Characterization of Extracellular Polymeric Substances (Eps) Produced by \textit{Cloacibacterium normanense} Isolated from Wastewater Sludge for Sludge Settling and Dewatering

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\textbf{Abstract}

In this study, extracellular polymeric substances (EPSs) producing strain was isolated from municipal wastewater sludge (MWWS). Growth profile and the EPS production by \textit{Cloacibacterium normanense} using wastewater sludge as raw material in shake flask fermentation for 96 h were investigated. The highest concentration of S-EPS (13.0 ± 0.8 g/L) and C-EPS (0.3 ± 0.1 g/L) were attained at 48 h of fermentation. S-EPS revealed higher flocculation activity (94.2%) and dewaterability (59.9%) than other types of EPS in kaolin suspension. The dewaterability of MWWS with 2 g suspended solids (SS)/L was improved by 37.6% using 0.02 ± 0.01 g/L of S-EPS and 600 mg/L of Al\textsubscript{2}(SO\textsubscript{4})\textsubscript{3}. The study showed a promising approach of new isolated strain to produce high concentration EPS in sludge with high flocculation activity as well as good settling.

\textbf{Keywords:} Bioflocculants; Bacterial polymers; Extracellular polymeric substances; Pollution control; Sludge; Wastewater

\textbf{Abbreviations:} APHA: American Public Health Association; B – EPS: Broth EPS; BS: Bacterial Strain; BSA: Bovine Serum Albumin; TP/TTC ratio: Protein to Carbohydrate ratio; C-EPS: Capsular EPS; CFU: Colony Forming Units; CST: Capillary Suction Time; EPS: Extracellular Polymeric Substances; FA: Flocculation Activity; LB EPS: Loosely Bound EPS; mg EPS/g of Kaolin: mg of EPS Added Per Gram of Kaolin Suspension in Water; MWWS: Municipal Wastewater sludge; PCA: Plate Count Agar; PPS: : Pulp and Paper Sludge; S-EPS: Slimy EPS; SS: Suspended Solids; SVI: Sludge Volume Index; TBEPS: Tightly Bound EPS; TSB: Tryptic Soy Broth; \(\zeta\): Zeta Potential

\textbf{Introduction}

Sludge settling and dewatering are the most important steps of wastewater treatment and sludge management. Better dewaterability leads to a sludge economical disposal, reuse as a soil conditioner in agriculture, bricks for construction, and raw material for growing industrial microorganisms [1]. In recent years, researchers have been venturing into bioflocculation of sludge using microorganisms [2]. Bioflocculation is defined as an aggregation of bacterial flocs and it is utmost important for efficient separation of microorganisms from the treated effluent. A typical floc is formed by different types of bacteria together with other microorganisms (protozoa, fungi, filamentous microorganisms etc) and viruses along with some abiotic suspended materials. Flocs are held together in a polymeric network of extracellular polymeric substances (EPSs). The microbial EPS plays an important role in bioflocculation by interacting with the sludge solids [3]. The bacterial growth is often accompanied by the production of EPS, which has ecological and physiological functions [4]. EPSs are organic macromolecules that are formed by polymerization of similar or identical building blocks that may be arranged as repeated units within the polymer. The major organic fractions of EPS are carbohydrates and proteins [5]. EPSs also act as excellent emulsifying agents and this property is attributed to the diversity in bacteria [6]. The bacterial EPSs are usually acidic heteropolysaccharides possessing different functional groups (e.g., hydroxyl, carboxyl and phosphoric acid), which exhibit high affinity towards certain metal ions. Many physical and chemical properties of microbial EPSs have led to a wide range of field applications, e.g., adhesion, chelation of heavy metals, coagulation and flocculation, detoxification of toxic compounds, nutrient sequestration, protection against osmotic shock, stabilizers, thickeners, gelling, film-forming and water-retention capability (in detergents, textiles, adhesives, paper, paint, food and beverage industries), oil recovery, mining industry and petroleum industries [2]. A wide range of bacteria from various environmental habitats are known to produce complex and diverse EPS occurring as capsular polysaccharides (C-EPS, strongly associated with the cell surface) or as slime polysaccharides (S-EPS, loosely associated with the cell). Recently, there has been growing interest in the isolation and characterization of microbial EPS owing to their practical importance. Different microorganisms produce various types of EPS with diverse characteristics and concentration. For economic reasons it is essential to find a high EPS yielding microbial strain with high flocculation activity per unit weight of EPS. Therefore, the present study aimed at: i) isolation and identification of high concentration EPS producing bacteria, ii) chemical and physical characterization of the EPS produced by the strain and iii) to evaluate the potential of produced EPS with respect to flocculation activity and dewaterability.

\textbf{Materials and Methods}

\textbf{Bacterial strain isolation and identification}

Wastewater sludge samples were collected from Communauté Urbaine du Québec (CUQ, Quebec, Canada). EPS producing strain \textit{Cloacibacterium Normanense} (NK6, accession number KF673204) was isolated from sludge samples using standard plate Count Agar (PCA). The strain was identified based on 16S rDNA sequencing.

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Isolated genomic DNA from the individual bacterial strains was subjected to PCR amplification of 16S rDNA using universal primers [7]. Amplified products were purified using the Qiagen gel extraction kit and subsequently were sequenced [8]. The obtained 16S rDNA gene sequences were submitted into the internet for similarity search.

**EPS production**

The sludge was first settled by gravity for 1 h and the settled (concentrated) sludge was collected by discarding the supernatant. *Cloacibacterium normanense* was inoculated in Tryptic soy broth (TSB) (100 mL sterilized TSB in 500 mL flask) and incubated for 48 h. After 48 h incubation, the culture broth was used as inoculum to inoculate the sterilized (12°C for 30 min) sludge (25 g suspended solids-SS/L, pH 7, 150 mL sludge in a 500 mL capacity flask). The flask was incubated in a shaking incubator at 180 rpm and 30°C for 24 h. This culture (with an approximate cell concentration 6.7 × 10⁶ colony forming units-CFU/mL) was used to inoculate (3% v/v) flask containing sterilized sludge (25 g/L SS, 150 mL sludge, pH 7). The flasks were incubated in a shaker at 180 rpm and 30°C for 96 h for EPS production. Samples were withdrawn at each 12 h interval to measure the cell concentration, and each 24 h to measure the EPS concentration, flocculation activity and dewaterability. All the samples were serially diluted with saline solution and the cell concentration was measured as CFU employing standard agar-plate technique. All the measurements were carried out in triplicates and the average of the results was presented.

**Extraction of EPS**

After incubation, the fermented broth was centrifuged at 6000 g, 4°C for 20 min to obtain supernatant (containing slime- EPS (S-EPS) and the biomass pellet was re-suspended in deionized water to the initial volume (100 mL of the broth) and incubated for 48 h. After 48 h incubation, the culture broth was used as inoculum to inoculate the sterilized (12°C for 30 min) sludge (25 g suspended solids-SS/L, pH 7, 150 mL sludge in a 500 mL capacity flask). The flask was incubated in a shaking incubator at 180 rpm and 30°C for 24 h. This culture (with an approximate cell concentration 6.7 × 10⁶ colony forming units-CFU/mL) was used to inoculate (3% v/v) flask containing sterilized sludge (25 g/L SS, 150 mL sludge, pH 7). The flasks were incubated in a shaker at 180 rpm and 30°C for 96 h for EPS production. Samples were withdrawn at each 12 h interval to measure the cell concentration, and each 24 h to measure the EPS concentration, flocculation activity and dewaterability. All the samples were serially diluted with saline solution and the cell concentration was measured as CFU employing standard agar-plate technique. All the measurements were carried out in triplicates and the average of the results was presented.

**Chemical characterization of EPS**

After precipitation, the extracted C-EPS and S-EPS were dissolved in distilled water, 150 mg/L of Ca²⁺ was added to the kaolin suspension and pH was adjusted to 7. The desired concentration of different types of EPS was added (in terms of volume of the sample range from 0.25 mL to 4 mL collected at different times of fermentation) to kaolin suspension and rapidly mixed at 180 rpm for an initial 5 min then slowly mixed at 70 rpm for an additional 30 min. After mixing, samples were transferred to a 500 mL cylinder where they were allowed to settle for 30 min. The supernatant of each sample was then collected to measure the turbidity using turbidimeter (Micro 100 turbidimeter, Scientific Inc.). Flocculation activity was measured using the formula [100*(B-A)/B] where ‘A’ is the turbidity of the sample (treated with S-EPS, C-EPS or B-EPS) and ‘B’ is the turbidity of the control sample (in which equal volume of EPS solution was replaced with distilled water). All the tests were conducted in triplicates and the average values were presented.

**Sludge dewaterability**

The capillary suction time (CST) was used to evaluate the dewaterability of the flocs in kaolin solution, MWWS and PPS using *Cloacibacterium normanense*. The CST of the control (without addition of the S-EPS) was also measured. The samples were prepared similar to SVI measurement. The sediment from each flocculation activity test was used to measure the CST by a CST instrument (Triton, Model 304 M, UK) [9]. A high value of CST usually implies a poor filterability and dewaterability.

**Results and Discussion**

**EPS production and characterization**

Growth and EPS production profiles of *Cloacibacterium*...
Cloacibacterium normanense sp. are presented in Figure 1. Exponential growth phase was observed between 12 h and 24 h of fermentation. The maximum cell concentration (7.5 x 10^8 CFU/mL) reached at 48 h. The concentration of B-EPS increased with the fermentation time and reached maximum (13.3 ± 0.9 g/L) at 48 h where the cell concentration was also maximum (Figure 1). The EPS concentration increased from 1.9 to 2.8 g/L during lag phase (from 0 to 12 h). The EPS production occurred mainly during the exponential growth phase but significant EPS production was also observed during declining phase i.e., between 36 h and 48 h of fermentation (Figure 1). The research of More et al. [2] investigated the effect of fermentation time on EPS production in activated sludge and found that the EPS content was proportional to the bacterial growth. A decrease in B-EPS concentration (from 13.3 to 11.4 g/L) was observed between 48 and 96 h. This decrease could be due to the fact that the bacteria may have consumed EPS when carbon limitation occurred in the medium. This phenomenon was also observed by other researchers using pure or mixed culture in sludge or synthetic medium [2,16]. According to More et al. [17], the highest EPS concentration achieved was 3.4 g/L in 72 h with sterilized sludge as a growth medium employing Serratia sp., which was much lower than the B-EPS concentration (13.3 g/L) observed in the present study (Table 1). The EPS synthesis by microorganisms depends on the carbon and nitrogen availability in the culture medium. Most of the EPS producing microorganisms use carbohydrates as their carbon and energy source and either ammonium salts or amino acids or both as their nitrogen source [18]. Therefore, the higher EPS concentration obtained in this work than those reported in the literature can be due to the fact that Cloacibacterium normanense strain may have a wider range of carbon and nitrogen utilization ability that eventually helped it to use available complex carbon and nitrogen sources present in sludge for its growth and EPS production.

### Protein and carbohydrate content

Carbohydrate and protein content of S-EPS (LB-EPS) and C-EPS (TB-EPS) and their concentrations in the broth are presented in Figures 2a-2d. The total protein (TP) and the total carbohydrate (TC) content of the EPS and their concentration in the medium increased with fermentation time and reached maximum at 48 h. The protein content (219.9 ± 5.3 mg BSA/g of extracted EPS) and carbohydrate content (128.5 ± 6.3 mg carbohydrate/g of extracted EPS) of S-EPS was higher than the protein content (145.6 ± 4.7 mg of BSA/g of extracted EPS) and carbohydrate content (104.0 ± 5.5 mg carbohydrate/g of extracted EPS) of C-EPS at 48 h fermentation. The protein and carbohydrate content of the EPS produced by the strain was higher than the control sample or the EPS extracted from the sterilized sludge (72.9 ± 1.1 mg BSA/g of S-EPS and 28.0 ± 2.1 mg carbohydrate/g of S-EPS).

The protein and carbohydrate content of the EPS increased in exponential and declining phase (i.e., until 48 h) followed by a decrease during the stationary phase (after 48 h). The decrease in B-EPS concentration after 48 h (Figure 1) was due to degradation of proteins and carbohydrate of EPS by the bacterial strain. The stationary phase corresponded to the beginning of nutrient depletion in the medium and the accumulation of waste products limiting the growth. The culture grew exponentially followed by a slow growth until the maximum cell density was reached (at 48 h), and eventually the growth ceased due to the cell lysis caused by the decrease in the integrity and stability of the cell surface. This process leads to a reduction or even complete cessation of extracellular product synthesis by microorganisms. Under this condition, the microorganism shift to use the carbohydrates and proteins of EPS to fulfill the demand of carbon and nitrogen sources. Zhang and Bishop [19] performed a comparative study to examine the

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**Table 1: Production of B-EPS by different strains in present study and reported in the literature.**

<table>
<thead>
<tr>
<th>Strains Name</th>
<th>Medium type</th>
<th>B-EPS (g/L)</th>
<th>Fermentation condition (shake flask)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus sp.2</td>
<td>Sludge (10 g/L)</td>
<td>1.27</td>
<td>250 rpm, 25°C, 72 h</td>
<td>More et al., [31]</td>
</tr>
<tr>
<td>Bacillus sp.3</td>
<td>Sludge (10 g/L)</td>
<td>1.68</td>
<td>250 rpm, 25°C, 72 h</td>
<td>More et al., [31]</td>
</tr>
<tr>
<td>Bacillus sp.4</td>
<td>Sludge (10 g/L)</td>
<td>1.28</td>
<td>250 rpm, 25°C, 72 h</td>
<td>More et al., [31]</td>
</tr>
<tr>
<td>Bacillus sp.5</td>
<td>Sludge (10 g/L)</td>
<td>1.24</td>
<td>250 rpm, 25°C, 72 h</td>
<td>More et al., [31]</td>
</tr>
<tr>
<td>Bacillus sp.6</td>
<td>Sludge (10 g/L)</td>
<td>1.45</td>
<td>250 rpm, 25°C, 72 h</td>
<td>More et al., [31]</td>
</tr>
<tr>
<td>Bacillus sp.7</td>
<td>Sludge (10 g/L)</td>
<td>1.56</td>
<td>250 rpm, 25°C, 72 h</td>
<td>More et al., [31]</td>
</tr>
<tr>
<td>Bacillus sp.8</td>
<td>Sludge (10 g/L)</td>
<td>1.65</td>
<td>250 rpm, 25°C, 72 h</td>
<td>More et al., [31]</td>
</tr>
<tr>
<td>Bacillus sp.9</td>
<td>Sludge (10 g/L)</td>
<td>1.23</td>
<td>250 rpm, 25°C, 72 h</td>
<td>More et al., [31]</td>
</tr>
<tr>
<td>Serratia sp.1</td>
<td>Sludge (17 g/L)</td>
<td>3.4</td>
<td>250 rpm, 25°C, 72 h</td>
<td>More et al., [2]</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>Mineral medium (25 g/L glucose, 0.2 g/L MgSO₄)</td>
<td>2.3</td>
<td>250 rpm, 25°C, 72 h</td>
<td>Bala subramanian et al., [10]</td>
</tr>
<tr>
<td>Serratia sp BS8</td>
<td>Mineral medium (25 g/L glucose, 0.2 g/L MgSO₄)</td>
<td>3</td>
<td>250 rpm, 25°C, 72 h</td>
<td>Bala subramanian et al., [10]</td>
</tr>
<tr>
<td>Bacillus sp BS9</td>
<td>Mineral medium (25 g/L glucose, 0.2 g/L MgSO₄)</td>
<td>2.4</td>
<td>250 rpm, 25°C, 72 h</td>
<td>Bala subramanian et al., [10]</td>
</tr>
<tr>
<td>Yersinia sp BS11</td>
<td>Mineral medium (25 g/L glucose, 0.2 g/L MgSO₄)</td>
<td>2.5</td>
<td>250 rpm, 25°C, 72 h</td>
<td>Bala subramanian et al., [10]</td>
</tr>
<tr>
<td>Microbacterium (BS15)</td>
<td>Mineral medium (25 g/L glucose, 0.2 g/L MgSO₄)</td>
<td>2.1</td>
<td>250 rpm, 25°C, 72 h</td>
<td>Bala subramanian et al., [10]</td>
</tr>
<tr>
<td>Staphylococcus aureus (A22)</td>
<td>Glycerol and Ethanol</td>
<td>10.8</td>
<td>150 rpm, 28°C, 48 h</td>
<td>Buthelezi et al., [32]</td>
</tr>
<tr>
<td>Pseudomonas plecoglossisida (A14)</td>
<td>Glycerol and Ethanol</td>
<td>8.3</td>
<td>150 rpm, 28°C, 48 h</td>
<td>Buthelezi et al., [32]</td>
</tr>
<tr>
<td>Cloacibacterium normanense</td>
<td>Sludge (25gL)</td>
<td>13.3</td>
<td>180 rpm, 30°C, 48 h</td>
<td>Present work</td>
</tr>
</tbody>
</table>

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**References**

biodegradability of EPS by microorganisms from the original biofilm (its own producers) and it was found that the cells consumed the newly produced EPS and microbial activity gradually stopped. Similarly, the present study also suggested that the EPS (protein and carbohydrate) could be used as a substrate. The protein content of the EPS was higher than the carbohydrate content (Figure 2) as observed by other researchers [20]. According to these authors, the protein was the main component and polysaccharides or carbohydrates were the secondary component of the EPS matrix in sludge. The bacterial strain used in this study was isolated from sludge and also grown in sterilized sludge (as raw material), therefore, EPS contained higher protein similar to that observed in activated sludge process. The total protein/total carbohydrate ratio of B-EPS varied from 1.48 to 1.85 with fermentation time (Figure 2e). This variation can explain the distinct nature of B-EPS produced at different times of fermentation. The total protein/total carbohydrate ratio of B-EPS observed in this study was higher than reported in previous studies (0.34) in case of Serratia sp.1 [17] (Table 2). Thus, the EPS composition (i.e., protein and carbohydrate content) synthesised by the new strain was different than those reported by others [17]. In general, the composition of EPS is heterogeneous and varies based on many factors such as bacterial strain, growth phase, the EPS extraction method and different EPS production process parameters (temperature, pH, agitation speed, cultivation time, medium composition, medium pre-treatment etc.) [16].

**Flocculation activity**

The results of flocculation activity (FA) of B-EPS, S-EPS and C-EPS are presented in Figure 3. The flocculation activity for B-EPS and S-EPS decreased with an increase in EPS concentration. The highest FA (48 h sample) was 94.2% ± 1.3 for S-EPS (1.3 ± 0.1 mg S-EPS/g kaolin, Figure 3b), 86.8% ± 3.5 for B-EPS (2.6 ± 0.2 mg B-EPS/g kaolin, Figure 3c) and 79.4% ± 1.4 for C-EPS (0.50 ± 0.02 mg C-EPS /g of kaolin, Figure 3a). After attaining maximum value, a decrease in FA with EPS concentration was due to an over dosage of the polymer that caused re-suspension or instability of kaolin particles (flocs) leading to a high turbidity [21]. An equal volume of the samples

![Figure 2](image-url)  
*Figure 2: EPS composition in terms of protein and total carbohydrates at different incubation time: (a) Total carbohydrates (mg/L) in the medium; (b) Carbohydrate content of EPS (mg carbohydrate/g EPS), (c) Total protein mg/L in the medium; (d) Protein content of EPS (mg BSA/g EPS), (e) Total protein/Total carbohydrates ratio of B-EPS, S-EPS and C-EPS.*

![Figure 3](image-url)  
*Figure 3: Effect of EPS concentration (mg EPS/g kaolin) on flocculation activity (a) C-EPS, (b) S-EPS and (c) B-EPS, respectively.*
taken at different fermentation time exhibited different FA because of the variation in EPS concentration (Figure 3). The maximum FA of 86.8% ± 3.5 using 2.6 ± 0.2 mg B-EPS/g Kaolin observed in the present study was higher than the maximum FA (79.1%) obtained using 0.7 mg B-EPS/g kaolin in case of *Serratia* sp.[17] Higher FA achieved using S-EPS in the present study is due to the specific structure of EPS. The difference in results of FA for different types of EPS (B-EPS, S-EPS and C-EPS) could be due to the presence of diverse nature of proteins and carbohydrates. Proteins and carbohydrates are complex materials and may contain structurally different components (or functional groups), which may change with fermentation time (Figure 2) and thus affecting the FA of the EPS [22]. The protein content and type could play a dominant role in flocculation through hydrophobic interactions and polyvalent cations bridging, which increases the floc binding strength and hence enhancing the stability of the biopolymer network. Moreover, the hydrogen bonding capacity of carbohydrates also helps in flocculation [23]. The flocculation activity of B-EPS was lower than S-EPS. The difference in flocculation activity between S-EPS and B-EPS could be due to the fact that B-EPS contains both the C-EPS and S-EPS. C-EPS could hamper the efficiency of the S-EPS when two EPS were present together in the broth. C-EPS contained abundant hydrophilic compounds (hydroxyl group) that interacted with molecules of water hindering the combination of S-EPS (which contain hydrophobic compounds) with C-EPS or other hydrophobic particles [24]. Moreover, B-EPS have the negative surface charge and contains both EPS (S-EPS & C-EPS) as well as other substances such as colloidal and residual matter (cells, organic and inorganic material etc.). Increase in volume of the B-EPS in kaolin solution (the assay solution) also increases the negative surface charge due to increase in colloidal content, which could destabilize the flocs and thus decreases the flocculation activity. The charge of EPS can affect the flocculation activity. The zeta potential of fresh sludge was -89.1 ± 0.8 mV, with the addition of Ca²⁺, zeta potential increased to -45.9 ± 1.4 mV. The zeta potential of S-EPS (-47.9 ± 0.4 mV) is higher than that of C-EPS (-62.7 ± 0.9 mV) and that of B-EPS (-71.9 ± 1.2 mV). The higher zeta potential of S-EPS implies the degree of repulsion between the EPS molecules is less, which tend to improve the bio-flocculation.

The zeta potential of fresh sludge was -89.1 ± 0.8 mV, with the addition of Ca²⁺, zeta potential increased to -45.9 ± 1.4 mV. The zeta potential of kaolin suspensions (5 g/L) without Ca²⁺ and EPS was -38.4 ± 1.5 mV, and it was increased to -17.1 ± 0.5 mV by the addition of 150 mg of Ca²⁺/L. The addition of EPS, after Ca²⁺, to kaolin suspension had very small change in the charge. Therefore, charge neutralization of the kaolin particles was achieved mostly by the addition of Ca²⁺. However, the EPS addition after Ca²⁺ revealed high flocculation activity and enhanced dewaterability (discussed in the next section). These results suggest that the specific interactions of EPS and calcium with kaolin particles can be supported by adsorption and bridging mechanism.

**Sludge settling**

S-EPS exhibited better kaolin FA than other types of EPS. Therefore, S-EPS was used to estimate the sludge settling characteristics of pulp and paper industry activated sludge (PPS) (Figure 4) and municipal wastewater secondary sludge (MWWS) (Figure 5) at different suspended solids concentrations (1, 2, 5 and 7 g/L). The SVI was below 100 ± 1.5 mL/g after 30 min settling. For a good sludge settling, SVI ≤100 is required (APHA, 2005). The addition of cations (600 mg/L of Al[SO₄]₂⁻) without EPS slightly improved the SVI value of the control samples (SVI decreased from 140 to 110 mL/g in case of PPS with 7 g SS/L, and SVI decreased from 200 to 40 mL/g in case of MWWS with 5 g SS/L). The reduction of SVI in control is due to the coagulation effect of Al[SO₄]₂⁻ in combinations with the native EPS of sludge (1.6 ± 0.3 g EPS/L in fresh PPS and of 1.2 ± 0.5 g EPS/L in MWWS). Further, SVI
could explain the lower value of SVI at lower concentration of S-EPS. The higher protein content of EPS would improve bioflocculation and settling [20]. The protein probably is more important than the carbohydrates; the protein contents of EPS were found to have a positive relationship with SVI [18]. At SS 5 g/L, the lowest SVI was 60 mL/g using S-EPS concentration of 2 mg/L (400 mg S-EPS/g SS) produced by the bacterial strain BS8 (s.). Thus, the biopolymer produced by Bacillus sp.7 and Serratia sp.1 was more effective in sludge settling at a very low concentration (0.02 g/L or 4.0 ± 0.5 mg S-EPS/g SS) and SVI of the control was 50 ± 1 mL/g (kaolin). Consequently, an increase in EPS concentration above 1.3 ± 0.1 mg EPS/g of kaolin increased the amount of surface bound water by EPS, and thus the required CST value for a good dewaterability is 20 s [10]. The CST value did not vary much (changed from 21.4 ± 0.3 s to 21.9 ± 0.1 s) by increasing the S-EPS concentration from 1.3 ± 0.1 mg S-EPS/g Kaolin to 3.9 ± 0.5 mg S-EPS/g Kaolin; whereas the CST value increased from 23.2 ± 0.2 s to 29.4 ± 0.3 s by increasing the B-EPS concentration from 2.6 ± 0.2 mg B-EPS/g kaolin to 4.0 ± 0.5 mg B-EPS/g kaolin. Thus, S-EPS was more efficient than B-EPS. Poor dewaterability was observed in case of C-EPS. The CST value increased from 22.7 ± 0.5 s to 26.2 ± 0.4 s by increasing C-EPS concentration from 0.03 ± 0.01 mg C-EPS/g Kaolin to 0.09 ± 0.02 mg C-EPS/g Kaolin. Excessive EPS concentration might deteriorate cell attachment and weaken the floc structure, which in turn lead to a poor sludge settling and dewaterability [11]. In this work, S-EPS and B-EPS exhibited higher dewaterability than C-EPS in kaolin solution. This was due to the formation of bigger flocs caused by higher protein content of S-EPS and B-EPS (sample collected at 48 hr) compared to C-EPS. It was widely reported that an increase of EPS concentration would lower the sludge dewaterability [25,28]. Houghton et. al. [29] found that an increase in dewaterability with EPS (at low concentration of EPS) was due to the enhancement of flocculation. The increase in flocculation resulted in an increase in floc size and thus improved the sludge dewaterability. In the present case, an increase in EPS concentration above 1.3 ± 0.1 mg EPS/g of kaolin increased the amount of surface bound water by EPS, and thus decreased the kaolin dewaterability. Contrary to these findings, Jin et. al. [30] found that the concentration of the individual polymers and was substantially improved by the addition of S-EPS (Figures 4 and 5). The SVI varies with the concentration of sludge SS, the type of sludge and the added concentration of S-EPS. In this study, different volumes of S-EPS were used to obtain different concentrations of EPS. It was found that 1.5 mL (or 0.02 ± 0.01 g S-EPS/L) of S-EPS revealed the best settling compared to a lower or higher concentration of the EPS. An increase in SVI value with EPS concentration greater than 0.02 g/L (4 mg of S-EPS/g SS) was due to bound water increase into the aggregates, which produced highly porous flocs with low density [25]. Therefore, 0.02 g/L S-EPS concentration was used to evaluate the variation of SVI at different SS concentrations. In case of PPS at SS 5 g/L, the lowest SVI value was 20 ± 2 mL/g with an optimum concentration of EPS (1.5 mL or 0.02 ± 0.01 g S-EPS/L) and SVI of the control was 50 ± 1 mL/g. In case of MWWS at SS 5 g/L and same concentration of the EPS, the SVI value (20 ± 2 mL/g) was similar to the SVI value of the PPS; however, SVI of the control was different (40 ± 2.5 mL/g). The different SVI of the control of PPS and MWWS could be because of the difference of organic matter present in different sludges, which affected the bioflocculation process. These results of SVI are better than those reported by Bala subramanian et. al. [10] (Table 3). In case of MWWS at SS 5 g/L, the lowest SVI was 60 mL/g using S-EPS concentration of 2 g/L (400 mg S-EPS/g SS) produced by the bacterial strain BS8 (Serratia sp.). Thus, the biopolymer produced by Bacillus coagulans was more effective in sludge settling at a very low concentration (0.02 g/L or 4.0 ± 0.5 mg S-EPS/g SS) than the biopolymer produced by BS8 (Serratia sp.) [10]. The carbohydrate and protein contents of EPS were found to have a positive relationship with SVI [20]. The protein probably is more important than the carbohydrates; the high protein content of S-EPS would improve bioflocculation and settling property of activated sludge [26]. The higher protein content of the EPS produced by the present strain than that produced by Serratia sp. [17] could explain the lower value of SVI at lower concentration of S-EPS.

### Sludge dewaterability

The minimum CST value of kaolin solution was 23.2 ± 0.3 s (ΔCST = 16.7 ± 0.4 s), 21.4 ± 2.4 s (ΔCST = 18.5 ± 0.9 s) and 22.7 ± 0.7 s (ΔCST = 17.2 ± 1.2 s) with the addition of 2.6 ± 0.2 mg B-EPS/g Kaolin, 1.3 ± 0.1 mg S-EPS/g Kaolin and 0.03 ± 0.01 mg C-EPS/g Kaolin, respectively. These values were lower compared to the control sample (without addition of EPS) (39.9 ± 0.8 s). This result is better than that reported by other researchers [27] who found that the minimum CST value of the kaolin solution was 23.7 s (∆CST = 6.8 s) and 24.5 s (∆CST = 8.1 s) with the addition of B-EPS and S-EPS, respectively with a dose of 3.44 ± 0.05 B-EPS/g kaolin and 1.70 ± 0.05 S-EPS/g kaolin, respectively. However, the required CST value for a good dewaterability is 20 s [10]. The CST value increased with the increase in EPS concentration. The CST value did not vary much (changed from 21.4 ± 0.3 s to 21.9 ± 0.1 s) by increasing the S-EPS concentration from 1.3 ± 0.1 mg S-EPS/g Kaolin to 3.9 ± 0.5 mg S-EPS/g Kaolin; whereas the CST value increased from 23.2 ± 0.2 s to 29.4 ± 0.3 s by increasing the B-EPS concentration from 2.6 ± 0.2 mg B-EPS/g kaolin to 4.0 ± 0.5 mg B-EPS/g kaolin. Thus, S-EPS was more efficient than B-EPS. Poor dewaterability was observed in case of C-EPS. The CST value increased from 22.7 ± 0.5 s to 26.2 ± 0.4 s by increasing C-EPS concentration from 0.03 ± 0.01 mg C-EPS/g Kaolin to 0.09 ± 0.02 mg C-EPS/g Kaolin. Excessive EPS concentration might deteriorate cell attachment and weaken the floc structure, which in turn lead to a poor sludge settling and dewaterability [11]. In this work, S-EPS and B-EPS exhibited higher dewaterability than C-EPS in kaolin solution. This was due to the formation of bigger flocs caused by higher protein content of S-EPS and B-EPS (sample collected at 48 hr) compared to C-EPS. It was widely reported that an increase of EPS concentration would lower the sludge dewaterability [25,28]. Houghton et. al. [29] found that an increase in dewaterability with EPS (at low concentration of EPS) was due to the enhancement of flocculation. The increase in flocculation resulted in an increase in floc size and thus improved the sludge dewaterability. In the present case, an increase in EPS concentration above 1.3 ± 0.1 mg EPS/g of kaolin increased the amount of surface bound water by EPS, and thus decreased the kaolin dewaterability. Contrary to these findings, Jin et. al. [30] found that the concentration of the individual polymers and

### Table 2: Characterization of extracted EPS in terms of total protein and carbohydrates.

<table>
<thead>
<tr>
<th>Strains</th>
<th>B-EPS (g/L)</th>
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<th>Flocculation activity</th>
<th>Dewaterability of Kaolin</th>
<th>Dewaterability of sludge</th>
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<td>2.5</td>
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<td>77.38</td>
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<td>Sludge (25 g/L)</td>
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<td>1.3</td>
<td>59.9</td>
<td>5.2</td>
<td>37.6</td>
</tr>
</tbody>
</table>

Note: *TC- total carbohydrate; *TP- total protein. *Car- carbohydrates.

### Table 3: Comparison of flocculation activity, dewaterability and settling results.

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total EPS had negative correlations with CST. The results obtained for dewaterability on municipal and pulp and paper secondary sludge using S-EPS were presented (Figures 6a and 6b). At 2 g/L SS of MWWS, the CST value was decreased from 130 ± 2 s (in the control sample) to 81 ± 1 s with the addition of 0.02 ± 0.01 g/L of S-EPS (Figure 6b). A good dewaterability of PPS was achieved at 5 g/L SS; the CST value decreased from 10.0 ± 0.4 s (control) to 8.0 ± 0.1 s (with S-EPS dose of 0.02 ± 0.01 g/L) (Figure 6a). The EPS produced by *Cloacibacterium normanense* strain (this work) was more effective in lowering the CST value than the EPS present in sludge (1.6 g/L of PPS and 1.2 g/L of MWWS). In this work, reduction in CST was more effective at low EPS concentration compared to the results reported by Bala subramanian et al. [10], where the CST value decreased from 130 s (control) to 36.4 s after addition of 400 mg S-EPS /g SS produced by BS8 strain (Table 3). The dewaterability improvement was higher in MWWS than in PPS (Figure 6). This might be due to the difference in characteristics of organic matter in MWWS and PPS as well as the structure of protein and carbohydrate of EPS and their content in sludge [31,32].

**Conclusions**

High concentration (13 ± 0.8 g/L) of S-EPS and 0.3 ± 0.1 g/L C-EPS of extracellular polymeric substances (EPSs) was produced by *Cloacibacterium normanense* in sterilized sludge with 25 g/L suspended solids. EPS combined with Ca²⁺ demonstrated to be a good bioflocculant. Slime EPS exhibited higher flocculation activity (94.2%) and better dewaterability (59.9%) compared to the capsular EPS and the broth EPS in kaolin solution. The maximum dewaterability of municipal wastewater sludge (with suspended solids 2 g/L) achieved was 37.6% with the use of 0.02 ± 0.01 g/L of slime-EPS and 600 mg/L of Al₂(SO₄)₃. The study showed a promising approach of new isolated strain, which produced high concentration of EPS in sludge with high flocculation activity as well as good settling.

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**References**


