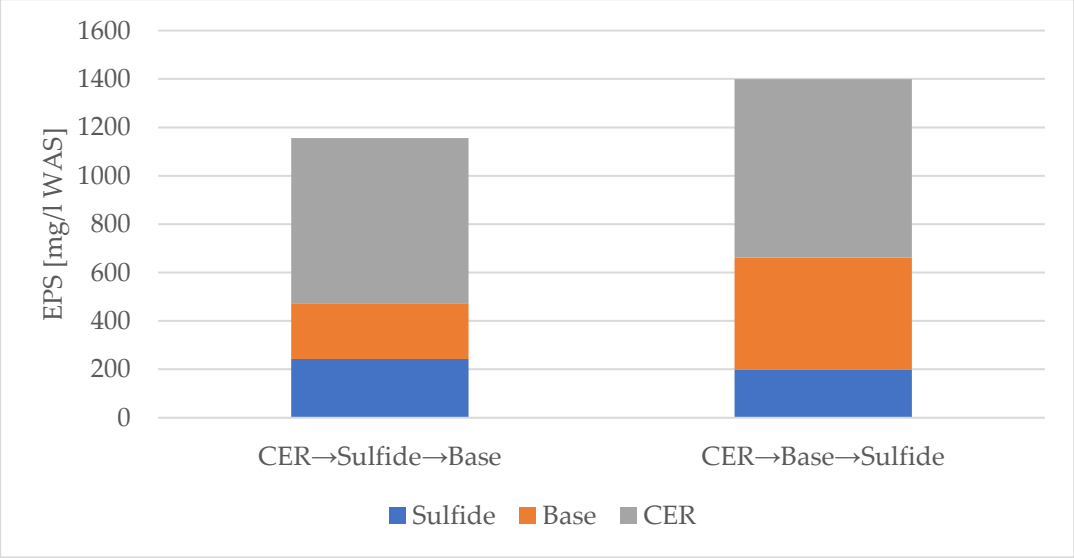


Figure 1. Average EPS extracted by respective series (n=2)

The TF/SC and MBR plants were further analyzed using the CER-to-base-to-sulfide series extraction in order to analyze changes in EPS yields. The sum of the three extraction methods was defined as the total amount of extracted EPS. Table 2 shows that there was a significant increase in EPS extracted beyond what the CER alone could extract though the CER was the major fraction of the total amount. The fraction of organics as EPS nearly doubled for each plant. Figure 2 shows the chemical compositions breakdown of the extracted EPS from each extraction method at each plant and also compares it to the breakdown of the total EPS extracted. Minimal compositional differences between the CER and total EPS distributions were observed when comparing them though this is likely attributed to the CER constituting more than half of the total EPS extracted. Park et. al. suggested that lectin-like proteins linked to polysaccharides were bridged by calcium and magnesium, targeted by the CER, organic materials were linked with aluminum, which can be solubilized at high pH with the base extraction, and biopolymers were bound to iron, which could be removed from the flocs by formation of FeS in the sulfide extraction [8]. The differing composition

of the sludges using the same extraction method implies that the materials bound in the specific ways stated are not compositionally similar and are likely unique to the sludge.

Table 2. Extraction yields from the varying methods ran in series (Honouliuli; n=5, Wahiawa; n=5)

			mg/gVSS			
WWTP	Process	EPS/VSS (%)	Total	CER	Base	Sulfide
Honouliuli	TF/SC	51±2.7	507±26.5	261±24.7	169±6.8	78±9.8
Wahiawa	MBR	26±1.0	256±10.0	143±4.0	67±6.5	46±3.3

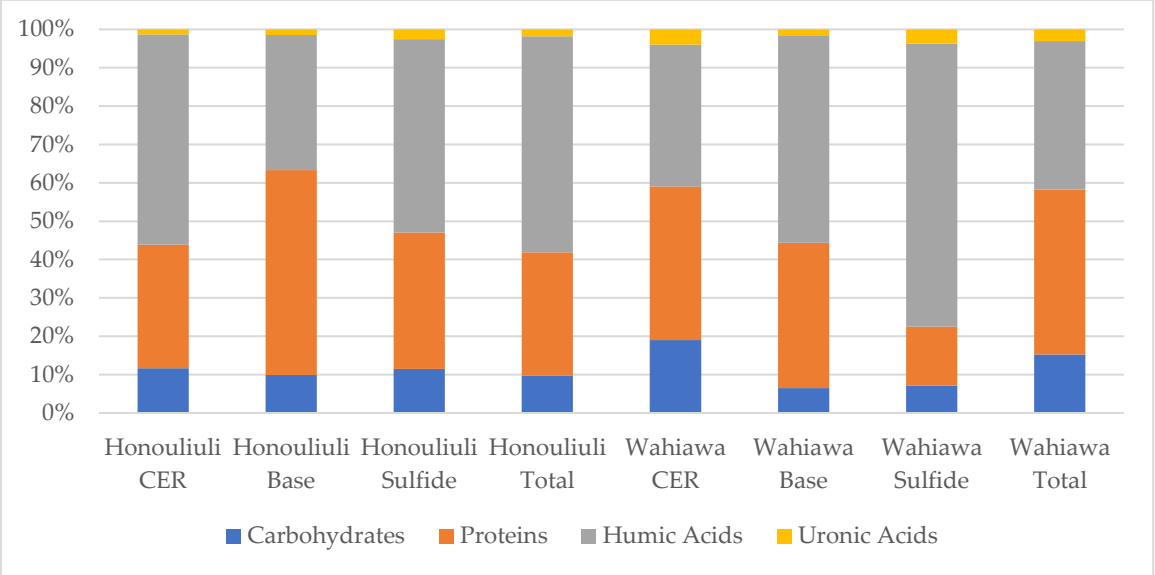
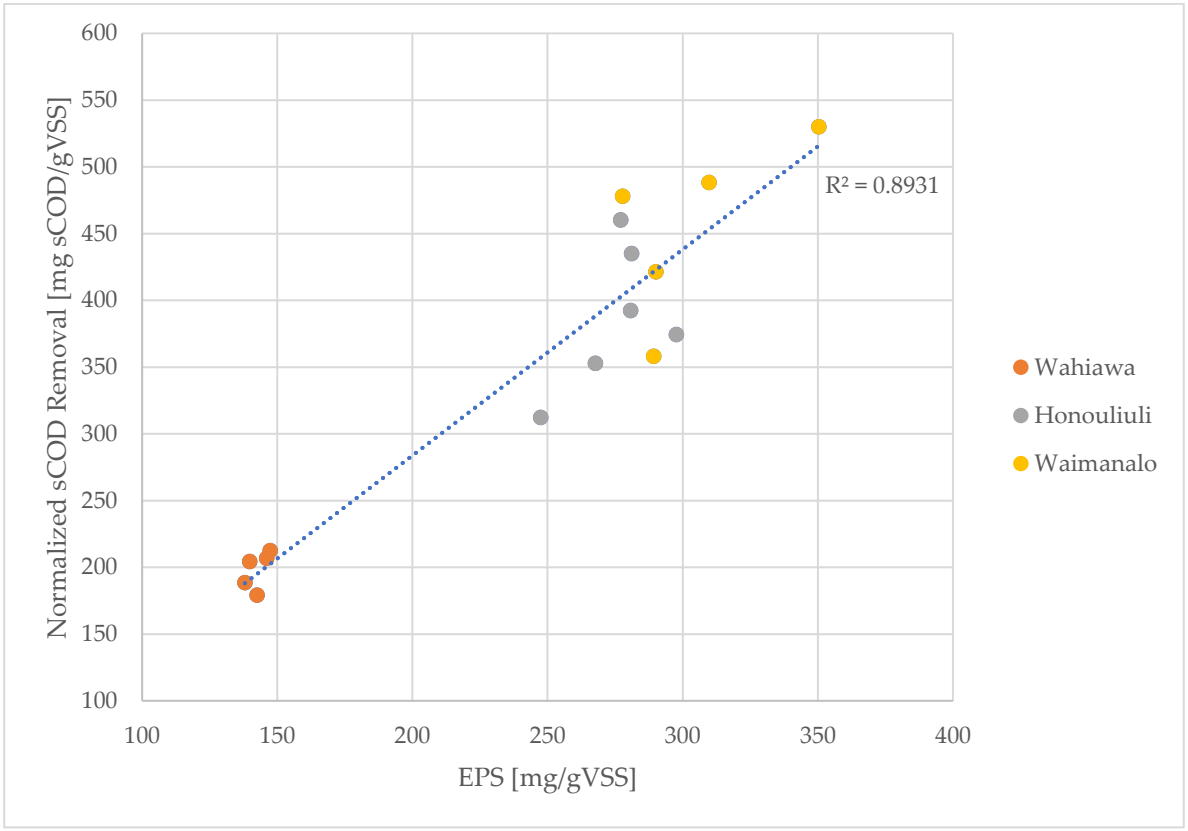


Figure 2. Chemical composition of each extraction at the two studied plants expressed as percent of respective extracted amount (n=5 for all tests)

3.2 Biosorption Correlation

Using the extraction data from the previous section with the results of the biosorption testing, relationships between the ability of the WAS to conduct biosorption and its respective EPS characteristics could be observed. For the first comparison, normalized soluble and truly soluble organic removals were compared with the normalized amount of total EPS generated via the CER method across the three WWTP and can be seen in Figure 3 and Figure 4, respectively. In terms of both removals and EPS extracted, Wahiawa, the MBR, exhibited a very tight spread of data. Individually, each plant exhibited a loose trend that more EPS suggests more removal. When looked at as one data set rather than analyzing them by plants a stronger relationship could be developed for the trend suggesting that more EPS available in the organic fraction of the WAS leads to better biosorption properties.



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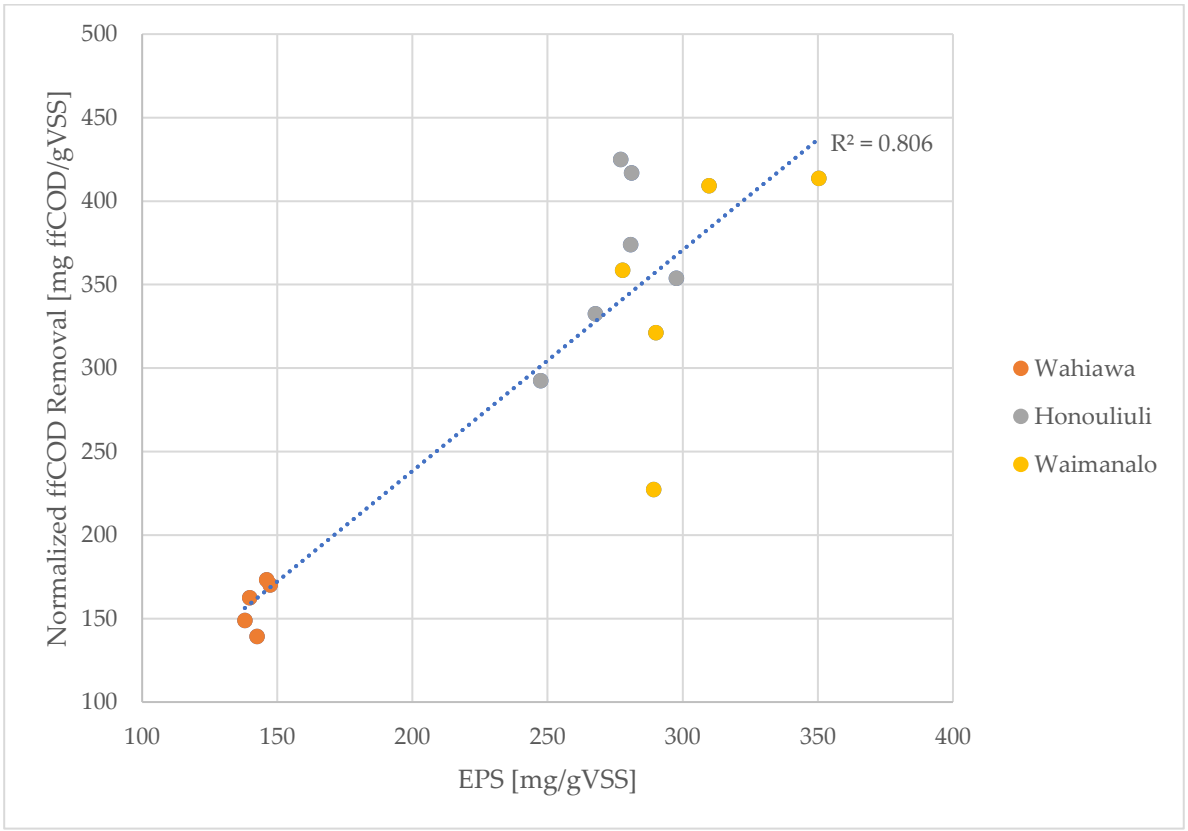
186 **Figure 3.** Normalized soluble organics removal correlation with the amount of CER extracted EPS present
187 in the organic fraction of WAS from the three studied WWTP



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189 **Figure 4.** Normalized truly soluble organics removal correlation with the amount of CER extracted EPS
190 present in the organic fraction of WAS from the three studied WWTP

Regardless of the treatment process the WAS originated from, the EPS fraction within them all exhibited similar removal capabilities. Table 3 shows quantified values of removal in relation to the amount of EPS present in the organics of the WAS. Since there was a large difference in total organics in the different WAS samples (7,500-8,000 mg/l VSS for the MBR and ~3,000 mg/l for the other two plants) having similar removals when normalized in this way highly suggests that the EPS is the fraction of the organics responsible for biosorption and that the total amount of organics present is not a good indicator of the biosorption capability of the sludge.

Table 3. Amount of soluble and truly soluble organic matter removed during biosorption per amount of EPS extracted via the CER method (Honouliuli; n=6, Waimanalo; n=5, Wahiawa; n=5)

		mg Removed/mg CER EPS	
WWTP	Process	sCOD	ffCOD
Honouliuli	TF/SC	1.41±0.16	1.33±0.15
Waimanalo	CAS	1.50±0.18	1.14±0.21
Wahiawa	MBR	1.39±0.08	1.11±0.08
Total		1.43±0.15	1.20±0.18

Further analysis to see if any of the individual chemical constituents of the total EPS played more of a role in the biosorption process was also conducted. Figure 5 and 6 shows the individual chemical constituents of the EPS as well as the total EPS in relation to the normalized removals. Minimal correlation between uronic acids and removal was observed though the other analyzed fractions had stronger, yet still varying, relations. It is likely that the chemical composition of the EPS is not the driving factor behind the ability of a sludge to conduct biosorption.

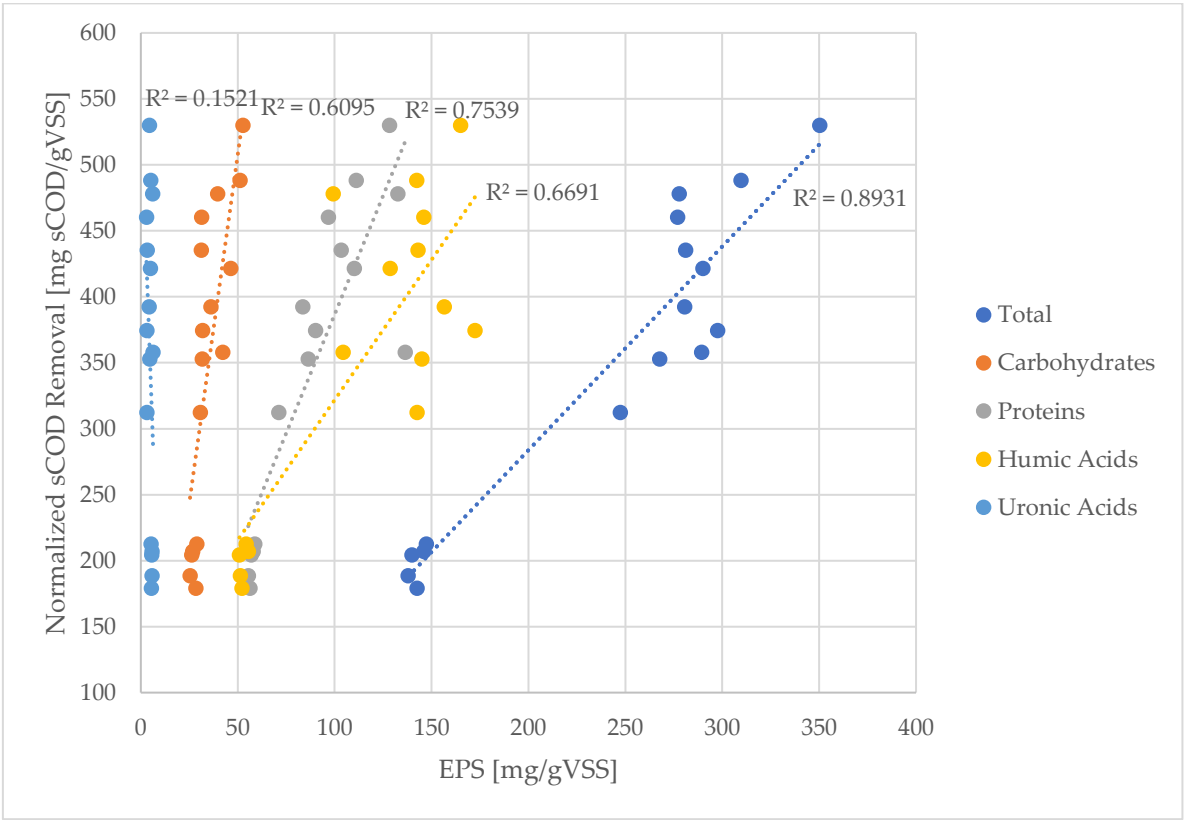

Figure 5. Normalized soluble organics removal correlation with the chemical constituent fractions of CER extracted EPS present in the organic fraction of WAS from the three studied WWTP

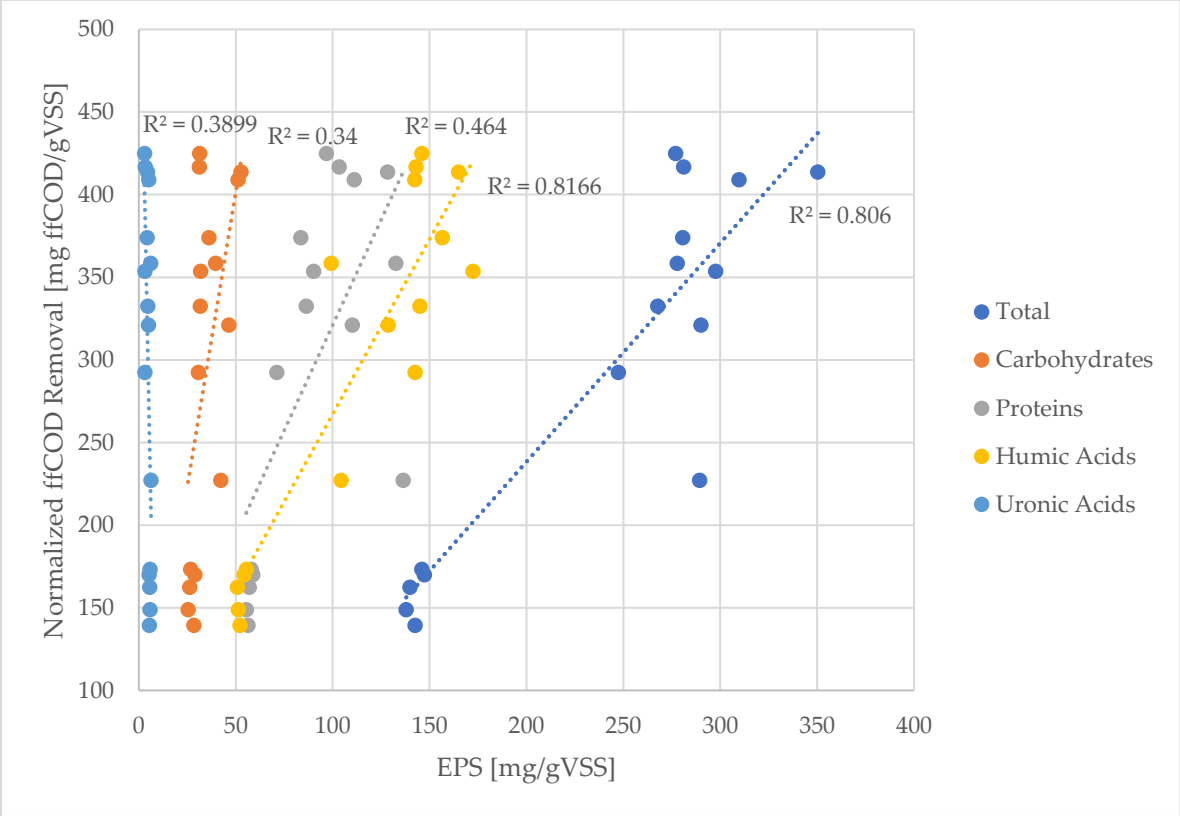

Figure 6. Normalized truly soluble organics removal correlation with the chemical constituent fractions of CER extracted EPS present in the organic fraction of WAS from the three studied WWTP

There was the potential that the trend of larger EPS fractions indicating higher removals only existed when looking at the CER extracted EPS thus, the series extractions that were conducted on the two plants were compared to their respective biosorption removals in order to see if the trends persisted with more extracted EPS available and with respect to the other extraction methods. Figure 7 and Figure 8 show these comparisons. The series extractions allowed for higher identification percentages of organics as EPS and was closer to the true fraction of EPS in the WAS than the standalone CER extraction. As seen in the figures, with higher amounts of extracted EPS, the trend persisted nearly equally or better than the CER relationship and all of the other extractions. This data suggests that EPS from one type of extraction is not necessarily more related to biosorption properties than the others but rather that all EPS from any of the studied sources had a strong correlation with biosorption capabilities. Moreover, having a larger fraction of the organics identified as EPS and exhibiting good correlation with biosorption properties further emphasizes the trend that biosorption is related to the amount of organics as EPS regardless of its origin within the cell matrix or chemical composition.

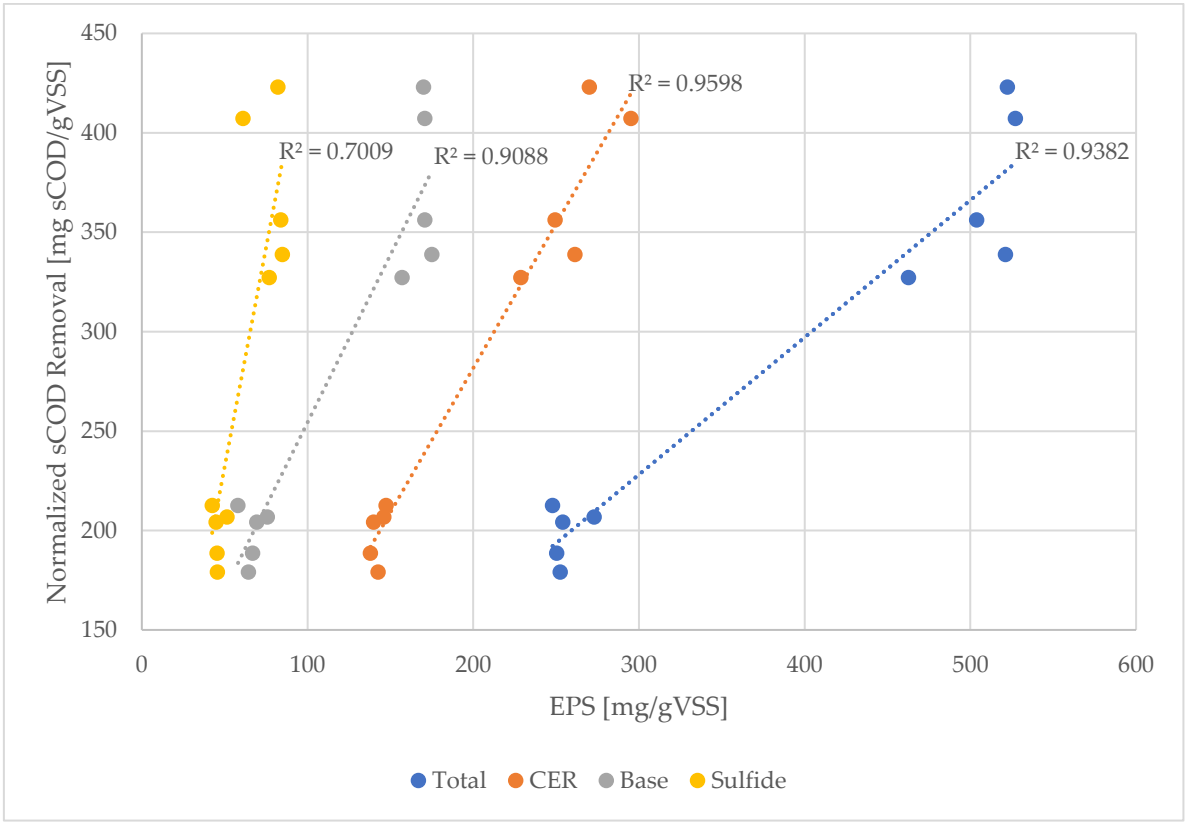

Figure 7. Normalized soluble organics removal correlation with extracted EPS from the three extractions as well as the total extracted amount from the two WWTP studied in series

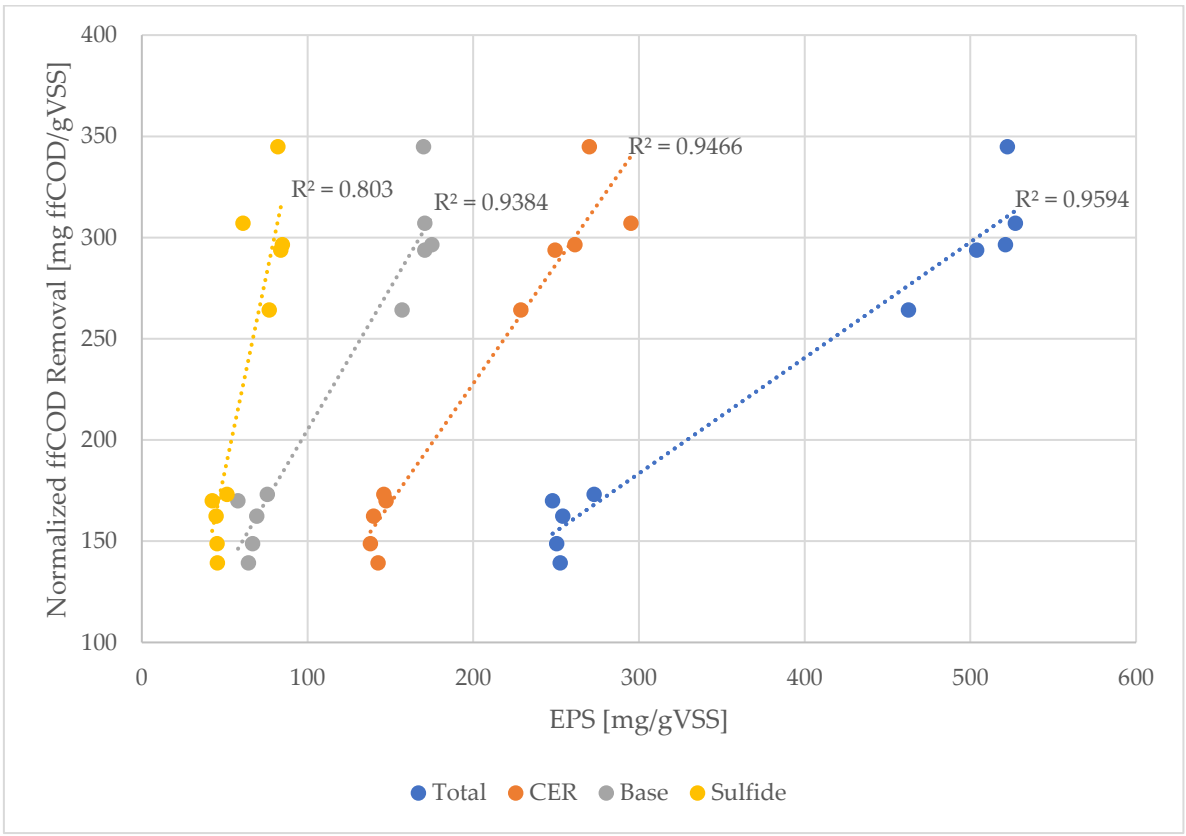

Figure 8. Normalized truly soluble organics removal correlation with extracted EPS from the three extractions as well as the total extracted amount from the two WWTP studied in series

4. Conclusions

The studied WAS exhibited varying chemical compositions and expressed differing fractions of their organic material as EPS. Significantly larger fractions of the EPS could be extracted from the WAS by using the CER method, the base method, and the sulfide method in series. The differing chemical compositions of the EPS extracted by the same extraction technique at different plants suggest that EPS bound in the matrix in similar ways are not necessarily compositionally similar.

A positive correlation was found between the amount of EPS present in the organic fraction of the sludges and the normalized removal of soluble organic matter. Furthermore, the EPS, regardless of its chemical composition, remove nearly the same amount of soluble and truly soluble organics removing 1.43±0.15 (n=16) mg of soluble chemical oxygen demand, and 1.20±0.18 (n=16) mg of truly soluble chemical oxygen demand, per mg of cation exchange resin extracted extracellular polymeric substances. When analyzing the series extractions with biosorption trend was shown to persist beyond just the CER extracted fraction suggesting that EPS, regardless of how it was bound within the cell matrix, influences the biosorption capabilities.

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