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Characterization of Proton Binding Properties of Extracellular Polymeric Substances in an Expanded Granular Sludge Bed Using Linear Programming Analysis

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Authors' contributions

This work was carried out in collaboration between all authors. Authors YL, QG and RC conducted the experiments. Author FMD developed the linear programming analysis code. Author YL and SC wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Extracellular polymeric substances (EPS) produced in biological wastewater treatment processes are from substrate metabolisms and biomass decay and mainly consist of proteins, polysaccharides, and nucleic acids. The chemical functional groups associated with these polymeric substances determine the charge, hydrophobicity, and chelating capacity of EPS. In spite of the fundamental importance of the proton binding properties of EPS, very limited studies have, to date, been conducted to explore the proton binding properties of colloidal and soluble EPS in biological wastewater treatment systems. The aims of this paper are to demonstrate the application of linear programming analysis of titration data in characterization of the pK_a, intensity, and chemical functional properties of proton binding sites of EPS in biological wastewater treatment systems.
Methodology: In this study, EPS has been sampled from a full-scale expanded granular sludge bed (EGSB) and a combined chemical composition analysis, linear programming analysis of titration data, and FT-IR analysis method was used to characterize the proton binding properties of

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the EGSB EPS.
Results: The linear programing analysis determined that the EGSB EPS has 4 proton binding sites with pK_a values of 6.4, 7.3, 7.6, and 9.7 at intensities of 1.26, 0.14, 0.25, and 3.12 mmol/g-EPS, respectively. The chemical composition analysis showed that the main acid/base reaction species in the titration sample are proteins, polysaccharides, and phosphates. The FT-IR analysis confirmed that the identified sites could be associated with carboxyl, phosphate, and amine chemical functional groups.
Conclusions: This study illustrated that the combination of chemical composition analysis, the linear programming analysis of titration data, and FT-IR analysis is an effective approach to determine the pK_a, intensity, and chemical functional properties of proton binding sites of EPS. Determining the chemical functional properties of EPS is of great importance for studying the fate of heavy metals in biological wastewater treatment processes, sludge bio-flocculation principles, and EPS membrane fouling in wastewater treatment membrane bioreactors (MBRs).

Keywords: Extracellular polymeric substances; proton binding sites; linear programming analysis; chemical functional properties; titration; granular sludge; expanded granular sludge bed.

1. INTRODUCTION

Effluents from biological wastewater treatment processes contain extracellular polymeric substances (EPS) which are released from substrate metabolisms and biomass decay. EPS is a pool of organics consisting of polysaccharides, proteins, nucleic acids, and humic and fulvic acids. These polymeric substances contain proton binding chemical functional groups, such as, carboxyl, phosphate, and amine. These chemical function groups determine the properties of charge, hydrophobicity, and metal chelating of EPS and enable EPS to react with heavy metals, nutrients, and other organics in the wastewater or receiving water. Thus, understanding the proton binding properties of EPS is of great importance to study the fate of heavy metals and emerging trace contaminants in biological wastewater treatment systems [1].

A variety of methods, including colorimetric spectroscopy [2,3], confocal laser scanning microscopy [4], and size exclusion chromatography [5] etc., have been used to characterize the properties of EPS in biological wastewater treatment systems, however, most of these methods can only reveal the chemical composition of EPS and relative molecular weight distributions but shed no light on the proton binding properties. The EPS proton binding property is characterized by the pK_as, intensities, and chemical functional properties of proton binding sites. Brassard et al. [6] developed a linear programming method to quantify the pK_a values and intensities of proton binding sites using data obtained from acid-base titration. This method had been successfully used to characterize the concentration and equilibrium constants of proton binding sites of Kaolinite [7], natural organic materials [8], and bacterial surfaces [9]. However, only limited studies have, to date, been conducted on quantifying the properties of proton binding sites of isolated or soluble EPS by using the potentiometric titration and linear programming analysis method [10,11,12].

In this study, we illustrate a combined chemical composition analysis, titration data linear programing analysis, and FT-IR characterization method to determine proton binding and chemical functional properties of EPS recovered from a full-scale expanded granular sludge bed (EGSB) reactor treating brewery wastewater. The result showed that the titration data linear program analysis is an effective method to determine the pK_as and intensities of proton binding sites; chemical composition analysis is essential to determine the main species affecting the titration behavior of the samples; and the FT-IR analysis is critical to elucidate the chemical functional properties of proton binding sites.

2. MATERIALS AND METHODS

2.1 EPS Sample Source and Characterization

The EPS samples examined in this study were obtained from the effluent of a full-scale EGSB system that treated brewery wastewater at an organic loading rate ranging from 10 to 15 $kgCOD/m^3/day$. The concentrations of COD, ammonium nitrogen (NH_4^+ -N), total phosphorus (TP), total volatile fatty acids (VFA), and alkalinity

(ALK) of the raw brewery wastewater and effluent were measured using methods specified in Standard Methods [13] to characterize the treatment capacity of the granular sludge in the full-scale EGSB. The activity of the granular sludge was assessed by batch respiration experiments using a 500 mL bench-scale anaerobic respirometer. The colorimetric and spectroscopic methods [2,3] were used to determine the concentration of carbohydrate and protein components using a Hach DR5000 spectrophotometer.

The EPS tested was recovered from the effluent of the EGSB reactor. The EGSB effluent was first filtered by 1.5 μm filter paper (GF/F, Whatman, USA) and, then, filtered by 0.45 μm membrane filter (Nylon, Millipore). The filter paper filtrate contained both particulate and soluble EPS, while the 0.45 μm membrane filtrate contained soluble EPS. The titration samples were prepared by treating the filtrate of 1.5 μm filter paper using dialysis membrane tubes with a molecular weight cut off (MWCO) of 3.5 kDa (Thermo, Snake Skin® Dialysis Tubing) for 28 hours. The retained of the dialysis membrane is supposed to contain EPS with molecular weight higher than 3.5 kDa.

The dialysis membrane treated effluent was lyophilized at -50°C by a Labconco one liter bench-top freezing drier (Labconco, USA) for the FT-IR analysis. FT-IR spectrum analysis was carried out on a Golden-gate Diamond single reflectance ATR in an FTS 7000 FT-IR spectrometer equipped with a DTGS detector (DIGILAB, Randolph, MA). The spectrum for lyophilized EPS was recorded at absorbance mode from 3800 to 600 cm^{-1} at a resolution of 4 cm^{-1} with 128 co-added scans. The metal contents in the raw and dialysis membrane treated effluent were measured using an ICP-MS system by using a certified external ALS environmental lab located in Waterloo, Ontario, Canada.

2.2 Titration Procedures

0.1N HCl and 0.1 N CO_2-free NaOH solutions (Fisher Scientific, Canada) were used for acid and base titration. A Radiometer combined conductivity and potentiometric bi-burette titration workstation (Titralab 870, Radiometer) was used for the titration operation. The pH electrode coupled with the titration station was a red rod combined electrode (pHc2085, Radiometer) with a resolution of 0.001 pH. The pH electrode was calibrated at pH 4, 7, and 10 prior to each titration experiment. The temperature during the titration was maintained at 20±0.3°C by circulating 20°C water through the water jacket of a thermostat titration cell. The thermostat titration cell was sealed by a self-constructed lid to maintain the anaerobic titration environment that was established by nitrogen gas stripping prior to the titration experiments. A monotonic titration protocol, which was characterized by titrating the sample step by step with an equal amount of titrant added at each step, was used to generate the titration data for the linear programming analysis of the EPS proton binding sites. The titration procedures included adjusting pH of the 30 mL titration sample to 2; N_2 gas bubbling for 10 minutes to remove carbonate alkalinity residual; adjusting pH to 10.7; and then, titrating the solution from pH 10.7 to 2.5 step by step by adding 0.1 N HCl at 0.01 mL per step.

2.3 Linear Programming Analysis of Titration Data

The linear programming analysis method developed by Brassard et al. [6] was used to analyze the acid-base equilibrium constants (pK_a) of the proton binding sites and site intensities. This method assumes that the heterogeneous mixture of binding sites of EPS consists of n monoprotic acid/base sites with pK_a values from $(pK_a)_1$ to $(pK_a)_n$. For the arbitrary i^{th} monoprotic acid/base site (HL_i/L_i^-), the acid-base reaction can be expressed as:

$$HL_i = L_i^- + H^+$$

where HL_i and L_i^- represent the conjugate acid and base pair associated with site L_i.

The following mass balance (Eq 1) and equilibrium relation (Eq 2) can be applied to the monoprotic acid HL_i

$$L_{ic} = [L_i^-] + [HL_i] \quad (1)$$

$$K_i = \frac{[L_i^-][H^+]}{[HL_i]} \quad (2)$$

where L_{ic} is the total molar concentration of the i_{th} site L_i (mmole/L); K_i is the acidity constant of HL_i; $[L_i^-]$ is the molar concentration of the deprotonated L_i sites (mmole/L); and $[HL_i]$ is the

molar concentration of protonated L_i sites (mmole/L).

Combining equation (1) and (2), the following equation can be derived to express $[L_i^-]$ as a function of K_i and L_{ic}

$$[L_i^-] = \frac{L_{ic} K_i}{[H^+] + K_i} \quad (3)$$

In this study, an acidic titration with pH between 10.7 and 2.5 was performed on the dialysis membrane treated EGSB EPS sample and the carbonate alkalinity was removed prior to the titration. Thus, the acidic titration ionic balance can be formulated as:

$$\sum_{i=1}^{n} [L_i^-]_j + C_{aj} + [OH^-]_j - [H^+]_j = ANC_0 \quad (4)$$

where ANC_0 is the initial acid neutralization capacity of the solution (mmole/L); C_{aj} is the cumulative concentration of the acid titrant added at the j_{th} step of titration (mmole/L); $[H^+]_j$ and $[OH^-]_j$ are the proton and hydroxyl concentrations after the j_{th} titration (mmole/L).

Combining equation (3) with equation (4), the following equation can be derived:

$$\sum_{i=1}^{n} \left(\frac{L_{ic} K_i}{K_i + [H^+]_j} \right) - ANC_0 = -C_{aj} + [H^+]_j - [OH]_j \quad (5)$$

All the parameters in Equation (5) are measurable except the site concentration of L_{ic} and the acidic constant K_i. The solution of the titration mass balance (Eq. 5) using the linear programming method developed by Brassard et al. [6] involves: (a) assuming n pre-assumed proton binding sites (K_1 to K_n) to reduce equation (5) into a linear equation containing n+1 variables (L_{1c} to L_{nc} and ANC_0); (b) establishing m equations based on the pH measured at each of the m titration steps (m > n); and (c) solving the m linear equations using the LPA method. In this study, 51 discrete pK_i ($K_i = 10^{pKi}$) values ranging from 2 to 12 in a step of 0.2 were set as the pre-assumed monoprotic sites and a linear programming solution code was developed to solve the linear equation group established based on the titration data using LINGO11.0 software.

3. RESULTS AND DISCUSSION

3.1 Characterization of the EGSB Sludge

The removal capacity of the anaerobic granular sludge tested in this study was characterized by comparing the wastewater characteristic parameters of the wastewater treated by the EGSB and those in the effluent discharged from the EGSB. As shown in Table 1, the raw brewery wastewater tested in this study contained a 8160.0 mg/L COD, 7390.0 mg/L soluble COD (sCOD), 27.1 mg/L total phosphorus, 0.6 mg/L NH_4^+-N, 739.0 mg/L volatile fatty acids, and 153.0 mg/L of alkalinity as $CaCO_3$. After the treatment of the EGSB reactor, the total COD and soluble COD in the wastewater were reduced to 838.0 m/L and 212.0 mg/L, respectively, representing a 90% total COD and a 97% sCOD removal. However, the effluent still contained around 100 mg/L total VFA. The NH_4^+-N and alkalinity concentrations of the effluent were significantly higher than those of the raw wastewater. The high NH_4^+-N concentration in the effluent was an indication of degradation of protein type organics, while the high alkalinity was resulted from the addition of alkalinity required to maintain the desired operation pH.

The biogas production capacity of the EGSB granular sludge tested was assessed by batch respiration experiments using a 500 mL bench-scale respirometer at an organic loading rate 2 g/L/day. Fig. 1 shows that the recorded biogas production rate over a 5-hour test period for three batch respiration tests repeated in three consecutive days with the same granular sample. The average COD reduction for these tests over the five-hour testing period was 86 $\pm$ 2 % with a COD to biogas conversion factor of 0.5 L biogas /gCOD. The respiration tests showed the granular sludge tested in this study was biologically active and stable.

3.2 Chemical Constitutes of the Dialysis Membrane Treated Effluent

The chemical analysis determined that the protein contents in the 1.5 μm filer paper filtrate, the 0.45 μm membrane filtrate, and the retained of the dialysis membrane were 209.2 mg/L, 70.6 mg/L, and 196.6 mg/L, respectively; while the respective polysaccharide concentrations were 60.8 mg/L, 20.9 mg/L, and 60.7 mg/L (Table 2). The 0.45 μm membrane filter exhibited a significant rejection to both particulate proteins

and polysaccharides which can be supposed in a size range between 0.45 and 1.5 μm. The dialysis membrane showed a nearly complete retention to the proteins and polysaccharides in the 1.5 μm filter paper filtrate of the EGSB effluent. The dialysis treated samples were used for the titration experiments to determine the protein binding properties via the titration linear programming.

As shown in Table 2, the dialysis membrane treatment removed nearly 98% of NH_4^+-N and around 59% TN. The remaining nitrogen in the dialysis treated sample can be associated with the organic nitrogen of the EPS origin. The total phosphorus (TP) concentration in the 1.5 μm filter paper filtrate was 29.6 mg/L, among which around 5.6 mg/L was retained by the 0.45 μm membrane and 13.6 mg/L was retained by the dialysis membrane. The difference in TP concentrations between the dialysis membrane and 0.45 μm retained, which is around 8 mg/L, could be polyphosphates retained by the dialysis membrane.

Table 3 summarizes the main detectable metal species in the EGSB effluent before and after the dialysis treatment. The main detected metal

Table 1. Characteristics of the raw brewery wastewater and EGSB effluent tested.

	COD mg/L	sCOD mg/L	PO_4^{3-}-P mg/L	NH_4^+-N mg/L	VFA mg/L	ALK mg/L as $CaCO_3$	pH
Wastewater	8160.0	7390.0	27.1	0.6	739.0	153.0	4.8
Effluent	838.0	212.0	24.0	15.0	99.8	1741.0	7.3

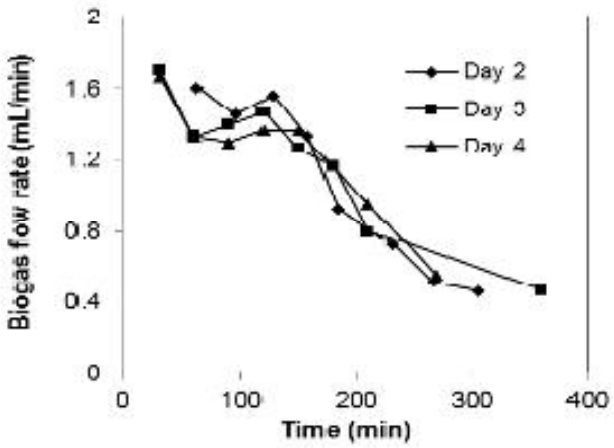

Fig. 1. Biogas production rate-time profiles of the EGSB granular sludge tested

Table 2. Compositions of the 1.5 μm filter paper filtrate, 0.45 μm membrane filtrate, and the 3.5 kDa dialysis membrane retained of the EGSB effluent

	1.5 μm filtrate (mg/l)	0.45 μm filtrate (mg/L)	Dialysis membrane retained (mg/L)
COD	405.0	212.0	276.0
TP	29.6	24.0	13.6
TN	32.6	21.6	13.2
NH_4^+-N	15.0	14.0	0.3
VFA	99.8	92.3	ND
Proteins	209.2	70.2	196.6
Carbohydrates	60.8	20.9	60.7
Alkalinity	1741.0 as $CaCO_3$	-	100.0 as $CaCO_3$

species in the EGSB effluent tested in this study included potassium (24 mg/L), sodium (1160 mg/L), calcium (128 mg/L), and magnesium (47.2 mg/L). After 28-hour dialysis membrane treatment, the potassium, sodium, calcium, and magnesium concentrations were reduced to < 1 mg/L, 25.8 mg/L, 25 mg/L, and 9.2 mg/L, respectively. The effect of the monovalent metal ions on acid-base titration would be insignificantly. The pK_a values for conjugate acid/base pairs of $Ca^{2+}/Ca(OH)^{+}$ and $Mg^{2+}/Mg(OH)^{+}$ are 12.78 and 11.44, respectively [14], implying that the residual of Ca^{2+} and Mg^{2+} ions in the dialysis membrane treated sample will remain in hydrolyzed forms in the pH range of the titration conducted in this study.

The treatment by 3.5 kDa dialysis membranes for 28 hours resulted in a 94% reduction in alkalinity but there was still 100 mg/L as $CaCO_3$ of alkalinity remained in the dialysis membrane treated sample. The alkalinity is the sum of the concentration of the chemical species that consume proton at pH above 4.5. The main contributors to the alkalinity could include bicarbonate alkalinity and proton binding sites with the mediate pK_a values. The bicarbonate alkalinity can be removed by nitrogen gas stripping at low pH condition. In this duty, a 10-minute N_2 gas stripping at pH 2 was determined to be sufficient to remove bicarbonate alkalinity prior to the monotonic titration conducted for the proton binding site analysis.

Table 3. Metal ions in the EGSB effluent and 3.5 kDa dialysis membrane treated samples

		EGSB effluent	Dialysis retained
Aluminum	mg/L	<0.10	0.08
Barium	mg/L	0.045	0.030
Calcium (total)	mg/L	128.0	25.0
Iron	mg/L	< 0.50	0.087
Magnesium (total)	mg/L	47.2	9.2
Magnesium (Mn)	mg/L	0.0210	0.0115
Potassium	mg/L	24.0	< 1.0
Sodium	mg/L	1160.0	25.8
Strontium	mg/L	3.690	0.672
Conductivity	μs/cm	6360.0	258.0

3.3 Linear Programming Analysis of the Titration Data

The intensities of the proton binding sites were determined as the ratio of the binding site concentration (L_{ic}, mmole/L) determined by the linear programming analysis to the total concentration of the protein and carbohydrate determined by the chemical composition analysis. The linear programing analysis identified 4 proton binding sites with pK_a values of 6.4, 7.3, 7.6, and 9.7 at intensities of 1.26, 0.14, 0.25, and 3.12 mmol/g-EPS, respectively (Fig. 2). In addition to these proton binding sites, the linear programming analysis also identified

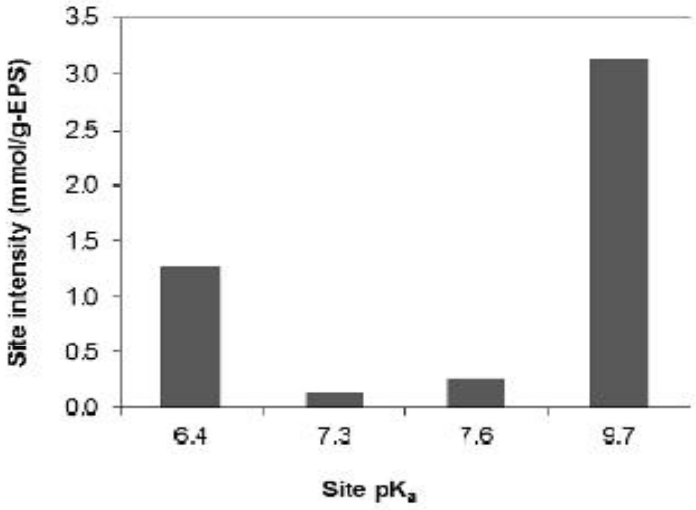

Fig. 2. The pK_a values and intensities of proton binding sites of the EPS in the EGSB effluent tested

a strong acidic site with a pK_a value of 2 at an intensity of 18.7 mmol/g-EPS. However, as pointed out by Smith et al. [8], acid-base titration data beyond the pH range of 4 -10 is not accurate enough for the linear programming analysis. Given that proton binding sites will exhibit evident acid/base reaction capacity in the pH around the $pK_a \pm 1$, the linear programming analysis is expected to determine sites with pK_a values ranging from 3 to 11. Thus, the intensity of 18.7 mmol/g-EPS identified by the linear programming analysis for pK_a 2 can be considered as the total intensities of the proton binding sites with pK_a values lower than 3. The limitations of the linear programming analysis in the low and high pH range are not a critical concern because the proton binding sites with pK_a values beyond the range of 3 - 11 would maintain a consistent charge property in the normal pH range related to biological wastewater treatment.

The accuracy of the linear programming analysis was examined by comparing the experimentally determined titration curve with that simulated based on the pK_a values determined by the linear programming analysis. The alkalinity balance for the titration with consideration of change in sample volume caused by the addition of titrant can be derived from Equation (7) as below:

$$ANC_0 V_0 = C_a V_t + (V_0 + V_t)\left\{\sum_{i=1}^{n}\left(\frac{L_{ic}K_i}{K_i + [H^+]_t}\right) + [OH^-]_t - [H^+]_t\right\} \quad (7)$$

where V_0 is the initial solution volume in the titration cell and V_t is the titrant added into the cell.

From equation (7), the following V_t - $[H^+]_t$ relationship can be derived:

$$V_t = \frac{\left\{ANC_0 - \left\{\sum_{i=1}^{n}\left(\frac{L_{ic}K_i}{K_i + [H^+]_t}\right) + [OH^-]_t - [H^+]_t\right\}\right\}V_0}{C_a + \sum_{i=1}^{n}\left(\frac{L_{ic}K_i}{K_i + [H^+]_t}\right) + [OH^-]_t - [H^+]_t} \quad (8)$$

The theoretical titration curve was simulated by using Equation (8) with the linear programming determined K_i, L_{ic}, and ANC_0. The results which are shown in Fig. 3 indicate an excellent match-up between the simulated titration curve and the experimental titration data with a R-squared value of 0.9998, suggesting that the titration behavior can be well characterized by the linear-programming analysis determined proton binding sites.

Table 4 compares the pK_a values and intensities of the proton binding sites of the EGSB EPS determined in this study with those reported by Liu and Fang [10] for the hydrogen-producing sludge (HPS) and sulfate-reducing biofilm (SRB) sludge using the titration and linear programming analysis methods, which is the only reference we could found in the literature on the titration linear programing analysis of proton binding sites conducted on the isolated EPS. The total intensity of the sites with pK_a values ranging from 5 to 10 was reported as 3.02 mmol/g-EPS for the HPS and 5.07 mmol/g-EPS for the SRB, which are comparable to the 4.77 mmol/g-EPS determined for the EGSB EPS in this study. The total site intensity, including the sites with pK_a values lower than 3, for the EGSB EPS was 22.78 mmol/g-EPS, which is also comparable to 10.88 mmol/g EPS for the HPS sludge and 16.44 mmol/g EPS for the SRB sludge reported by Liu and Fang [10]; 4-24 mg/gEPS for natural organic matter [8]; and 16-23 mmol/g-EPS for activated sludge [15].

3.4 Characterization of the Chemical Properties of the Proton Binding Sites

The chemical functional properties of the freezing dried EGSB EPS sample were characterized by Fourier transferred infrared (FT-IR) analysis. Fig. 4 shows the FT-IR spectrum of the EGSB samples. The strong presence of proteins was confirmed by the typical amide band I and amide

band II peaks at 1645.3 cm^{-1} and 1512.2 cm^{-1}, respectively. The presence of polysaccharide was confirmed by the peak at 1047.3 cm^{-1}, which is associated with C-O and C-O-C vibrations of polysaccharides [16,17]. The FT-IR also confirmed a strong presence of carboxylates by the absorbance at 1409.9 cm^{-1}, which could be derived from both protein and polysaccharides [16,18]. Although amine is a common side-chain functional groups of amino acids, the absorption of amine, which is usually associated with the broad band in 3000-3400 cm^{-1} (N-H stretching) and the peak at 1630 cm^{-1} (N-H bending), was not unambiguously identified by FT-IR due to its adsorptions overlapping with other side chain groups and polypeptide backbone [16]. The region between the wave number of 1114 and 1250 cm^{-1} holds characteristic bands of phosphate [17,19]. Since the strong presence of the characteristic band of polysaccharides in this region, only a weak peak at 1257.6 cm^{-1}, which could be associated with the phosphate group in nucleic acids [19], was detected.

The linear programming analysis identified four proton binding sites with pK_a values of 6.4, 7.3, 7.6, and 9.7 and sites with pK_a values lower than 3. The binding sites with pK_a values lower than 3 could be associated with carboxyl and phosphate groups. The carboxyl groups of EPS can be related to the side-chain functional groups of amino acids of proteins and to uronic acids of polysaccharides, both of which were confirmed for the sample tested in this study by the IR adsorption analysis. The pK_a values of carboxyl groups associated with amino acids are in the range of 1.83 – 2.83 [20], while the carboxyl group of uronic acid has a pK_a value in the range of 2.0 – 3.0 [21]. The residual phosphate in the dialysis treated samples can contribute to the intensity of the binding sites with pK_a ranging from 0.5 up to 12.35. Orthophosphate has pK_a

Table 4. Identified proton binding sites in this study and those reported by Liu and Fang [10]

EPS in HPS [10]		EPS in SRB [10]		EPS in EGSB (This study)	
pK_a	**Concentration (mmole/g-EPS)**	**pK_a**	**Concentration (mmole/g-EPS)**	**pK_a**	**Concentration (mmole/g-EPS)**
4.8	2.81	4.4	6.49	< 3	18.0
6.0	0.76	6.0	2.35	6.4	1.26
7.0	0.26	7.4	0.79	7.3	0.14
9.8	2.00	8.2	0.55	7.6	0.25
11.0	5.05	9.4	1.38	9.7	3.12
		11.0	4.88		-
Total	10.88		16.44		22.77

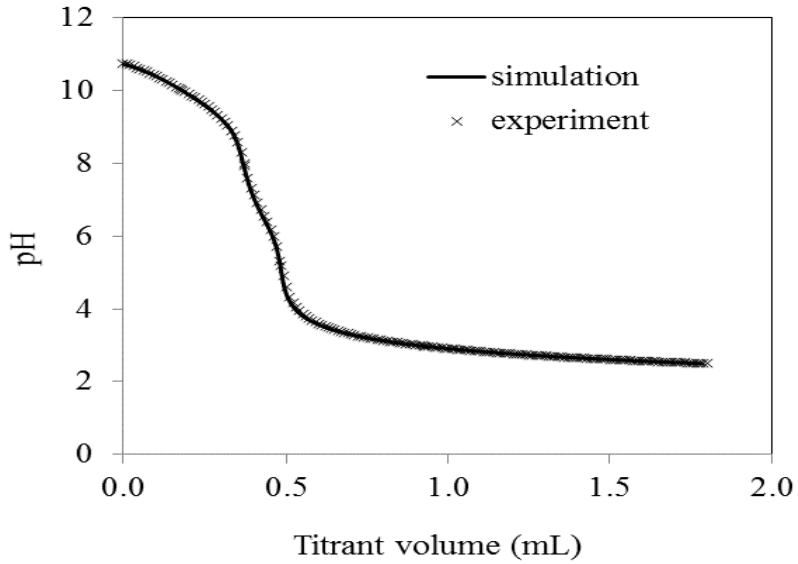

Fig. 3. Comparison of the measured and simulated titration curves

values of 2.15, 7.20, and 12.35 and polyphosphates with different phosphorus numbers were reported having pK_a values ranging from 0.5 to 9.4 [22]. Long chain polyphosphates have strongly acidic hydrogens with pK_a values of 1 – 2 bound to each residue of phosphate with two weakly acidic hydrogens with pK_a of 7.2 – 8.2 at the ends of the chain [23]. Thus, the phosphate can contribute to the proton binding sites with pK_a values lower than 3 and those with pK_a values of 6.4, 7.3, and 7.6. The sites with the pK_a value of 9.7 are likely related to amine group given the strong presence of proteins which is confirmed by FT-IR and chemical analysis. The pK_a value of amine could be in the range of 8.8 – 10.6 but it is usually below 10 when neighbored with a carboxyl group [9,19]. The primary and secondary amides have a pK_a value around 15 so amide groups would not exhibit acidic or basic behavior during the titration conducted in this study.

Table 5 summarizes the potential chemical functional groups associated with the proton binding sites of different pK_a ranges reported by various researchers. The strong acidic sites (pK_a < 5) are commonly attributed to carboxyl and phosphate groups, while the weak acidic sites

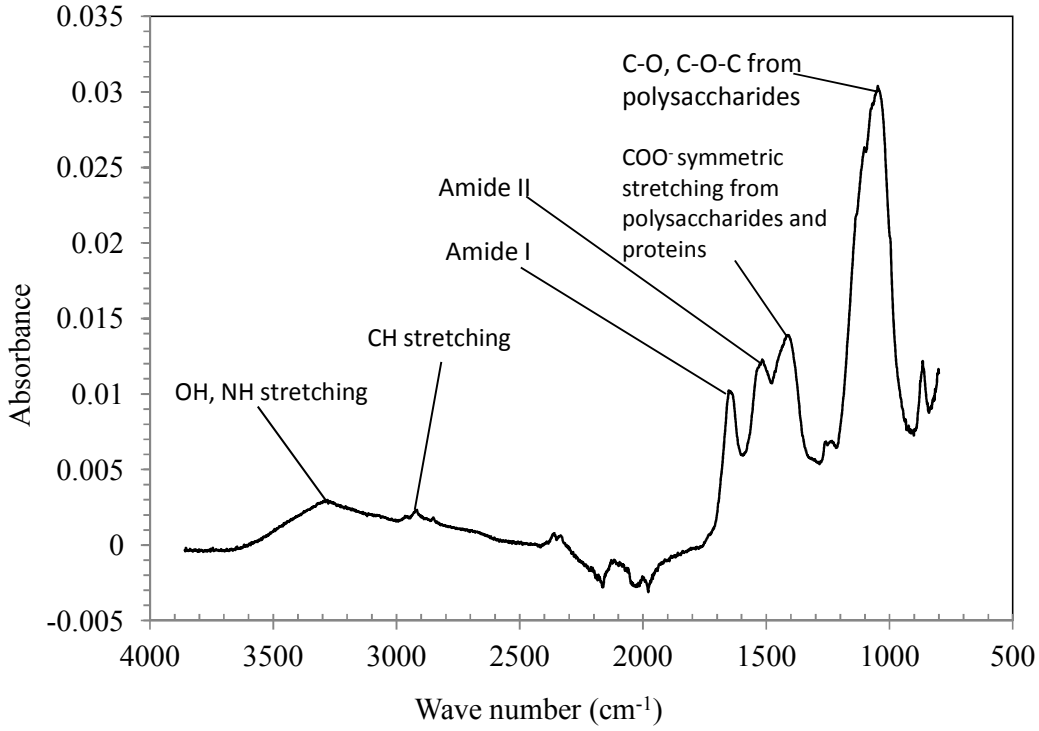

Fig. 4. FT-IR spectrum of the freezing dry EPS samples

Table 5. Functional groups suggested for proton binding sites with pK_a values of different ranges

pK_a ranges	Liu and Fang [10] (EPS)	Cox et al. [9] (bacterial surfaces)	Smith & Kramer [8] (natural organic matters)	This study (EGSB EPS)
< 5	Carboxyl	Carboxyl, phosphodiesters, phosphoric,	Carboxyl	Carboxyl, phosphodiesters phosphoric,
5.1 – 7.5	Carboxyl, phosphoric	Carboxyl, phosphoric,	β-dicarbonyl, enols, alcohol, inorganic substances	phosphates
7.6 – 9.2	Sulfhydryl	Phenolic		phosphates
> 9.3	Amine, phenolic, Hydroxyl	Amine, phenolic	Phenolic	Amine

(pK_a > 9.3) are often referred to amine and phenolic groups. The intermediate acidic sites which have pK_a values between 5 and 9.2 could be attributed to a wide variety of groups, including carboxyl, phosphates, sulfhydryl, and phenolic groups. Based on the likely functional groups associated with the proton binding sites, it is anticipated that the majority of the proton binding sites will exhibit a negative or neutral charge under normal pH condition, except that amine group will exhibit a positive charge under pH below 8. The intensity of negatively charged sites will increase with pH due to the increased trend of deprotonation.

4. CONCLUSION

This study demonstrated that the combination of linear programing analysis of titration data, chemical composition analysis, and FT-IR spectrum analysis is an effective approach to identify the proton binding sites of EPS and their chemical properties. In this study, the linear programming analysis determined that the EPS sampled from a full-scale EGSB have proton binding sites with pK_a values of 6.3, 7.3, 7.6, and 9.7 and sites with pK_a value lower than 3. The chemical composition analysis showed that the main acid/base reaction species in the titration sample are proteins, polysaccharides, and phosphates. The FT-IR analysis confirmed that the identified sites could be associated with carboxyl, phosphate, and amine chemical functional groups. The determination of the intensities and chemical properties of proton binding sites is of significance for understanding the environmental impact of EPS in the effluent of biological wastewater treatment processes.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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