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Dewaterability and Degradability

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Article

Deciphering the Dual Roles of an Alginate-Based Biodegradable Flocculant in Anaerobic Fermentation of Waste Activated Sludge: Dewaterability and Degradability

Yi-Bo Wang,[‖] Jie Tang,[‖] Dan-Di Ran, Xiao-Mei Zhu, Si-Jie Zheng, Si-Di Hong, Shan-Fei Fu, Mark C. M. van Loosdrecht, Raymond Jianxiong Zeng,* Kun Dai,* and Fang Zhang*

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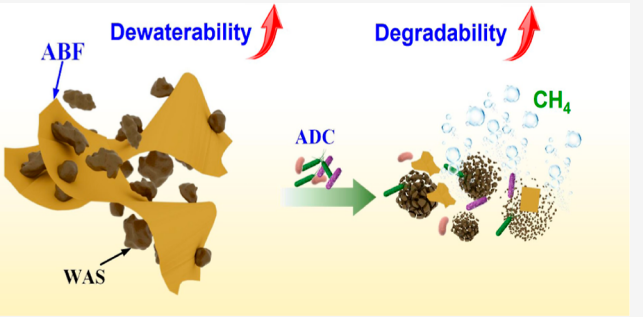
ABSTRACT: Biodegradable flocculants are rarely used in waste activated sludge (WAS) fermentation. This study introduces an alginate-based biodegradable flocculant (ABF) to enhance both the dewatering and degradation of WAS during its fermentation. Alginate was identified in structural extracellular polymeric substances (St-EPS) of WAS, with alginate-producing bacteria comprising ∼4.2% of the total bacterial population in WAS. Owing to its larger floc size, higher contact angle, and lower free energy resulting from the Lewis acid-base interaction, the addition of the prepared ABF with a network structure significantly improved the dewaterability of WAS and reduced capillary suction time (CST) by 72%. The utilization of ABF by an enriched alginate-degrading consortium (ADC) resulted in a 35.5% increase in the WAS methane yield owing to its higher hydrolytic activity on both ABF and St-EPS. Additionally, after a 30 day fermentation, CST decreased by 62% owing to the enhanced degradation of St-EPS (74.4%) and lower viscosity in the WAS + ABF + ADC group. The genus *Bacteroides*, comprising 12% of ADC, used alginate lyase (EC 4.2.2.3) and pectate lyase (EC 4.2.2.2 and EC 4.2.2.9) to degrade alginate and polygalacturonate in St-EPS, respectively. Therefore, this study introduces a new flocculant and elucidates its dual roles in enhancing both the dewaterability and degradability of WAS. These advancements improve WAS fermentation, resulting in higher methane production and lower CSTs.



KEYWORDS: *waste activated sludge, anaerobic digestion, alginate-based biodegradable flocculant, dewaterability, degradability*

1. 1. INTRODUCTION

The waste activated sludge (WAS) has emerged as a significant and undesirable byproduct of wastewater treatment plants (WWTPs).[1−3] In 2019, China produced over 60 million tons of WAS, with a water content of ∼80%.[3,4] Anaerobic digestion (AD) is a biological process that converts organic wastes into methane using volatile fatty acids (VFAs, such as acetate and butyrate) as intermediates.[1,2] However, in addition to the organic matter present in wastewater, WAS also contains numerous biological and chemical additives introduced during the wastewater treatment and sludge dewatering processes in WWTPs.[5,6] Inorganic coagulants and synthetic organic polymeric flocculants are widely used to effectively enhance solid−liquid separation.[6,7] For example, the concentration of cationic polyacrylamide (cPAM) in dewatered sludge typically ranges from 2.5 to 10 mg/g total suspended solids (TSS).[5] The presence of cPAM in WAS decelerates the AD process, leading to a reduction in methane production from 139 to 67 mL/g volatile suspended solids (VSS) and in VFAs from 3.4 to 2.4 g COD/L.[5,8] Therefore, these additives negatively affect sludge conversion and methane production, limiting the applicability of WAS fermentation.

Natural polymer-based flocculants, such as chitosan, starch, and cellulose, are widely available, environmentally friendly, and biodegradable.[9,10] However, these materials are often chemically modified to improve their dewatering properties, which can reduce the degradability of the resulting flocculants.[9,10] Therefore, developing biodegradable flocculants suitable for WAS fermentation is crucial to enhance methane production and dewatering. Extracellular polymeric substances (EPS), produced by bacteria in WWTPs, have been considered alternatives to traditional chemical-based flocculants.[11−13] A common gel-forming substance, previously known as alginate-like exopolysaccharides (ALE) but now termed structural EPS

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(St-EPS), has been extracted from WAS flocs, typically comprising 10−30% of the total composition.[14,15] Owing to its nontoxicity and minimal secondary pollution, alginate has been used as a flocculant in wastewater treatment.[17] Alginate, a linear natural polymer, exhibits a strong affinity for multivalent metal ions (e.g., Ca^{2+} and Mg^{2+}). Additionally, alginate is commonly used in the food industry to increase viscosity and serve as an emulsifier.[16] Therefore, this study aimed to investigate the role of alginate-based biodegradable flocculation (ABF) in enhancing both the dewatering and degradation of WAS.

The presence of alginate in WAS, such as the alginate-producing bacteria, remains unclear. Moreover, the mechanisms underlying the effect of ABF on the flocculation and anaerobic fermentation of WAS have been underexplored. Factors that may influence WAS treatment with ABF include changes in gel strength, metabolite concentration, and shifts in microbial diversity.[17] WAS flocculation may correlate with the network structure of the prepared ABF and the change in interfacial interaction energy between WAS and water.[18] Rheological analysis can identify changes in gel strength through assessments of sludge viscosity, elasticity, and the ratio of loss modulus to storage modulus.[19] Zhang et al. reported the enrichment of an alginate-degrading consortium (ADC) enriched from WWTPs, which could fully utilize alginate in St-EPS and enhance WAS fermentation.[4] However, the presence of multivalent metal ions (e.g., Ca^{2+} and Mg^{2+}) might play structural skeletal functions and hinder the degradation of alginate in prepared ABF.[20] For example, Lin et al.[6] reported that sludge treated with aluminum coagulants posed greater challenges in degradation owing to reduced efficiency of organic hydrolysis and acidogenesis. Furthermore, high-throughput sequencing and multiomic analyses provided comprehensive insights into the diversity and metabolic functions of enriched microbial consortia, particularly for the less abundant bacteria.[21,22]

In this study, the presence of alginate in extracted St-EPS was identified through chemical, high-throughput sequencing, and metagenomic analyses. Moreover, ABF was prepared, and its ability to enhance both the dewaterability and degradability of WAS was assessed using enriched ADC. Additionally, the mechanisms by which dosing with ABF influenced the dewatering and fermentation of WAS were investigated through interfacial interaction energy analysis, rheological testing, and metagenomic analyses. This study introduces a cheap and easily accessible flocculant to enhance WAS fermentation, leading to higher methane production and improved dewaterability efficiency.

2. MATERIALS AND METHODS

2.1. WAS Parameters and Enrichment of ADC. WAS was obtained from the Jinshan WWTPs (Fuzhou City, China). Table S1 (Supporting Information) summarizes typical values of pH, TSS, VSS, total COD (TCOD), soluble protein, and soluble polysaccharide in the collected WAS. An inoculum to enrich ADC bacteria was obtained from a batch reactor supplied with alginate and sparged with N_2 (>99.99%) for 20 min[23] Subsequently, a mesophilic (35 °C) chemostat with a working volume of 2.2 L was established, operating at a stirring velocity of 250 rpm and a hydraulic retention time of 4.3 days. The alginate concentration in the influent was maintained at 10 g/L, and the composition of the inorganic medium remained consistent with the previously reported formulation.[23] Methane, hydrogen, and VFA levels were assessed at least twice weekly for 60 days, while alginate and biomass concentrations in the effluent were analyzed weekly.

2.2. Preparation and Dewaterability of ABF. Owing to the correlation between the molecular weight of a macromolecular flocculant and its viscosity,[16] $CaCl_2$ was used to cross-link with alginate to prepare ABF (Figure S1). First, varying volumes of $CaCl_2$ (5 g/L) 0, 1, 2, 3, 4, and 5 mL were individually added to 30 mL of the alginate solution (5 g/L), and the mixture was stirred at 500 rpm and 25 °C. Subsequently, the viscosity of the alginate solution was determined. The prepared ABF with a high viscosity was introduced into 100 mL of WAS at a dosage of 29 mg/g TSS and stirred for 5 min before dewaterability tests were performed. Additionally, the extracted St-EPS and xanthan were used to prepare biodegradable flocculants under the same conditions as those for ABF, and the dosage for WAS dewatering was 19 and 6 mg/g TSS, respectively. Finally, a minimal amount of $FeCl_3$ (8.9 mg/g TSS[24]) was added to ABF to improve its dewatering performance based on capillary suction time (CST) values.

2.3. Effect of ABF on the Activity of ADC and Extracellular Enzymes. Four groups—control, alginate, ABF, and ABF + Fe—were used to assess the effect of ABF (at a dosage of 7.5 mL) on ADC activity and methane production. Further details are provided in Supporting Information. ADC was extracted from the effluent of the chemostat and centrifuged at 8000 rpm. The metabolites of methane and VFAs were determined. Moreover, four groups—control, alginate, ABF, and ABF + Fe—were prepared to evaluate the effect of ABF (at a dosage of 7.5 mL) on the activity of the ADC extracellular enzymes. These serum bottles were cultured under neutral pH conditions (7 ± 0.2) and maintained at 35 °C. The activity of the extracellular enzymes was determined using UV254 nm. Additionally, typical uronic acid compounds (3 g/L each), including polygalacturonate, xanthan, and hyaluronate, were used to assess methane production by the enriched ADC.

2.4. Anaerobic Fermentation of WAS with ABF and Enriched ADC. Two final groups, WAS and WAS + ABF + ADC (n = 3 for each) were prepared in 1 L reactors (with a working volume of 600 mL) to investigate the role of ABF in WAS fermentation. First, 500 mL of WAS and 100 mL of an inorganic medium were added to the WAS group. ADC was centrifuged to remove the supernatant. Subsequently, 500 mL of WAS, 75 mL of ABF, collected ADC (at a ratio of 0.1 g VSS/g VSS to WAS), and 25 mL of an inorganic medium were added to the WAS + ABF + ADC group. These reactors were cultured for 30 days under the same conditions described in Section 2.3. The concentrations of metabolites, changes in the EPS layers (referred to as S-EPS, LB-EPS, and TB-EPS layers), St-EPS, VSS, zeta potential, and rheological properties were all determined. Finally, the polyaluminum chloride (PAC, 0.037 g Al/g TSS[24]) was added to WAS to improve its dewaterability.

2.5. Analysis. The CH_4 and H_2 contents were determined via gas chromatography (SP7890, Lunan, CN). VFA concentrations were measured via gas chromatography (7890, Agilent, CA). The alginate concentration was measured through the carbazole-sulfuric acid method. Three EPS layers, S-EPS, LB-EPS, and TB-EPS, were extracted from WAS, and 3D-EEM fluorescence spectra were obtained using a fluorescence spectrophotometer (Cary Eclipse G9800A, Agilent Technologies, USA). Additionally, St-EPS was

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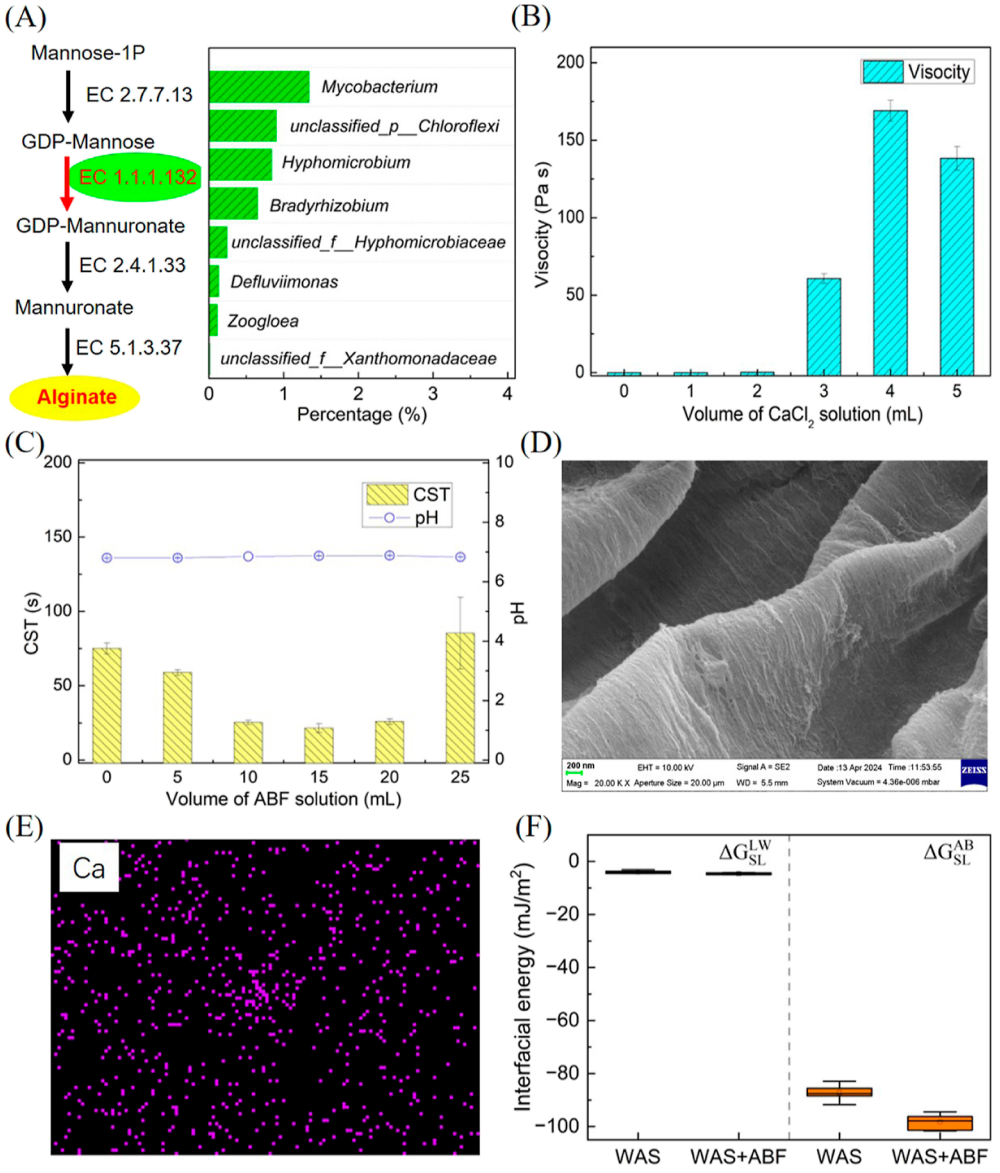

Figure 1. (A) Alginate-producing bacteria identified in the collected WAS; (B) viscosity of ABF; (C) CST and pH values of WAS dosed with ABF; (D) SEM and (E) EDS of prepared ABF; (F) interfacial thermodynamic analysis.

extracted from WAS through the heated Na_2CO_3 method (with details provided in Supporting Information).[25] The activity of ADC extracellular enzymes was determined using a UV−visible spectrophotometer (A560, AOE Instruments, CN). The zeta potential was analyzed using a particle size analyzer (Nanosizer ZS instrument, Malvern Co., UK). The CST value (n = 3) was measured using a CST instrument (model 319, Trion, London, UK). Moreover, the bound water content in WAS was measured through the centrifugal method (n = 3). The rheological properties were determined using a rheometer (MCR 301, Anton Paar Physica, Austria). The microstructure and elemental distribution (C, O, and Ca) were analyzed via scanning electron microscopy (SEM, Zeiss) and energy dispersive spectrometer (EDS), respectively (Merlin Compact, Germany). Contact angles of WAS and WAS−ABF samples were measured using a contact angle analyzer (JC2000C, Powereach, Shanghai, China) to obtain parameters for the interfacial thermodynamic analysis (details in Supporting Information). The centrifugal stability of WAS samples was measured using an analytical centrifuge (LUMiFuge, L.U.M, Germany).

2.6. Illumina Miseq High-Throughput Sequencing and Metagenomic Analyses. Four DNA samples were extracted from WAS, enriched ADC, WAS fermentation, and WAS + ABF + ADC fermentation on day 30. High-throughput sequencing was conducted to determine bacterial diversities in WAS fermentation (Majorbio Corporation, Shanghai, China). Metagenomic analysis was conducted on WAS and ADC samples to construct metabolic pathways for alginate production and degradation using the NovaSeq 6000 platform (Majorbio, China, with details provided in Supporting Information).

3. RESULTS

3.1. Identifying the Presence of Alginate in WAS and Assessing ABF Dewatering Properties. Figure 1A shows the comprehensive pathway identified for alginate production. The pathway indicated GDP-mannuronate as the precursor

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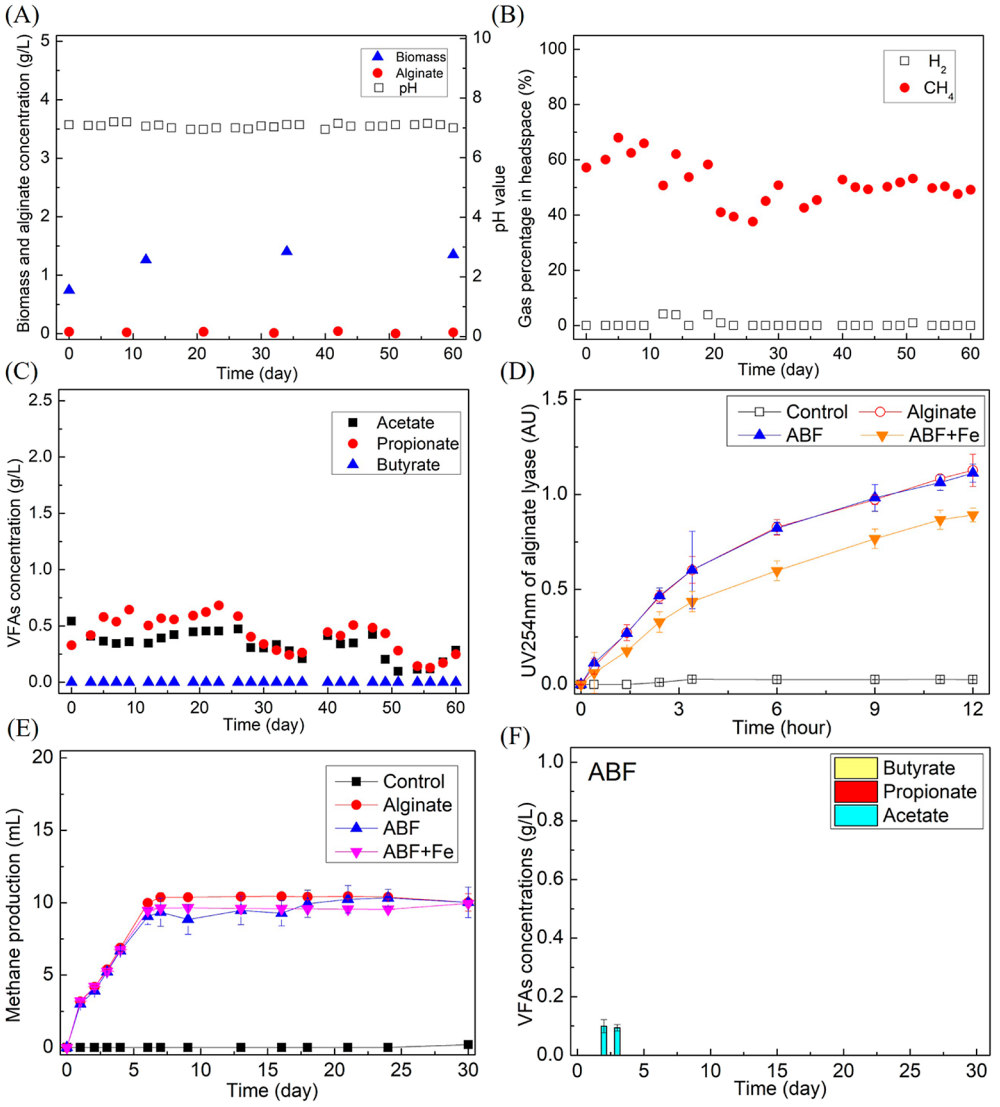

Figure 2. (A) pH values and biomass; (B) methane production; (C) VFA production in a mesophilic chemostat enriched with ADC; (D) extracellular alginate lyase activity; (E) methane production; (F) VFA accumulation from the prepared ABF.

responsible for generating alginate and other mannuronate-containing polysaccharides in the EPS of the WAS sample (Figures S2−S5 and Tables S2−S5). Approximately 4.2% of the bacteria, comprising *Mycobacterium* (1.34%) and *Zoogloea* (0.11%), were identified as potential alginate-producing bacteria based on the presence of the key enzyme EC 1.1.1.132. Notably, ∼7.0% of these bacteria could produce a uronic acid-rich polymer, polygalacturonate (Table S6). The WAS sample exhibited an St-EPS content of 46.8 ± 3.9 mg/g VSS, consistent with results reported by Qian (101 mg/g VSS) and Lin (72 mg/g VSS).[14,23] These results confirm the presence of uronic acids in St-EPS, including alginate and polygalacturonate, produced by the dominant bacteria in WAS.

Alginate exhibited a significantly high affinity for multivalent metal ions.[16] Thus, as the $CaCl_2$ concentration increased to 4 mL, the viscosity reached the maximum level at 169.0 ± 6.8 Pa·S and then decreased to 138.3 ± 7.6 Pa·S with higher Ca content (Figure 1B). The addition of the prepared ABF with the highest viscosity to 100 mL of WAS did not alter the pH (∼6.8) (Figure 1C). However, upon dosing 0.028 g of ABF/g TSS, the CST value significantly decreased by 72%, from 75.2 ± 3.7 to 21.5 ± 3.1 s. Moreover, a higher dosage of ABF deteriorated the dewatering of WAS, with the CST value increasing to 85.3 ± 24.2 s. SEM−EDS images revealed the formation of a network structure of ABF from the powdered alginate through cross-linking with Ca^{2+} (Figures 1D,E, and S6). Consequently, dosing the prepared ABF into WAS resulted in the formation of a thin film on the WAS surface (Figure S6), ensuring a more stable structure (Figure S7) and an increase in particle size from 96.1 to 128.9 μm. The addition of ABF led to a decrease in the hydrophilicity of WAS, as indicated by the contact angle increasing from 87.7° to 90.0° (Table S7). The free energy resulting from the Lewis acid−base interaction (ΔG_{adh}^{AB}, −83.1 vs −98.2 mJ/m^2) was the main factor determining the interfacial interaction energy (ΔG_{adh}, −85.3 vs −102.9 mJ/m^2) between the sludge flocs and water (Figure 1F and Table S7). However, the free energy generated through the van der Waals interaction (ΔG_{adh}^{LW}) remained unchanged (−4.0 vs −4.6 mJ/m^2). Thus, all these positive factors contributed to the good dewaterability of ABF.

The ABF method can also be used to prepare two other biodegradable flocculants from xanthan and extracted St-EPS, resulting in CST values decreasing by 47.4 and 61.3%, respectively (Figure S8). With the minimal addition of Fe^{3+}

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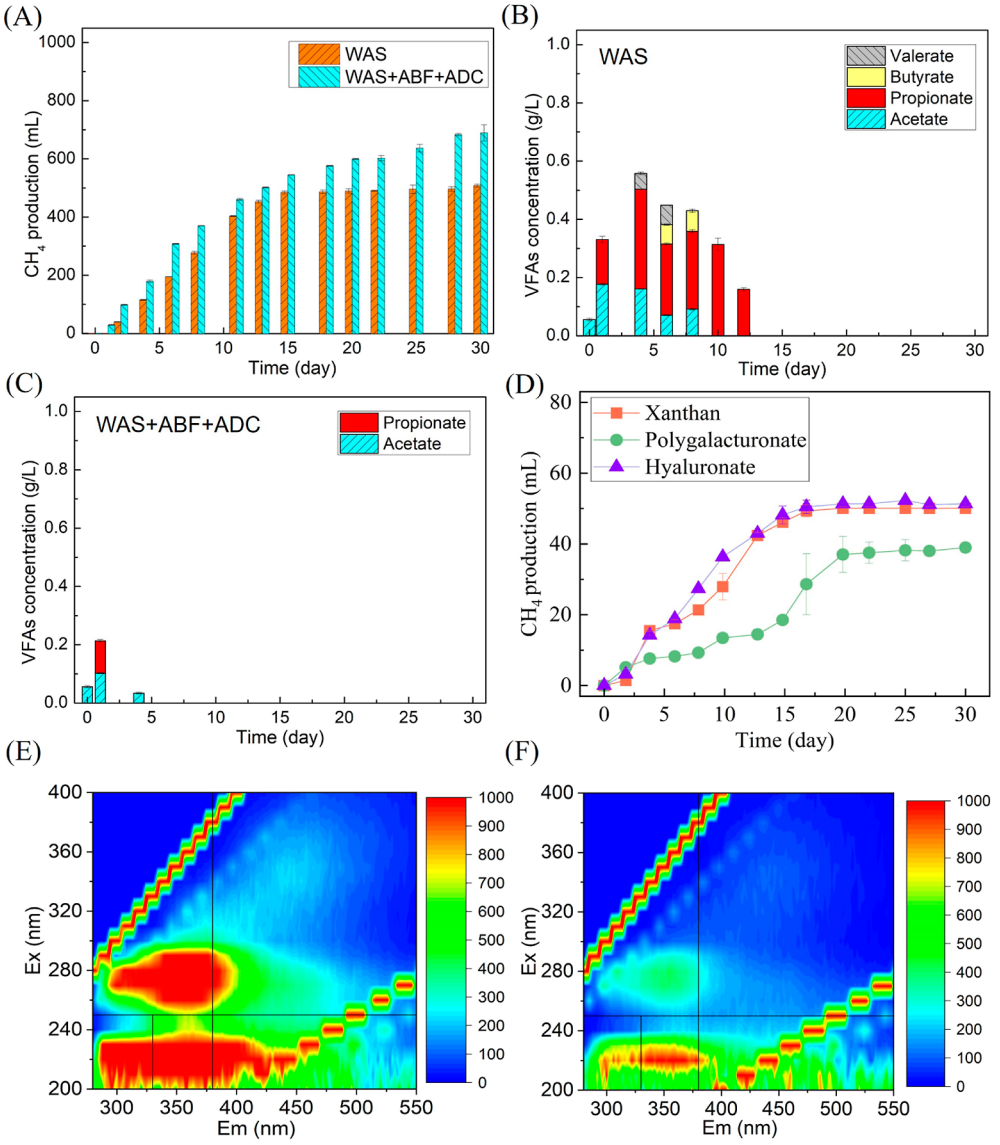

Figure 3. WAS fermentation with the addition of ABF. (A) Methane production; (B) VFA production in the WAS group; (C) VFA production in the WAS + ABF + ADC group; (D) methane production from three model compounds of uronic acids, and 3D-EEM fluorescence spectra of the TB-EPS layer in the WAS + ABF + ADC group on (E) days 0 and (F) 30.

(8.9 mg/g TSS), the CST value further decreased to 14.1 ± 1.0 s, indicating an 81.3% increase in the dewatering rate of ABF compared with the WAS group (Table S8). Additionally, upon dosing WAS with ABF, the water content of the sludge decreased to 90.6%, indicating a high dewatering rate for ABF. The degradation of ABF was investigated in the following sections.

3.2. Anaerobic Degradation of ABF by Enriched ADC.

Figure 2 illustrates the enrichment of ADC in a mesophilic chemostat using alginate (10 g/L) as the sole carbon substrate. The alginate concentration in the effluent consistently remained below 0.1 g/L, indicating that over 99% of alginate was degraded (Figure 2A). The biomass concentration ranged from 0.7 to 1.4 g VSS/L. Methane was the main metabolite in the headspace, with a production rate of 558 ± 77 mL/day (n = 12, Figure 2B). The H_2 consistently remained below 5% and even below 0.01%. Intermediates, such as acetate (<0.5 g/L), propionate (<0.7 g/L), and a trace amount of butyrate (<0.01 g/L), were detected in the liquid phase (Figure 2C). The average COD balance exceeded 90%, consistent with the strong activity of the enriched ADC.

The enzymatic activity of alginate lyase was examined (Figure 2D), with the alginate and ABF groups exhibiting final values of 1.1 ± 0.08 and 1.1 ± 0.05, respectively. The addition of minor Fe^{3+} (8.9 mg/g TSS) might influence the hydrolysis and anaerobic degradability of alginate, leading to a slight inhibition of alginate lyase activity, as reflected in the final UV254 nm values recorded as 0.9 ± 0.04. In the subsequent anaerobic degradation process, methane production remained below 1.9 mL on day 30 in the control group. Additionally, with the addition of only Ca^{2+} or Fe^{3+} to ADC, methane was not detected. After the addition of substrates either alginate or ABF, both alginate and ABF + Fe groups exhibited similar final methane production levels on day 30, with volumes of 10.0 ± 0.6 and 9.9 ± 0.02 mL, respectively (Figure 2E). During the initial 5 days, VFAs such as acetate, propionate, and butyrate were present in the alginate and ABF + Fe, ABF, and control groups (Figures 2F and S9), indicating a good COD balance of

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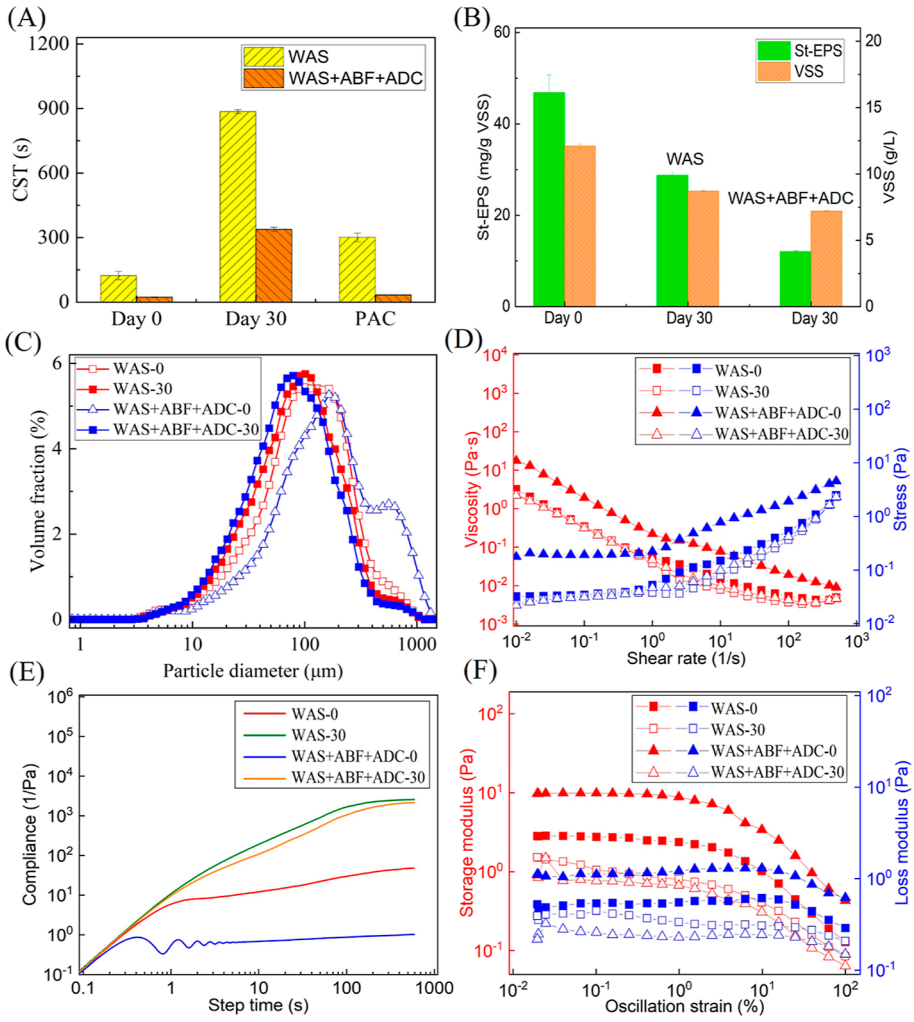

Figure 4. Changes in WAS properties after dosing with ABF. (A) CST values; (B) St-EPS and VSS contents; (C) particle diameters; (D) dynamic strain sweep; (E) creep tests; (F) typical rheogram.

90 to 96%. In previous studies, a high dosage of Fe^{3+} (16−30 mg/g TSS) may inhibit VFAs and methane production.[6,26] Therefore, the addition of Ca^{2+} and a minimal amount of Fe^{3+} to the prepared ABF did not adversely affect alginate degradation and methane production by the enriched ADC.

3.3. Anaerobic Degradation of WAS after Dosing with ABF and Enriched ADC. Figure 3 illustrates the WAS fermentation with the addition of ABF to the 1 L reactors. On day 30, methane production in WAS fermentation was measured at 507.9 ± 4.9 mL (Figure 3A). Upon dosing with enriched ADC in the WAS + ABF + ADC group, methane production significantly increased to 689.5 ± 27.7 mL, representing a 35.5% increase compared with the WAS group. According to the methane yield in Figure 2E, the methane production from ABF was 99 mL. Consequently, the methane production originating from St-EPS in WAS was calculated to be 82.6 mL. However, H_2 was not detected throughout the observation period. During the initial 12 days, acetate (<0.2 g/L), propionate (0.2−0.4 g/L), minor traces of butyrate (<0.1 g/L), and valerate (<0.1 g/L) were detected in the WAS group (Figure 3B). Moreover, in the WAS + ABF + ADC group, only acetate (<0.1 g/L) and propionate (<0.1 g/L) were detected. However, these compounds were completely consumed within the initial 4 days (Figure 3C).

The enriched ADC could produce methane from other model substrates, including xanthan (50.1 ± 0.5 mL), polygalacturonate (39.0 ± 0.5 mL), and hyaluronate (51.3 ± 0.2 mL), while no H_2 was detected (Figure 3D). After day 20, all VFAs generated were consumed (Figure S10). The 3D-EEM fluorescence spectra of the extracted EPS (Figures 3 and S11 and S12 and Table S9) confirmed that biodegradable materials such as protein were utilized by enriched ADC to produce methane, consistent with the results presented in Figure 3A. Overall, our results support the hypothesis that the prepared ABF can facilitate methane production from the enriched ADC, thereby enhancing WAS fermentation.

3.4. Dewaterability and Changes in St-EPS after Dosing WAS Fermentation with ABF and ADC. Figure 4A shows that the final CST value in the WAS group increased to 886 s on day 30. Similarly, Liu et al.[17] reported that CST values increased from 70 to 1418.6 s owing to higher viscosity at the end of WAS fermentation. Additionally, Liu et al.[27] reported a higher CST value of 1600 s during WAS fermentation. However, the final CST in the WAS + ABF + ADC group was 338 s, representing a 62% reduction compared with that in the WAS group (886 s). This reduction may be attributed to the degradation of ABF and St-EPS. Moreover, the WAS + ABF + ADC group (12.0 ± 0.2 mg/g VSS) exhibited a significantly lower extracted St-EPS content than

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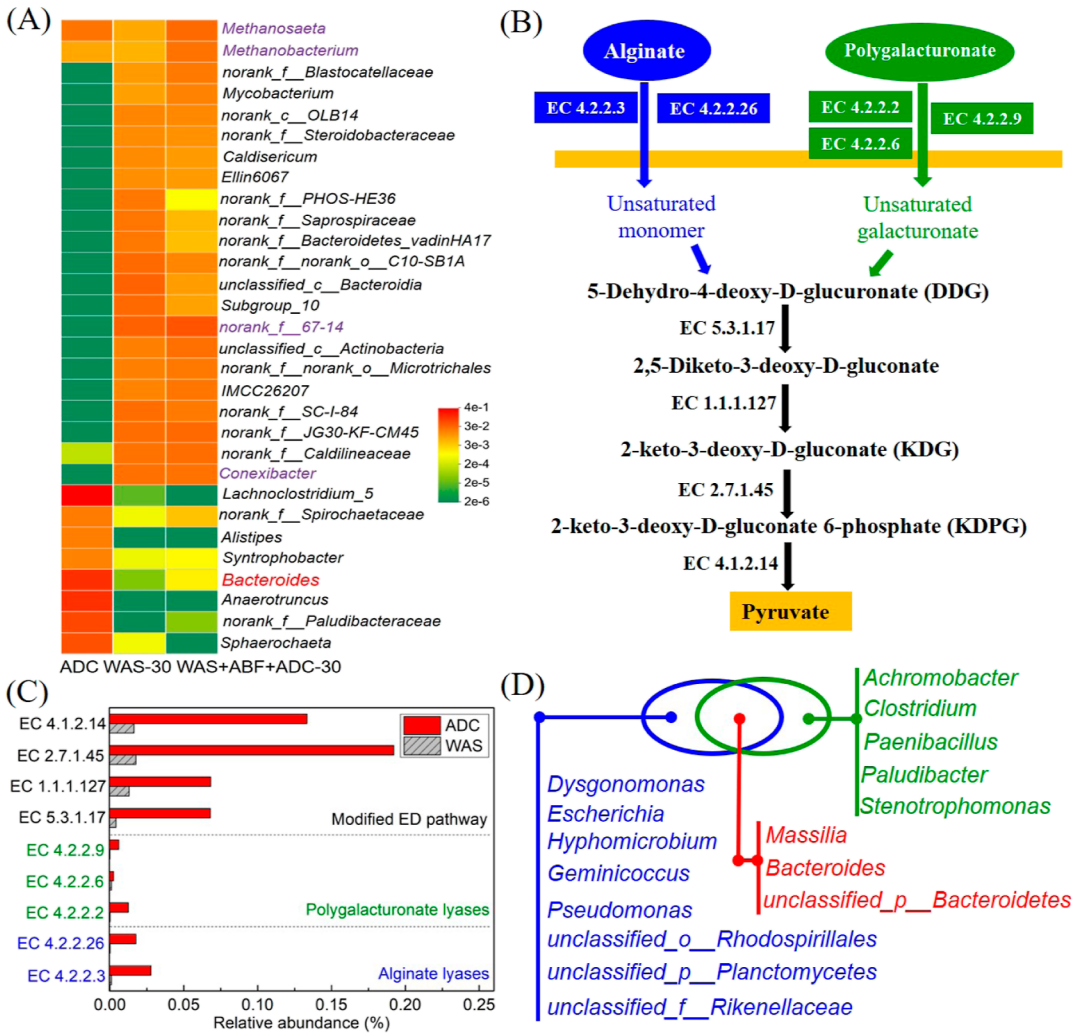

Figure 5. (A) Top 30 enriched bacteria at the genus level in WAS fermentation after dosing with ABF. (B) Constructed metabolic pathway. (C) Relative abundance of genes for identified enzymes. (D) Bacteria identified in the degradation of alginate and polygalacturonate.

the WAS group (28.7 ± 1.1 mg/g VSS) (Figure 4B). This corresponded to a degradation percentage of 74.4%, which was significantly higher than the 41% degradation reported by Guo et al.[28] in mesophilic anaerobic batch reactors without the enrichment of St-EPS degrading bacteria. St-EPS degradation may facilitate the breakdown of WAS flocs and enhance the dewaterability of WAS. For example, VSS levels in the WAS + ABF + ADC group significantly decreased, with a final VSS concentration of 7.2 g/L, compared with 8.7 g/L in the WAS group. Furthermore, the addition of PAC (0.037 g Al/g TSS) to the WAS + ABF + ADC group led to a significant decrease in the CST values to 33.2 s, which was beneficial for the subsequent dewatering process (Figure 4A). Thus, degradation of ABF and St-EPS can prevent any significant deterioration in WAS dewatering.

Additionally, St-EPS degradation may lead to lower particle size, zeta potential, and viscosity, all of which are beneficial for WAS dewatering. On day 30, the WAS group exhibited a particle size of 73.4 μm and a Zeta potential of −4.8 ± 0.5 mV. However, the WAS + ABF + ADC group exhibited a lower particle size (76.1 μm in Figure 4C) and higher Zeta potential (−22.5 ± 0.5 mV, Figure S13). Dosing with enriched ADC in the WAS + ABF + ADC group reduced both the apparent viscosity and shear stress, indicating the degradability of ABF and St-EPS in WAS dosed with ADC (Figure 4D). The creep tests (Figure 4E) revealed that the degradation of St-EPS led to the breakdown of WAS and further facilitated the deformation of WAS flocs (Figure 4A). Moreover, the dynamic strain sweep curve (Figure 4F) indicated that the maximum value of the angle tangent in the WAS sample decreased from 9.9 (on day 0) to 1.0 (on day 30). This reduction indicates that both the network strength and cohesive force of the WAS flocs were reduced by the enriched ADC. Therefore, these results indicate improved performance in both methane production and dewaterability with the addition of ADC to the WAS + ABF + ADC fermentation.

3.5. Microbial Diversity and Metabolism of ABF Degradation. Figure 5 shows the microbial diversity, metabolic pathway, and key bacteria involved in ABF degradation. The Shannon index curve, rarefaction curve, and main sequencing indices (Figure S14 and Tables S2 and S10) indicated that the sequencing results comprehensively represented the entire enriched consortia. At the domain level (Figure S14), the bacterial percentage in all three groups exceeded 95%. Figure 5A summarizes the percentages of the top 30 enriched bacteria at the genus level. In the presence of alginate as the sole carbon source, *Lachnoclostridium*_5 (41.0%), *Bacteroides* (12.7%), and *Anaerotruncus* (12.6%)

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were the main enriched genera in ADC. In the WAS group, the three main genera comprised norank_f_67−14 (5.64%), *Conexibacter* (4.44%), and norank_*Saprospiraceae* (3.59%). Similarly, in the WAS-30 and WAS + ABF + ADC-30 groups, the main genera consisted of norank_f__67−14 (4.3 and 5.4%), *Conexibacter* (2.9 and 2.9%). Additionally, the proportions of *Lachnoclostridium*_5, *Bacteroides*, and *Anaerotruncus* were below 0.01% in the WAS + ABF + ADC-30 group. The proportions of alginate producers, namely *Mycobacterium* and *Zoogloea*, in the WAS group decreased to below 0.1% in both the WAS-30 and WAS + ABF + ADC-30 groups. Two archaeal genera, *Methanobacterium* (hydrogenotrophic *methanogens*) and *Methanosaeta* (acetoclastic *methanogens*), were identified as the main methane producers. Therefore, dosing WAS with ABF did not significantly alter the microbial diversity owing to the low ratio of inoculum (ADC) to WAS (0.1 g/g VSS) in the WAS + ABF + ADC-30 group.

Figure 5B presents the metabolic pathway of alginate degradation, which was further investigated through metagenomic analysis. Extracellular alginate lyases (EC 4.2.2.3 and EC 4.2.2.26) were detected in the metabolic pathway (Figures S15 and S17). This process was crucial for alginate degradation because these lyases hydrolyzed alginate into unsaturated monomers. All key enzymes involved in the conversion of produced monomers to pyruvate through modified Entner−Doudoroff and Embden−Meyerhof−Parnas pathways were identified. Because alginate and polygalacturonate were typical components of St-EPS in WAS,[29] the St-EPS contents decreased even during WAS fermentation in the absence of enriched ADC (Figure 4B). The metabolic pathway for the degradation of polygalacturonate, an isomer of alginate, is further elucidated in Figure 5B. The extracellular enzymes for polygalacturonate hydrolysis were identified as pectate lyases (EC 4.2.2.2, 4.2.2.6, and 4.2.2.9 in Figures S16 and S18).[30]

Figure 5C illustrates the genic relative abundance of identified enzymes, as shown in Figure 5B. Particularly, the alginate lyase exhibited genic percentages of 0.028% for EC 4.2.2.3 and 0.018% for EC 4.2.2.26 in the enriched ADC, which were 28 times and 180 times those in WAS (0.001 and 0.0001%). Moreover, Figure 5D shows the top ten genera were identified as key bacteria in enriched ADC in the presence of alginate lyase (Table S11). Additionally, eight genera were detected as polygalacturonate-degrading bacteria (GDB, Figure 5D) in the presence of pectate lyases (Table S12). Notably, three genera, *Bacteroides*, unclassified_p__*Bacteroidetes*, and Massilia, can degrade both alginate and polygalacturonate. Therefore, the enriched ADC exhibited higher hydrolytic activity in St-EPS conversion compared with WAS, thereby enhancing methane production in WAS fermentation.

4. DISCUSSION

4.1. Benefits of ABF Utilization in WAS Dewatering. To date, only few reports focused on the use of flocculants to enhance the dewaterability and degradability of WAS during its fermentation. ALE can form a gel matrix via cross-linking with Ca^{2+} to maintain the structural integrity of WAS.[31,32] In this study, a new flocculant, ABF, was synthesized from alginate via cross-linking with Ca^{2+} to enhance WAS dewatering for the first time. The significant 72% decrease in the CST value can be attributed to the efficient trapping effect of the network structure of ABF on WAS particles, leading to an increase in floc size. With the addition of ABF, the hydrophilicity of WAS decreased, evident from a higher contact angle of 90.0°. The reduced free energy resting from the Lewis acid−base interaction was the main factor influencing the interfacial interaction energy between the sludge flocs and water. These positive effects contributed to enhanced solid−liquid separation (Figure 1). Because alginate in St-EPS can be considered a gel-forming polysaccharide in WAS flocs,[15] the degradation of both ABF and St-EPS by enriched ADC may be crucial for breaking down the WAS structure and promoting dewatering. The WAS treated with ABF and enriched ADC exhibited a lower final St-EPS content than the control group, resulting in reduced viscosity and shear stress (Figure 4). The treated WAS exhibited a significantly lower CST value (∼380 s) than the untreated WAS (886 s). Previous studies have reported CST values exceeding 1600 s[27] Our data indicated that the addition of ABF and ADC can prevent the deterioration of WAS dewatering, which poses a significant challenge to efficient WAS fermentation.

Notably, the preparation method for ABF can also be applied to xanthan and extracted St-EPS to enhance WAS dewatering. The dosages of alginate (29 mg/g TSS), xanthan (6 mg/g TSS), and extracted St-EPS (18 mg/g TSS) were similar to that of cPAM (∼10 mg/g TSS[5]). According to information collected from Alibaba (https://www.alibaba.com), the cost of alginate (1000−1200 US$/ton) was lower than that of commercial cPAM (1800−2700 US$/ton). Brown seaweed comprises the largest marine algae source, with an annual global production of 15.8 million wet tons. Alginate is recognized as the main matrix component in the cell wall, comprising up to 60% of the dry weight.[33] Thus, the development of ABF from seaweed can provide a cheap and easily available flocculant for WAS fermentation. The cost of xanthan (2000−2800 US$/ton) was similar to that of PAM. Direct extraction of St-EPS from WAS provided a significantly lower-cost alternative. Additionally, the prepared ABF can be used to produce methane. Therefore, this study presents a promising method for preparing biodegradable flocculants. However, further research, including optimization of molecular weight and investigation into the addition of other polysaccharides, is required to improve the dewatering properties of ABF for future applications.

4.2. Environmental Implication of Applying ABF in WAS Fermentation. The addition of commonly used inorganic coagulants (i.e., PAC and Fe) and synthetic organic polymeric flocculants (i.e., cPAM) to WAS may reduce methane production.[5,17,34] Studies have shown that the presence of cPAM in WAS can reduce methane production by ∼50% and VFAs by ∼30%.[5] However, the ABF prepared in this study avoided these drawbacks, as the added flocculant can also serve as a substrate for methane production. This can be attributed to the multifunctional capability of enriched ADC to degrade alginate, xanthan, and polygalacturonate in St-EPS. The proportion of genus *Bacteroides* in WAS remained below 0.03%, which was significantly lower than that of the enriched ADC (12%). Lü et al.[35] found that dosing with polygalacturonase (EC 3.2.1.15) increased the release of total polysaccharides in EPS by 7-fold. Recent results have shown that dosing with ADC or GDB can promote methane production.[4,36] Thus, these findings support the assumption that a higher content of genus Bacteroides in WAS may promote St-EPS hydrolysis and the release of organics, which is beneficial for subsequent acidogenesis and methanogenesis. Moreover, WAS treated with ABF exhibited a lower final St-EPS content than the control group, leading to higher methane production

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during WAS fermentation. Moreover, no additional pretreatment was required for WAS fermentation, resulting in lower operational costs and a reduced need for skilled operators. Overall, these results indicate that EPS-derived biodegradable flocculant can serve as a promising alternative for WAS fermentation, which may enhance methane production.

An appropriate amount of cPAM (4 mg/g TSS) could mitigate the toxicity of ZnO nanoparticles in WAS fermentation by reducing levels of reactive oxygen species induced by the ZnO nanoparticles.[37] The potential role of ABF in detoxifying these external chemicals will be investigated in the future. Figure S19 illustrates the concept of applying ABF in an operational WWTP. Additionally, ALE was considered a potential resource with potentially high added value. For example, Kim et al. demonstrated the flame retardant property of flax fabrics coated with EPS recovered from both activated sludge and aerobic granular sludge.[38] The extraction of EPS for ALE from aerobic granular sludge has been extensively investigated and implemented in the Netherlands.[39] Furthermore, biopolymers can be extracted and purified from WAS, with concentrations ranging from 90 to 190 mg/g VSS.[40] Additionally, Xue et al. identified the key bioactivities of polysaccharides (such as fucoidan, carrageenan, and heparin), namely antiangiogenesis, anticoagulant, and antioxidant, in WAS.[41] Recently, Liu et al. demonstrated that combining polysaccharides with methane recovery from WAS can provide an efficient and sustainable approach to sludge treatment.[42] Therefore, extracting these valuable materials before applying ABF to WAS may enhance the efficiency of anaerobic fermentation and contribute to a more sustainable operation of WWTPs.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.4c05971.

> The Supporting Information is available free of chargeAdditional tables (Tables S1−S12) and figures (Figures S1−S19) about the WAS parameters, method to prepare ABF, ABF morphology and interfacial interaction energy, VFAs production, Zeta potential, microbial diversity by high-throughput sequencing, and metagenomic analysis of WAS and enriched ADC, and concept of applying ABF in WWTPs (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Toutian, V.; Barjenbruch, M.; Unger, T.; Loderer, C.; Remy, C. Effect of temperature on biogas yield increase and formation of refractory COD during thermal hydrolysis of waste activated sludge. *Water Res.* **2020**, *171*, 115383.

(2) Hu, Z.-Y.; Wang, S.; Geng, Z.-Q.; Dai, K.; Ji, W.-X.; Tian, Y.-C.; Li, W.-T.; Zeng, R. J.; Zhang, F. Controlling volatile fatty acids production from waste activated sludge by an alginate-degrading consortium. *Sci. Total Environ.* **2022**, *806*, 150730.

(3) Wu, B.; Dai, X.; Chai, X. Critical review on dewatering of sewage sludge: Influential mechanism, conditioning technologies and implications to sludge re-utilizations. *Water Res.* **2020**, *180*, 115912.

(4) Zhang, F.; Qian, D.-K.; Geng, Z.-Q.; Dai, K.; Zhang, W.; van Loosdrecht, M. C. M.; Zeng, R. J. Enhanced Methane Recovery from Waste-Activated Sludge by Alginate-Degrading Consortia: The Overlooked Role of Alginate in Extracellular Polymeric Substances. *Environ. Sci. Technol. Lett.* **2021**, *8* (1), 86−91.

(5) Wang, D.; Liu, X.; Zeng, G.; Zhao, J.; Liu, Y.; Wang, Q.; Chen, F.; Li, X.; Yang, Q. Understanding the impact of cationic polyacrylamide on anaerobic digestion of waste activated sludge. *Water Res.* **2018**, *130*, 281−290.

(6) Lin, L.; Li, R.-h.; Yang, Z.-y.; Li, X.-y. Effect of coagulant on acidogenic fermentation of sludge from enhanced primary sedimenta-

tion for resource recovery: Comparison between $FeCl_3$ and PACl. *Chem. Eng. J.* **2017**, *325*, 681−689.
(7) Akgul, D.; Abbott, T.; Eskicioglu, C. Assessing iron and aluminum-based coagulants for odour and pathogen reductions in sludge digesters and enhanced digestate dewaterability. *Sci. Total Environ.* **2017**, *598*, 881−888.
(8) Liu, X.; Xu, Q.; Wang, D.; Wu, Y.; Yang, Q.; Liu, Y.; Wang, Q.; Li, X.; Li, H.; Zeng, G.; Yang, G. Unveiling the mechanisms of how cationic polyacrylamide affects short-chain fatty acids accumulation during long-term anaerobic fermentation of waste activated sludge. *Water Res.* **2019**, *155*, 142−151.
(9) Yang, Z.; Hou, T.; Ma, J.; Yuan, B.; Tian, Z.; Yang, W.; Graham, N. J. D. Role of moderately hydrophobic chitosan flocculants in the removal of trace antibiotics from water and membrane fouling control. *Water Res.* **2020**, *177*, 115775.
(10) Salehizadeh, H.; Yan, N.; Farnood, R. Recent advances in polysaccharide bio-based flocculants. *Biotechnol. Adv.* **2018**, *36* (1), 92−119.
(11) Kant Bhatia, S.; Gurav, R.; Choi, Y.-K.; Choi, T.-R.; Kim, H.-j.; Song, H.-S.; Mi Lee, S.; Lee Park, S.; Soo Lee, H.; Kim, Y.-G.; Ahn, J.; Yang, Y.-H. Bioprospecting of exopolysaccharide from marine *Sphingobium yanoikuyae* BBL01: Production, characterization, and metal chelation activity. *Bioresour. Technol.* **2021**, *324*, 124674.
(12) Salehizadeh, H.; Yan, N. Recent advances in extracellular biopolymer flocculants. *Biotechnol. Adv.* **2014**, *32* (8), 1506−1522.
(13) Ding, G.; Li, X.; Lin, W.; Kimochi, Y.; Sudo, R. Enhanced flocculation of two bioflocculation-producing bacteria by secretion of Philodina erythrophthalma. *Water Res.* **2017**, *112*, 208−216.
(14) Lin, Y. M.; Sharma, P. K.; van Loosdrecht, M. C. M. The chemical and mechanical differences between alginate-like exopolysaccharides isolated from aerobic flocculent sludge and aerobic granular sludge. *Water Res.* **2013**, *47* (1), 57−65.
(15) Sarvajith, M.; Nancharaiah, Y. V. Properties of alginate-like exopolymers recovered from flocculent and granular microbial sludges of different biological treatment systems treating real municipal wastewater. *Sep. Purif. Technol.* **2023**, *313*, 123460.
(16) Felz, S.; Kleikamp, H.; Zlopasa, J.; van Loosdrecht, M. C. M.; Lin, Y. Impact of metal ions on structural EPS hydrogels from aerobic granular sludge. *Biofilm* **2020**, *2*, 100011.
(17) Liu, Q.; Li, Y.; Yang, F.; Liu, X.; Wang, D.; Xu, Q.; Zhang, Y.; Yang, Q. Understanding the mechanism of how anaerobic fermentation deteriorates sludge dewaterability. *Chem. Eng. J.* **2021**, *404*, 127026.
(18) Hou, X.; Liu, S.; Zhang, Z. Role of extracellular polymeric substance in determining the high aggregation ability of anammox sludge. *Water Res.* **2015**, *75*, 51−62.
(19) Wang, H.-F.; Ma, Y.-J.; Wang, H.-J.; Hu, H.; Yang, H.-Y.; Zeng, R. J. Applying rheological analysis to better understand the mechanism of acid conditioning on activated sludge dewatering. *Water Res.* **2017**, *122*, 398−406.
(20) Pang, H.; An, L.; Zhang, Y.; He, J.; Yan, Z.; Xie, B. Divalent cation chelation enhancing carbon migration and recovery from anaerobic fermentation of waste activated sludge. *Chem. Eng. J.* **2023**, *457*, 141374.
(21) Geng, Z.-Q.; Qian, D.-K.; Hu, Z.-Y.; Wang, S.; Yan, Y.; van Loosdrecht, M. C. M.; Zeng, R. J.; Zhang, F. Identification of Extracellular Key Enzyme and Intracellular Metabolic Pathway in Alginate-Degrading Consortia via an Integrated Metaproteomic/Metagenomic Analysis. *Environ. Sci. Technol.* **2021**, *55* (24), 16636−16645.
(22) Liu, C.; Huang, H.; Duan, X.; Chen, Y. Integrated Metagenomic and Metaproteomic Analyses Unravel Ammonia Toxicity to Active Methanogens and Syntrophs, Enzyme Synthesis, and Key Enzymes in Anaerobic Digestion. *Environ. Sci. Technol.* **2021**, *55* (21), 14817−14827.
(23) Qian, D.-K.; Geng, Z.-Q.; Tang, J.; Wang, S.; Hu, Z.-Y.; Dai, K.; van Loosdrecht, M. C. M.; Zeng, R. J.; Zhang, F. Highly Selective Fermentation of Waste-Activated Sludge by Alginate-Degrading Consortia. *ACS ES&T Eng.* **2021**, *1* (11), 1606−1617.
(24) Wang, H.-F.; Hu, H.; Wang, H.-J.; Zeng, R. J. Combined use of inorganic coagulants and cationic polyacrylamide for enhancing dewaterability of sewage sludge. *J. Clean. Pro* **2019**, *211*, 387−395.
(25) Felz, S.; Al-Zuhairy, S.; Aarstad, O. A.; van Loosdrecht, M. C. M.; Lin, Y. M. Extraction of Structural Extracellular Polymeric Substances from Aerobic Granular Sludge. *JoVE* **2016**, *115*, No. e54534.
(26) Zhan, W.; Li, L.; Tian, Y.; Lei, Y.; Zuo, W.; Zhang, J.; Jin, Y.; Xie, A.; Zhang, X.; Wang, P.; Li, Y.; Chen, X. Insight into the roles of ferric chloride on short-chain fatty acids production in anaerobic fermentation of waste activated sludge: Performance and mechanism. *Chem. Eng. J.* **2021**, *420*, 129809.
(27) Liu, H.; Wang, Y.; Wang, L.; Yu, T.; Fu, B.; Liu, H. Stepwise hydrolysis to improve carbon releasing efficiency from sludge. *Water Res.* **2017**, *119*, 225−233.
(28) Guo, H.; Felz, S.; Lin, Y.; van Lier, J. B.; de Kreuk, M. Structural extracellular polymeric substances determine the difference in digestibility between waste activated sludge and aerobic granules. *Water Res.* **2020**, *181*, 115924.
(29) Felz, S.; Vermeulen, P.; van Loosdrecht, M. C. M.; Lin, Y. M. Chemical characterization methods for the analysis of structural extracellular polymeric substances (EPS). *Water Res.* **2019**, *157*, 201−208.
(30) Garron, M.-L.; Cygler, M. Uronic polysaccharide degrading enzymes. *Curr. Opin. Struct. Biol.* **2014**, *28*, 87−95.
(31) Wilén, B. M.; Jin, B.; Lant, P. Relationship between flocculation of activated sludge and composition of extracellular polymeric substances. *Water Sci. Technol.* **2003**, *47* (12), 95−103.
(32) Dueholm, M. K. D.; Besteman, M.; Zeuner, E. J.; Riisgaard-Jensen, M.; Nielsen, M. E.; Vestergaard, S. Z.; Heidelbach, S.; Bekker, N. S.; Nielsen, P. H. Genetic potential for exopolysaccharide synthesis in activated sludge bacteria uncovered by genome-resolved metagenomics. *Water Res.* **2023**, *229*, 119485.
(33) Ghadiryanfar, M.; Rosentrater, K. A.; Keyhani, A.; Omid, M. A review of macroalgae production, with potential applications in biofuels and bioenergy. *Renew. Sustain. Energy Rev.* **2016**, *54*, 473−481.
(34) Zhan, W.; Tian, Y.; Zhang, J.; Zuo, W.; Li, L.; Jin, Y.; Lei, Y.; Xie, A.; Zhang, X. Mechanistic insights into the roles of ferric chloride on methane production in anaerobic digestion of waste activated sludge. *J. Clean. Pro* **2021**, *296*, 126527.
(35) Lü, F.; Wang, J.; Shao, L.; He, P. Enzyme disintegration with spatial resolution reveals different distributions of sludge extracellular polymer substances. *Biotechnol. Biofuels* **2016**, *9* (1), 29.
(36) Hu, Z.-Y.; Lin, Y.-P.; Wang, Q.-T.; Zhang, Y.-X.; Tang, J.; Hong, S.-D.; Dai, K.; Wang, S.; Lu, Y.-Z.; van Loosdrecht, M. C. M.; Wu, J.; Zeng, R. J.; Zhang, F. Identification and degradation of structural extracellular polymeric substances in waste activated sludge via a polygalacturonate-degrading consortium. *Water Res.* **2023**, *233*, 119800.
(37) Zhang, B.; Tang, X.; Fan, C.; Hao, W.; Zhao, Y.; Zeng, Y. Cationic polyacrylamide alleviated the inhibitory impact of ZnO nanoparticles on anaerobic digestion of waste activated sludge through reducing reactive oxygen species induced. *Water Res.* **2021**, *205*, 117651.
(38) Kim, N. K.; Mao, N.; Lin, R.; Bhattacharyya, D.; van Loosdrecht, M. C. M.; Lin, Y. Flame retardant property of flax fabrics coated by extracellular polymeric substances recovered from both activated sludge and aerobic granular sludge. *Water Res.* **2020**, *170*, 115344.
(39) Feng, C.; Lotti, T.; Lin, Y.; Malpei, F. Extracellular polymeric substances extraction and recovery from anammox granules: Evaluation of methods and protocol development. *Chem. Eng. J.* **2019**, *374*, 112−122.
(40) Li, J.; Hao, X.; Gan, W.; van Loosdrecht, M. C. M.; Wu, Y. Recovery of extracellular biopolymers from conventional activated sludge: Potential, characteristics and limitation. *Water Res.* **2021**, *205*, 117706.

https://doi.org/10.1021/acs.est.4c05971
Environ. Sci. Technol. 2024, 58, 14282−14292

(41) Xue, W.; Zeng, Q.; Lin, S.; Zan, F.; Hao, T.; Lin, Y.; van Loosdrecht, M. C. M.; Chen, G. Recovery of high-value and scarce resources from biological wastewater treatment: Sulfated polysaccharides. *Water Res.* **2019**, *163*, 114889.
(42) Liu, J.; Zhang, Z.; Deng, Y.; Sato, Y.; Wu, D.; Chen, G. Coupling methane and bioactive polysaccharide recovery from wasted activated sludge: A sustainable strategy for sludge treatment. *Water Res.* **2023**, *233*, 119775.

https://doi.org/10.1021/acs.est.4c05971
Environ. Sci. Technol. 2024, 58, 14282−14292