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Series of Combined Pretreatment Can Affect the Solubilization of Waste-Activated Sludge

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Abstract: Various pretreatment methods have been combined and employed for maximizing the solubilization of waste-activated sludge (WAS). However, the question "by changing the series of applied combined pretreatments (CPs), can the solubilization efficiency of WAS be affected?" has never been addressed. In this study, firstly, thermal (T), alkaline (A), and ultrasonic (U) pretreatments were individually applied at broad strengths (T = 80–120 °C for 30 min, A = pH 9–12, and U = 5–60 min at 300 W). Then, pretreatment conditions that caused similar solubilization (13.0%) (120 °C, pH 11, and 30 min for T, A, and U, respectively), were adopted for CP with reverse sequences of T&A, U&A, and T&U. A similar disintegration degree was observed in U→A and A→U, while a meaningful difference was found in T&A and T&U: T→A (28.3%), A→T (42.9%), T→U (22.9%), and U→T (27.1%). The difference in pretreatment series also affected the characteristics of soluble matters, which was analyzed by excitation emission matrix and molecular weight distribution. Due to these differences, the highest methane yield of 68.8% (based on (chemical oxygen demand) COD_{input}) was achieved at A→T, compared to T→A (62.3%). Our results suggested a simple strategy for increasing solubilization, at the same expense of energy, which might be beneficial in the following treatment process, such as dewatering and transportation.

Keywords: combined pretreatment; sequence; waste-activated sludge; solubilization; methane yield; anaerobic digestion

1. Introduction

By the development and increase in the number of wastewater treatment plants, the amount of sludge that is discharged is increasing [1]. Because of the non-biodegradable fraction contained in sludge and its high viscous characteristics, the management of the sludge costs more than half of the costs dedicated for wastewater treatment plants' operation [2,3]. There are largely two types of sludge: primary sludge and waste-activated sludge (WAS), which are made of organic matters, recalcitrant matters, alive microbes, and water. Primary sludge contains mainly easily biodegradable matters, then, it can be easily treated [4,5]. WAS can be simply defined as the aggregated flocs which contain extracellular polymeric substances, microbial cells, and hazardous pollutants [6,7]. Although WAS treatment is mandatory, it is still a problematic issue, because of the recalcitrant content found in

WAS. Among various technologies utilized for WAS treatment, anaerobic digestion (AD) is considered an effective technology, from economic and environmental standpoints [8].

AD is widely applied on WAS for valorization, stabilization, and pathogens' reduction [9,10]. Applying AD upon sludge has many advantages, e.g., (i) limiting sludge volume, which plays a major role in minimizing the costs associated to handling/disposing of sludge, (ii) reducing sludge-caused health problems [11], and (iii) harvesting renewable energy, which is harvested as methane (CH_4) [12]. However, applying AD on WAS still has some limitations, such as the need for long solid retention time (>20 days), and poor digestion efficiency (20–50%) [13]. For instance, when WAS is subjected to AD for 30 days, under mesophilic conditions, the generated CH_4 was found to be lower than 240 mL CH_4/g volatile solids (VS), which is much less than the theoretical range (450–600 mL CH_4/g VS) [14].

To overcome poor AD efficiency, WAS pretreatment is required. The goals of WAS pretreatment are flocs' disintegrating and cells' breaking [15]. As a result, a releasing for both intracellular and extracellular materials can be acquired [16]. Various pretreatment methods including thermal [17], alkaline [18], and ultrasonic [19] treatment have been employed. Pretreatments can be applied individually, but also sometimes in combination, to maximize WAS solubilization and/or CH_4 recovery in the subsequent AD [15]. The solubilization of WAS could be increased to over 60% by combined pretreatment (CP), which was limited to less than 30% in the individual pretreatment [20,21]. However, no previous research has highlighted the impact of altering the pretreatment series upon either solubilization or digestion performance.

On the other hand, many previous studies referred to the fact that the increased solubilization, after the pretreatment, is not always yielding an improved efficacy in the subsequent AD [20,22–24]. This might be assigned to some consequences that can happen as a result of harsh pretreatment conditions, such as the release of recalcitrant and inhibitory soluble organic compounds from the sludge flocs to liquid portion [20,24,25]. Therefore, pretreatment efficiency term should be more linked to the generated CH_4 in the AD process, than the achieved solubilization. Since some WAS soluble content is not biodegradable, investigating the change in the characteristics of the soluble content, found in WAS, can help in evaluating the efficiency of the WAS pretreatment method [26,27]. In this regard, excitation-emission matrix (EEM) and molecular weight distribution (MWD) can be employed for characterizing WAS soluble content, which helps in predicting the effectiveness of the applied pretreatment process.

The aim of this work is to answer the question "by changing the series of applied CP, can the solubilization efficiency and CH_4 recovery from WAS be affected?". Therefore, we firstly examined the efficacy of applying individual pretreatments (thermal, alkaline, and ultrasonic). Then, we tested six CP sets, (alkaline→thermal) vs. (thermal→alkaline), (thermal→ultrasonic) vs. (ultrasonic→thermal), and (ultrasonic→alkaline) vs. (alkaline→ultrasonic). After pretreatments, WAS disintegration degree (DD) was measured, and characteristics of pretreated WAS filtrate were identified by using EEM spectrum and MWD. Later, we conducted a biochemical CH_4 potential (BMP) test by using the whole and soluble fraction of pretreated WAS.

2. Materials and Methods

2.1. Feedstock and Inoculum

WAS was collected from the thickener line of a local sewage treatment plant, aerobic/anaerobic treatment plant, with a capacity of 27,500 ton/days, in the city of Incheon, Korea. Prior to use, a filtration was done using a sieve with pore size of 2 mm, in order to exclude suspended solids of big size, then saved at 4 °C to avoid an undesired microbial reaction. The characteristics of WAS were: total solids (TS): 23,380 ± 130 mg/L, volatile solids (VS): 17,140 ± 100 mg/L, total chemical oxygen demand (TCOD): 27,264 ± 1063 mg/L, soluble chemical oxygen demand (SCOD): 1070 ± 28 mg/L, and pH: 6.4 ± 0.1. The seeding sludge utilized in BMP experiments was from a lab-scale mesophilic AD reactor (10 L of effective volume), which has been operated and fed with food waste and WAS (1:1 on weight

basis) for three months, showing a reasonable CH_4 yield of 0.15–0.20 L CH_4/g (chemical oxygen demand) COD_{added}. Concentrations of TS, VS, TCOD, and pH of digester sludge were 20.0 g/L, 18.2 g/L, 24.6 g/L, and 7.7, respectively.

2.2. Pretreatment and BMP Test

The impact of WAS pretreatment, using individual pretreatment, i.e., alkaline (A), thermal (T), and ultrasonic (U), upon WAS solubilization was tested, whereas the tested strength range was (T = 80–120 °C for 30 min, A = pH 9–12, and U = 5–60 min at 300 W). The conditions that gave similar solubilization impact of around 13.0% (SCOD/TCOD) were found to be 120 °C, pH 11, and 30 min for T, A, and U respectively, and were selected for the subsequent CP approaches. During thermal pretreatment, heating has been done using a water bath, whereas the beaker was covered using aluminum foil in order to minimize the potential evaporation. While, alkaline pretreatment was carried out through raising the pH, by the addition of NaOH (6 N), and then alkalized WAS was stirred for one hour using a magnetic stirrer at agitation of 150 rpm. Thereafter, the impact of CP upon WAS solubilization was investigated using the aforementioned strengths, i.e., 120 °C, pH 11, and 30 min for T, A, and U, respectively. Six pretreatment sets, (A→T), (T→A), (T→U), (U→T), (U→A), and (A→U) referring to (alkaline→thermal), (thermal→alkaline), (thermal→ultrasonic), (ultrasonic→thermal), (ultrasonic→alkaline), and (alkaline→ultrasonic) respectively, were tested, whereas for all pretreatment sets, the first mentioned pretreatment is the first one applied, while the second mentioned pretreatment is the latter one applied.

The BMP of pretreated WAS was investigated using the total and the soluble fraction of pretreated WAS, for obtaining CH_4 yields (MY) of total (MYt) and soluble fraction (MYs), respectively. The test was conducted using serum bottles with working volume of 200 mL (a total volume of 270 mL). For each bottle, the substrate concentration and inoculum to substrate ratio were adjusted to be 2.0 g COD/L and 5.0 g volatile suspended solids (VSS)/g COD, respectively. Trace metals and nutrients were added to batch bottles [28,29]. Initial pH was adjusted to 7.5 ± 0.1 using KOH (2 N) and HCl (2 N) solutions, then purging was done for the serum bottles using 99.99% N_2 gas for 10 min for securing anaerobic condition. Thereafter, batch bottles were sealed by butyl rubber stoppers, and were put in an incubator under controlled agitation and temperature of 150 rpm and 37 ± 0.1 °C, respectively. The volume and composition of the generated biogas were checked periodically until almost no biogas was produced. All experiments were carried out in duplicate, and the averages of the results were taken.

2.3. Calculation and Analysis

The solubilization impact of any pretreatment was examined as a DD, which was calculated by the following Equation (1):

$$DD\ (\%) = (SCOD_{\text{after pretreatment}} - SCOD_{\text{initial}})/(TCOD_{\text{initial}} - SCOD_{\text{initial}}) \times 100 \quad (1)$$

MYt and MYs were calculated as the actual produced CH_4 divided by theoretical maximum produced CH_4 (Equation (2)), while CH_4 yield of particulate fraction of pretreated waste-activated sludge (MYp) was calculated by Equation (3). The solubilization refers to the percentage of SCOD to TCOD.

$$\text{MYt, MYs}\ (\%) = \frac{\text{Actual amount of generated } CH_4}{\text{Theoretical maximum amount of generated } CH_4} \times 100 \quad (2)$$

$$\text{MYp}\ (\%) = \frac{\text{MYt} - (\text{Solubilization} \times \text{MYs})}{100 - \text{Solubilization}} \times 100 \quad (3)$$

For investigating statistical significance, via *p*-value determination, analysis of variance (ANOVA) was implemented using Microsoft Excel 2019 software. TCOD, SCOD, TS, VS, VSS, and pH were measured according to Standard Methods [30], whereas for SCOD, a filtration was implemented using

a 0.45 µm membrane. For measuring the contents of CH_4 and CO_2, sampling was implemented from the headspaces of the batch bottles using a gas-tight micro-syringe. In order to analyze the collected gas samples, a gas chromatograph (Gow-Mac Series 580, Gow-Mac instrument Co., Bethlehem, PA, USA) with a thermal conductivity detector and a 1.8 m × 3.2 mm stainless-steel column was utilized. The temperatures of injector, detector, and column were kept at 50, 90, and 80 °C respectively, while N_2, with a flow rate of 30 mL/min, was used as a gas carrier. Sonication was implemented using a probe sonicator (STH-750S, Sonictopia, Cheongwon, Korea) with a frequency of 20 kHz. EEM and MWD were analyzed for the pretreatment resultant, whereas samples of the pretreated WAS were filtrated using a 0.45 µm membrane, then the concentrations were normalized to 60 mg COD/L. Afterwards, EEM was investigated using fluorescent spectroscopy (Shimadzu RF530, Shimadzu Co., Kyoto, Japan) at excitation wavelengths from 220 to 380 nm and emission from 250 to 600 nm [31]. MWD was analyzed using size exclusion chromatography connected with high-performance liquid chromatography (Younglin YL9101, YOUNG IN Chromass, Anyang, Korea) using polysaccharides (Dextran) as a standard. All the utilized chemicals were obtained from Sigma–Aldrich (St. Louis, MO, USA).

3. Results and Discussion

3.1. Solubilization

Table 1 shows DD values for individual and CP. Altering the sequence of the CP set affected the achieved DD; however, a variation in solubilization impact was noticed among various CP sets. Altering the sequence in cases of T&A and T&U could affect the solubilization of WAS, while altering the sequence in case of U&A did not lead to the significant change in the acquired solubilization. The highest solubilization effect of 42.9% ± 2.9% was achieved by (A→T), while (T→A) showed only 28.3% ± 1.6%. Also, a noticeable increase in the solubilization was observed when (T→U) (22.9% ± 1.4%) was altered to (U→T) (27.1% ± 1.2%). For each CP, the synergistic impact could be calculated as the difference between the actual DD and calculated DD, which is the summation of the DD resulted from each single pretreatment involved in the CP [15]. Synergistic impacts were observed because of combining any two pretreatments, except (T→U).

The big gap in DD, observed by altering (T→A) to (A→T), can be explained based on the relationship between the mechanism of pretreatment strategy and its timing, either applied first or second. Basically, the solubilization efficacy achieved by thermal treatment was found to decrease in the following order: carbohydrates > proteins > lipids [32]. That is why the lipidic content of WAS is less affected if the thermal treatment was applied in the beginning of pretreatment combination, as in (T→A). On the other hand, if the alkaline pretreatment was functioned as a first step in the CP, an efficient lipids saponification can be done by the strong OH^- attack [24]. Then, the subsequent thermal pretreatment would be more effective, since it can efficiently handle defragmented cell components, as in (A→T). However, to prove the above hypothesis, detailed analysis with more experiments is needed for identifying the impact of alkali upon lipids in WAS.

Further, by altering (T→U) to (U→T), a higher solubilization impact was noticed. The reason behind this increased solubilization could be the available solid content, which is subjected to sonication. The solid content in case of (U→T) was bulkier than that in (T→U) [33]. Previous research highlighted that sonication is more efficient if it is directed to mixture that has high solid content up to a certain value, since more hydro-mechanical shear force and cavitation sites are formed [34]. Moreover, in case of higher solid content, the statistical probability of solid to be affected by the formed mechanical jet streams, generated by implosion of sonication bubbles, is getting higher, leading to more floc rupture [35].

Table 1. Effect of different series of pretreatments on the solubilization of waste activated sludge, and CH_4 yield from different fractions of pretreated waste activated sludge.

Series of Pretreatments	DD * by Individual Pretreatment (%)			Calculated DD (%) (A = a + b + c)	Actual DD (%) (B)	Synergistic Impact (%) (C = B − A)	CH_4 Yield from Total (MYt, %)	CH_4 Yield from Soluble Fraction (MYs, %)	CH_4 Yield from Particulate Fraction (MYp, %)
	Alkaline (=a)	Thermal (=b)	Ultrasonic (=c)						
(A → T)	13.2 ± 1.0	12.7 ± 0.8	-	25.9	42.9 ± 2.9	17.0	68.8 ± 2.9	80.3 ± 4.7	60.2
(T → A)	13.2 ± 1.0	12.7 ± 0.8	-	25.9	28.3 ± 1.6	2.4	62.3 ± 3.6	82.4 ± 4.2	54.4
(T → U)	-	12.7 ± 0.8	12.8 ± 0.9	25.5	22.9 ± 1.4	−2.6	57.6 ± 2.4	88.3 ± 5.7	48.5
(U → T)	-	12.7 ± 0.8	12.8 ± 0.9	25.5	27.1 ± 1.2	1.6	56.7 ± 3.3	94.6 ± 4.0	42.6
(U → A)	13.2 ± 1.0	-	12.8 ± 0.9	26.0	30.7 ± 1.3	4.7	57.5 ± 3.6	84.8 ± 5.1	45.4
(A → U)	13.2 ± 1.0	-	12.8 ± 0.9	26.0	32.0 ± 2.1	6.0	58.6 ± 3.3	84.2 ± 5.8	46.6

* DD = disintegration degree.

Table 1 refers to the fact that (A→T), which showed the highest solubilization impact, in terms of DD, could not lead to harvesting relatively higher MYs. Then, it can be stated that DD has a certain limit, beyond which the further increase in DD will not necessarily lead to enhanced MYs anymore. Previous researchers also stated that the increased solubilization of excess sludge could not always guarantee improved digestion efficacy [20,24].

3.2. EEM and MWD

Figure 1 manifests the EEM spectrum for soluble filtrates of pretreated WAS, harvested after applying different CP sets. Based on the method provided in a previous study [36], the EEM spectrum was delineated into five main regions. The fluorescence regions, I ((excitation) Ex/(emission) Em wavelengths: 200–250 nm/280–330 nm), II (Ex/Em wavelengths: 200–250/330–380 nm), III (Ex/Em wavelengths: 200–250/>380 nm), IV (Ex/Em wavelengths: >250/280–380 nm), and V (Ex/Em wavelengths: >250/>380 nm), were caused by tyrosine-like substances, biological oxygen demand content (mainly aromatic protein II), fulvic acid-like substances, soluble microbial products (SMP)-like substance (tryptophan and protein-like group), and humic-like substances, respectively. The intensities of regions I and II were almost the same for all tested CP, while the intensity of the IV peak seems varied. Specifically, fluorescence intensity of regions I and II was higher than that for region IV in the cases of (T→A) and (T→U) (Figure 1b and c). These finding highlights that when thermal pretreatment was firstly applied, like in the cases of (T→A) and (T→U), only loosely bound-extracellular polymeric substances were released [37]. This result agrees with limited DD, achieved by (T→A) and (T→U). Further, since the conversion of compounds of high molecular weight into low molecular weight is considered as one of degradation efficiency indicators [38], MWD was analyzed for the filtrate (Figure 2). Apparently, altering the sequence altered the obtained MWD. An obvious MWD difference can be noticed between (A→T) and (T→A) (Figure 2a). However, a very slight difference was observed among the T&U pair (Figure 2b), while no difference at all could be noted between MWD of (U→A) and (A→U) (Figure 2c).

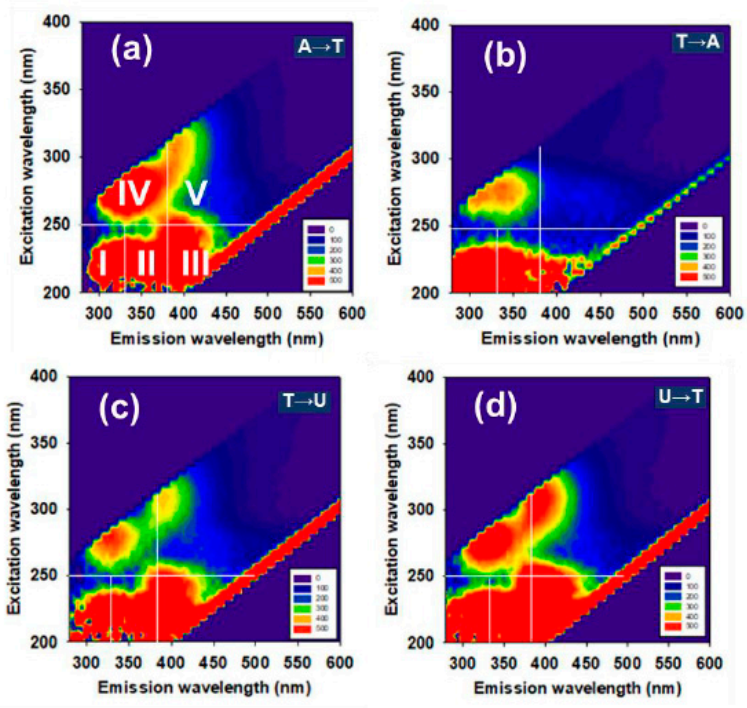

Figure 1. *Cont.*

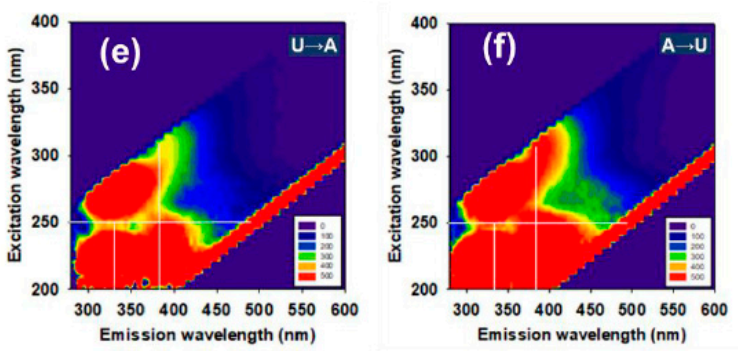

Figure 1. Excitation-emission matrix spectrum for the filtrate obtained from waste-activated sludge (WAS) pretreated by (**a**) alkaline→thermal (A→T), (**b**) thermal→alkaline (T→A), (**c**) thermal→ultrasonic (T→U), (**d**) ultrasonic→thermal (U→T), (**e**) ultrasonic→alkaline (U→A), and (**f**) alkaline→ultrasonic (A→U) pretreatments.

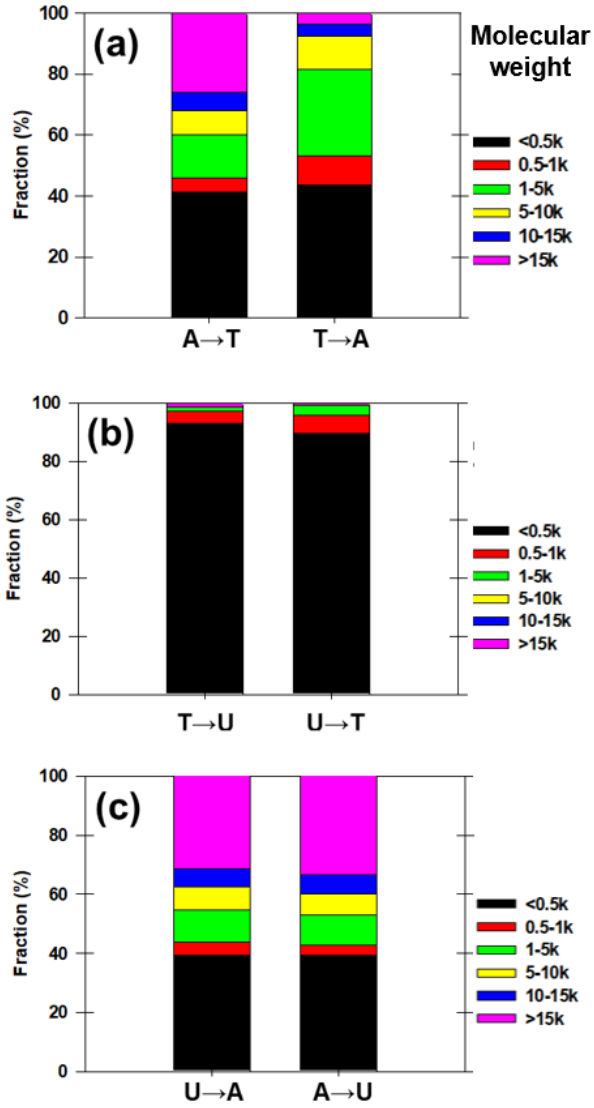

Figure 2. Molecular weight distribution comparison between filtrates of (**a**) alkaline→thermal vs. thermal→alkaline, (**b**) thermal→ultrasonic vs. ultrasonic→thermal, and (**c**) ultrasonic→alkaline vs. alkaline→ultrasonic pretreatments.

3.3. CH_4 Yield

The variation in EEM and MWD among various CP sets unveiled the possibility of obtaining different values for MYs. This could be confirmed by Table 1 data, where opposite CP yielded different MYs. Altering A&T and T&U sets led to variance in MYs values by 2.1% and 6.3% respectively, while MYs variation among the two U&A sets was negligible. The reason behind higher MYs from (U→T) compared to (T→U) could be the higher release of SMP (Region IV at Figure 1c, d). However, the EEM result in A&T sets is not consistent with MYs achieved, where a higher intensity for SMP release was observed in case of (A→T) than that of (T→A). This can be explained through the MWD result, in which more release for compounds of low molecular weight was acquired by (T→A), compared to (A→T) (Figure 2a,b). Previous studies referred to the effectiveness of using EEM [39] and MWD [38] as powerful tools for depicting MYs. Many previous works regarding EEM seem to agree that the higher intensity at region IV (tryptophan and protein-like group) warrant more effective WAS biodegradability and higher BMP results [26,40], while there has been an inconsistency regarding MWD results' connotation. A previous study mentioned that compounds with molecular weight lower than 27 kDa are the most beneficial for the digestion process [41]; however, in another study by the same group, the dominance of compounds with molecular weight of 5.6 kDa boosted CH_4 level from sludge [42]. Therefore, it is highly recommended to use both tools (EEM and MWD) in order to accurately explain the difference in MYs.

Different sequences also led to different values in MYt. Specifically, opposing (T→A) into (A→T) caused an increase in MYt by 6.5%, while MYt differences among other CP sets were negligible. Almost similar MYs with DD for (U→A) and (A→U) can be the reason behind why there was no big difference among them. In order to highlight the importance of the acquired increase in MYt (6.5%), it is worthy to refer to a previous study, where, boosting sludge temperature to 80 °C and raising the oxidation power of the used oxidizing agent (H_2O_2) by four times raised the harvested CH_4 yield only by 7.1% [43]. Altering the sequence of A&T and T&U caused the difference in MYp by 5.8% and 5.9% respectively, which was calculated by Equation (2). There have not been many previous papers addressing MYp, and it was found that MYp can be changed with the change in the applied pretreatment strategy and pretreatment strength [20]. Statistical analysis showed that MYt and MYs have statistically significant correlation with actual solubilization, with *p*-values of 0.04 and 0.02, respectively. However, *p*-values higher than 0.05 were found in the case of the relation between calculated solubilization vs. MYt and MYs. This seems to be logical since the calculated solubilization does not involve the synergistic/antagonistic impact, achieved after combing two pretreatments. On the other hand, neither calculated nor actual solubilization could correlate with MYp. This might be because MYp was not experimentally measured, rather, it was calculated.

Although altering the sequence in CP sets showed less impact upon AD efficiency compared to solubilization, it could be advantageous in the further process of WAS. For example, the reduction in the solid content could lead to lower viscosity of WAS, providing easier pumping and dewatering [44]. From an engineering standpoint, the finding of this study is, therefore, of importance, since it refers to the possibility of achieving higher solubilization and CH_4 yield just by altering the sequence of the utilized CP, which does not overload any additional energy burden. Interestingly, sequence changing does not require change in the infra-structure of the currently applied CP units. Further work needs to be implemented in order to evaluate the detailed impact of changing the pretreatment sequence upon overall economic efficiency of the WAS management process. By combing the results of Sections 3.2 and 3.3, it would be apparent that estimating MYs cannot be done through the individual usage of either EEM or MWD results, but rather, combing the two techniques is a more recommended strategy for estimating MYs.

4. Conclusions

Altering the sequence in CP could affect the solubilization of WAS, where the biggest differences in DD of 42.9% ± 2.9% and 28.3% ± 1.6% were achieved by (A→T) and (T→A), respectively. Reasons for

the differences can be the mechanism of action and the order of application, either firstly or secondly, for each step in CP, as well as the type of released substances. The opposite sequence also led to different EEM and MWD results, which can be linked to explaining different CH_4 yields.

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Abbreviations

Term:	Abbreviation:
Waste-activated sludge	WAS
Combined pretreatment	CP
Disintegration degree	DD
Alkaline followed by thermal pretreatment	A→T
Thermal followed by alkaline pretreatment	T→A
Thermal followed by ultrasonic pretreatment	T→U
Ultrasonic followed by thermal pretreatment	U→T
Ultrasonic followed by alkaline pretreatment	U→A
Alkaline followed by ultrasonic pretreatment	A→U
Soluble microbial products	SMP
Anaerobic digestion	AD
Volatile solids	VS
Volatile suspended solids	VSS
Total solids	TS
Total chemical oxygen demand	TCOD
Soluble chemical oxygen demand	SCOD
Excitation-emission matrix	EEM
Molecular weight distribution	MWD
Biochemical methane potential	BMP
CH_4 yield from total fraction of pretreated waste-activated sludge	MYt
CH_4 yield from soluble fraction of pretreated waste-activated sludge	MYs
CH_4 yield from particulate fraction of pretreated waste-activated sludge	MYp

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