

1           **Improved valorization of sewage sludge in the circular economy by**  
2           **anaerobic digestion: impact of an innovative pretreatment technology**

3    Nalok Dutta<sup>a#</sup>, Anthony T. Giduthuri<sup>a,c#</sup>, Muhammand Usman Khan<sup>a,e</sup>, Richard Garrison<sup>b</sup>,  
4    Birgitte K. Ahring<sup>a,c,d,\*</sup>

5    <sup>a</sup> Bioproducts, Sciences and Engineering Laboratory, Washington State University, Tri-Cities,  
6    Richland, WA 99354, United States

7    <sup>b</sup> Clean-Vantage LLC, 99354 Richland, WA, United States.

8    <sup>c</sup> The Gene and Linda Voiland School of Chemical Engineering and Bioengineering,  
9    Washington State University, Pullman, WA 99163, United States

10   <sup>d</sup> Department of Biological Systems Engineering, Washington State University, Pullman, WA  
11   99163, United States.

12   <sup>e</sup> Department of Energy Systems Engineering, University of Agriculture, Faisalabad, Pakistan.

13   <sup>#</sup> both authors contributed equally

14   <sup>\*</sup> Corresponding author: E-mail: [bka@wsu.edu](mailto:bka@wsu.edu) (Birgitte Kiaer Ahring)

15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25

26 **Abstract:**

27 Anaerobic digestion (AD) of sewage sludge shows low carbon conversion efficiency  
28 (CCE) due to the poor biodegradability of sewage sludge. The lack of digestibility is  
29 specifically linked to the waste-activated sludge (WAS) making up the majority of sewage  
30 sludge along with a smaller portion of primary sludge, depending on the wastewater treatment  
31 plant configuration. In this study, we examine the Advanced Wet Oxidation & Steam  
32 Explosion process (AWOEx) for improving the CCE of digested sewage sludge (DSS) by  
33 thermophilic AD. The effect of the pretreatment temperature in the range between 160 to  
34 185°C at a fixed residence time of 20 min with and without oxygen added at a dosage of 5%  
35 of the organics present was tested. Methane yield improved by 97.92% to  $183.91 \pm 4.93$  mL/g  
36 VS over the untreated DSS (control), whose methane yield was  $92.92 \pm 9.07$  mL/g VS. We  
37 have demonstrated for the first time that 84% of the organics in sewage sludge can  
38 successfully be transformed into biogas following AWOEx pretreatment, which can contribute  
39 significantly to the circular economy instead of greenhouse gas emissions from landfilling.

40 **Keywords:** Enhanced anaerobic digestion; sewage sludge pretreatment; Advanced wet-  
41 oxidation and steam explosion; thermophilic anaerobic digestion; optimization of  
42 pretreatment.

43

44

45

46

47

## 49 **1.Introduction**

50 More than 13.84 million US dry tons (12.56 million metric dry tons) of sewage is  
51 produced annually in the US from publicly owned treatment works or wastewater treatment  
52 facilities (WWTF) (Seiple et al., 2017; Venkatesan et al., 2015). Sewage sludge, also referred  
53 as biosolids, by the U.S. Environmental protection Agency (U.S. EPA) is a byproduct from  
54 the WWTFs that needs disposal. Also, it should be noted that sludge management may  
55 demand a significant share of the WWTF's overall energy requirement (Capodaglio & Olsson,  
56 2020). Though several technologies are being developed for valorizing sewage sludge,  
57 implementing these solutions at small-scale communities is still a challenge due to  
58 unfavorable economics. However, of late, under various parameter variables (season,  
59 temperature, and ambient humidity), a completely solar-powered greenhouse facility for  
60 sewage sludge drying was examined, and potential improvements for its efficiency were  
61 assessed (Boguniewicz-Zablocka et al., 2021). Due to its high organic content, digested  
62 sewage sludge (DSS) can be used to produce bioenergy and biofuels. Around 50% of the US's  
63 WWTFs, corresponding to 7,500 total WWTFs, convert their sewage sludge to biogas, a  
64 mixture of methane (ca. 60%) and carbon dioxide (ca. 40%) using anaerobic digestion (AD).  
65 Analyses have shown that the digestate produced after AD contains a large portion of  
66 organics, which needs further processing before conversion to valuable bioenergy or bio-  
67 products (Li et al., 2017; Nordell et al., 2021; Wu et al., 2020). Several thermal pretreatment  
68 technologies have been developed for enhancing AD of DSS (Cambi THP, Veolia Exelys)  
69 (Abu-Orf & Goss, 2012; Bora et al., 2020). However, these methods are tailored for upfront  
70 pretreatment of the sewage sludge and not on specific treatment of the recalcitrant fraction of

71 sewage sludge. Also, these technologies use far less severe operational conditions  
72 (temperatures of ca. 160°C), and the biogas yields are generally only improved by ca. 30%.  
73 (Nguyen et al., 2021; Seiple et al., 2020; Wang et al., 2021; Yan et al., 2022). Methane yields  
74 from the conventional AD of sewage sludge have been reported to range between 0.19 and  
75 0.24 Nm<sup>3</sup>/kg VS<sub>in</sub> (Di Capua et al., 2020) and the residual organics are generally concentrated  
76 and disposed of in landfills, on agricultural land and parks, or by incineration (Nguyen et al.,  
77 2021). However, some thermochemical processes like pyrolysis can recover nutrients from  
78 digestate by converting the residual organics into energy rich gas streams, promoting circular  
79 economy. Though this is more sustainable than incineration, it require further processing to  
80 close the carbon loop (Callegari & Capodaglio, 2018).

81 To overcome these shortcomings, we implemented Advanced Wet Oxidation and Steam  
82 Explosion (AWOEx) (a thermochemical procedure integrating wet oxidation and steam  
83 explosion) (Ahring & Munck, 2013; Miklos et al., 2018; Svensson et al., 2018; Wu et al.,  
84 2020). followed by a secondary AD (AD2).

85 In the current pretreatment process i.e., AWOEx, biomass materials are subjected to  
86 oxidizing agents such as air, hydrogen peroxide, or oxygen under high temperatures (over 160  
87 degrees Celsius) and high pressures (over 10 bars) for 15 to 45 minutes (Ahring & Munck,  
88 2013; Biswas et al., 2015; Biswas et al., 2020; M'Arimi et al., 2020). The AWOEx process  
89 significantly enhances the solubilization of lignin and hemicellulose in lignocellulosic  
90 biomass including the crystalline cellulose that is retained in the solid fraction (Biswas et al.,  
91 2020; Khan & Ahring, 2020). As a result of the AWOEx pretreatment, microbial cell wall is  
92 disrupted and complex structures are broken down. As a result the digestibility of the sludge  
93 improves resulting in better degradation and disruption of both extracellular and intracellular

94 organic matter during the hydrolysis step of AD. Thus after pretreatment, hydrolytic enzymes  
95 of the microbial population will be more effective leading to an increased methane yield (Pilli  
96 et al., 2020; Sudalyandi & Jeyakumar, 2022; Yadav et al., 2022). Additionally, acetic acid  
97 formed via de-esterification of the acetyl groups, affects the hemicellulosic dissolution into the  
98 aqueous solution (Biswas et al., 2020; Biswas et al., 2014). The AWOEx pretreatment also has  
99 several advantages besides its effectiveness, such as requiring no chemicals to be recovered,  
100 along with a lower formation of inhibitory by-products. It is possible to perform AWOEx  
101 pretreatment at dry matter concentrations up to 45%, lowering the cost of the pretreatment  
102 (Biswas et al., 2020). Despite extensive testing of AWOEx on various lignocellulosic biomass  
103 materials, the process has not been previously studied with sewage sludge as the raw material.  
104 In the current study, DSS that has already been digested using conventional single-staged AD  
105 before dewatering and the solids have been pretreated using the AWOEx pretreatment  
106 method. The pretreated sludge is then fed into a second AD step, resulting in a two-staged  
107 AD. This process provides a way to make the recalcitrant fraction of the sludge available for  
108 digestion.

109 Many researchers have investigated recycling and reuse of sludge from WWTPs as a  
110 viable sustainable choice since there is an increased focus on mitigating the potential negative  
111 effect of land applying sewage sludge as well as the need for reducing landfilling of wet  
112 organic waste materials (Gupta et al., 2021; Smol et al., 2015). In the circular economy  
113 perspective, the prime focus is on re-use of the waste as a raw material, which not only avoids  
114 the costs associated with waste disposal but instead valorizes the waste as the products and  
115 byproducts trickle into the economy. Therefore, sludge reuse with a focus on energy recovery  
116 is a prominent waste management option and a cornerstone of circular economy for WWTP

117 (Eliche-Quesada et al., 2011). In this study, we determined the effect of AWOEx pretreatment  
118 on DSS and optimized the pretreatment temperature for sustained high methane yield by  
119 thermophilic AD. To understand the impact of AWOEx on the specific components of the  
120 DSS, we performed composition analysis of the DSS out of the secondary AD (AD2).

## 121 **2. Materials and methods**

### 122 **2.1 Advanced wet oxidation & steam explosion pretreatment**

123 DSS was collected from the Walla Walla WWTP in Eastern Washington and then  
124 dewatered using a filter press before pretreatment. All samples of DSS before and after  
125 pretreatment were stored at 4°C before use.

126 The AWOEx pretreatment of DSS was carried out in a 100 L stainless steel pressure tank.  
127 In each run, 10 kg of dewatered DSS with initial total solids conc of ca. 11 wt % was fed into  
128 the reactor at a constant mixing speed of 50 rpm. The target temperature was achieved by  
129 steam addition into the pretreatment reactor while the temperature was kept constant using an  
130 external jacketed oil heater. Oxygen (O<sub>2</sub>) was purged into the reactor based on the amount of  
131 VS of DSS while increasing the mixing speed to 85 rpm. At the end of residence time, the  
132 material was flashed out of the tank into a 250 L flash tank. After cooling, the sample was  
133 collected and stored at 4°C for future experiments and analysis.

### 134 **2.2 Design of experiments and statistical analysis**

135 The Advanced Wet Oxidation & Steam Explosion (AWOEx) pretreatment of DSS was  
136 performed at temperatures ranging from 160 to 185°C, without and with oxygen (5 % of the  
137 VS) at a residence time of 20 min. Using the design of experiments (DOE) module (JMP Pro  
138 15.2.0 - SAS Institute Inc. Cary, NC, USA), 12 experiment conditions at temperatures from  
139 160°C to 185°C with 5 °C interval, without and with oxygen added (5% of the VS) and a

140 control without any pretreatment (**Table 1**) are defined based on the full factorial design for  
 141 combined testing of the following categorical factors: temperature (6 level – 160°C, 165 °C,  
 142 170 °C, 175 °C, 180 °C, 185 °C) and effect of oxygen addition (2 level – without oxygen and  
 143 with oxygen at 5% of VS) by recording the biogas produced for each condition.

144

Run	Temperature (°C)	Residence time (min)	O <sub>2</sub> dosage (%)
1	160	20	5
2	165	20	5
3	170	20	5
4	175	20	5
5	180	20	5
6	185	20	5
7	160	20	0
8	165	20	0
9	170	20	0
10	175	20	0
11	180	20	0
12	185	20	0
13	Dewatered AD Sludge (Without Pretreatment)		

145 **Table 1. AWOEx conditions for dewatered anaerobic DSS.**

146

### 147 **2.3 Anaerobic digestion of pretreated DSS**

148 Anaerobic digestion (AD) of DSS was conducted in a series of 1 L bioreactors (in  
 149 duplicates) operated in semi-continuous mode with a working volume of 400 mL and with a  
 150 water displacement setup to record the biogas volume produced from each reactor. The initial

151 inoculum used was the raw sludge procured from the Walla Walla WWTP and digested  
152 sludge from the same plant mixed at a ratio of 1:1. The VS loading was always maintained at  
153 70 wt % of the TS. The hydraulic retention time (HRT) was kept constant at 16 days  
154 throughout the experiments, and all bioreactors were fed every 48 hours with a recording of  
155 the biogas produced. One bioreactor was set as control and was fed with DSS without  
156 pretreatment. All the experiments were run at thermophilic conditions (55 °C) for total of three  
157 retention cycles (48 days). Upon stable experimental data, particularly volume and  
158 composition of biogas, AD experiments were considered complete at the end of the stable  
159 retention cycle. The methane yield data collected from the experiments was analyzed using  
160 two-way analysis of variance (ANOVA) test (JMP Pro 15.2.0, SAS Institute Inc. Cary, NC,  
161 USA) to determine the effect of pretreatment conditions and its significance on improving the  
162 methane yield. All the presented data and calculations are based on the experimental data of  
163 the stable retention cycle for all the 12 independent conditions in duplicates (repetitions).  
164 When applicable, the averages and standard deviation ( $\pm$  S.D) were calculated and reported.

## 165 **2.4 Composition analysis**

166 The moisture contents (MC), total solids (TS), volatile solids (VS), carbohydrates  
167 (cellulose and hemicellulose), lignin, and lipid content of all samples before and after AWOEx  
168 of DSS, DSS and AD were measured following the National Renewable Energy Laboratory  
169 (NREL) methods as previously described studies (Biswas et al., 2020; Hames et al., 2008;  
170 Van Wychen et al., 2016). Protein contents of all samples before and after AWOEx of DSS  
171 and DSS were measured by the Lowry method (Amin et al., 2021; González López et al.,  
172 2010). NREL standard method was used for determining total lignin (acid-soluble and acid-  
173 insoluble) and total cellulose and hemicellulose (Sluiter et al., 2008). Acid soluble lignin was

174 determined using a UV spectrophotometer at 205 nm (Korpinen et al., 2014), whereas total  
175 carbohydrates (sum of glucose, xylose, and arabinose) were determined after two-step acid  
176 hydrolysis (Jenway 6405 UV/Visible, NJ, USA) at a wavelength of 320 nm (Biswas et al.,  
177 2020; Khan & Ahring, 2020). Monomers were analyzed by using an UltiMate® 3000 HPLC  
178 system (Dionex, Sunnyvale, CA) with the Aminex® 87H Column 250 × 4.6 mm (Bio-Rad,  
179 Hercules, CA) with a Shodex RI-101 refractive index detector at 60 °C using 4 mM sulfuric  
180 acid as an eluent at a flow rate of 0.6 ml/min. All measurements of lignin and carbohydrates  
181 analysis were done in duplicates. The lipid extraction was performed following chloroform-  
182 methanol extraction method proposed by Bligh and Dyer (Bligh & Dyer, 1959). 1 g of dried  
183 sludge was mixed with 20 mL of mixed liquor (chloroform: methanol = 2:1, v/v) and then  
184 subjected to 60°C for 4 h. Thereafter, the mixture was placed in a centrifuge for 15 min at  
185 3000 rpm and the solvent phase was transferred to a glass vial. After evaporating the solvent  
186 at 60° C, the vial was weighed. The difference in weight was the total lipid content.

## 187 **2.6 Volatile fatty acids analysis**

188 The concentration of volatile fatty acids was determined in the influent and effluent  
189 samples for each bioreactor at the start and end of every retention cycle using high  
190 performance liquid chromatography (HPLC) as previously described (Khan & Ahring, 2020).  
191 2 mL of each sample was taken and centrifuged at 10, 000 RPM for 10 min. The supernatant  
192 was separated with pipette, diluted 2 times with MiliQ water and then filtered through 0.2 µm  
193 syringe filter. The samples were analyzed by UltiMate® 3000 HPLC system (Dionex,  
194 Sunnyvale, CA) with an Aminex® 87H column 250 × 4.6 mm (Bio-Rad, Hercules, CA) with a  
195 Shodex RI-101 refractive index detector using 4mM sulfuric acid as eluent.

## 196 **2.7 Biogas analysis**

197 The composition of methane and carbon dioxide from all bioreactors were determined  
198 using an Universal gas analyzer, UGA series (Stanford Research Systems, Sunnyvale, CA)  
199 (Khan & Ahring, 2020). 10 ml of gas sample was collected daily before the reactors were fed  
200 and injected into the gas analyzer using a peristaltic pump.

### 201 **3. Results and discussion**

#### 202 **3.1 Effect of the AWOEx pretreatment on DSS**

203 The characteristics of DSS are shown in **Table 2**. DSS contained 86.44 % of moisture  
204 contents (MS), 13.56 % of total solids (TS), and 5.78 % of volatile solids (VS) which is 77.8  
205 % of TS. The results were similar to previously reported data (Nordell et al., 2021; Usman et  
206 al., 2019). Further, it was found that DSS contained 2.3 % cellulose, 2.9 % hemicellulose, 0.9  
207 % lignin, 1.69 % of crude fat and 3.01 % of protein.

208

<b>Parameters (wt %)</b>	<b>Values</b>
Moisture (%)	86.4
Total solids (%)	13.6
Volatile solids (%)	5.78
Ash (%)	2.92
Cellulose (%)	2.3
Hemicellulose (%)	2.9
Lignin (%)	0.9
Crude Fat (%)	1.69
Protein (%)	3.01

209 **Table 2.** Calories (Kcal/100 g) 15

---

210 **Characteristics of dewatered anaerobic DSS.**

211

212

213

214

215

216

217

218

219

220

221

222 Based on the statistical analysis of the experimental data for both the tested categorical  
223 variables, addition of oxygen significantly improved methane yield for all the temperatures  
224 ranges of the pretreatment ( $p < 0.0003$ ). On the other hand, temperature improved methane  
225 yield ( $p < 0.057$ )- but not to an extent as seen in case of oxygen addition.

226 We calculated the Cohen's D to find the implication of the effect size on our study. If two  
227 groups are the same size and have equal standard deviations, Cohen's d is the suitable effect  
228 size measurement. If each group has a distinct standard deviation, an alternative metric is  
229 Glass's delta, which only considers the standard deviation of the control group. An option  
230 when there are diverse sample sizes is Hedges' g, which offers a measure of effect size

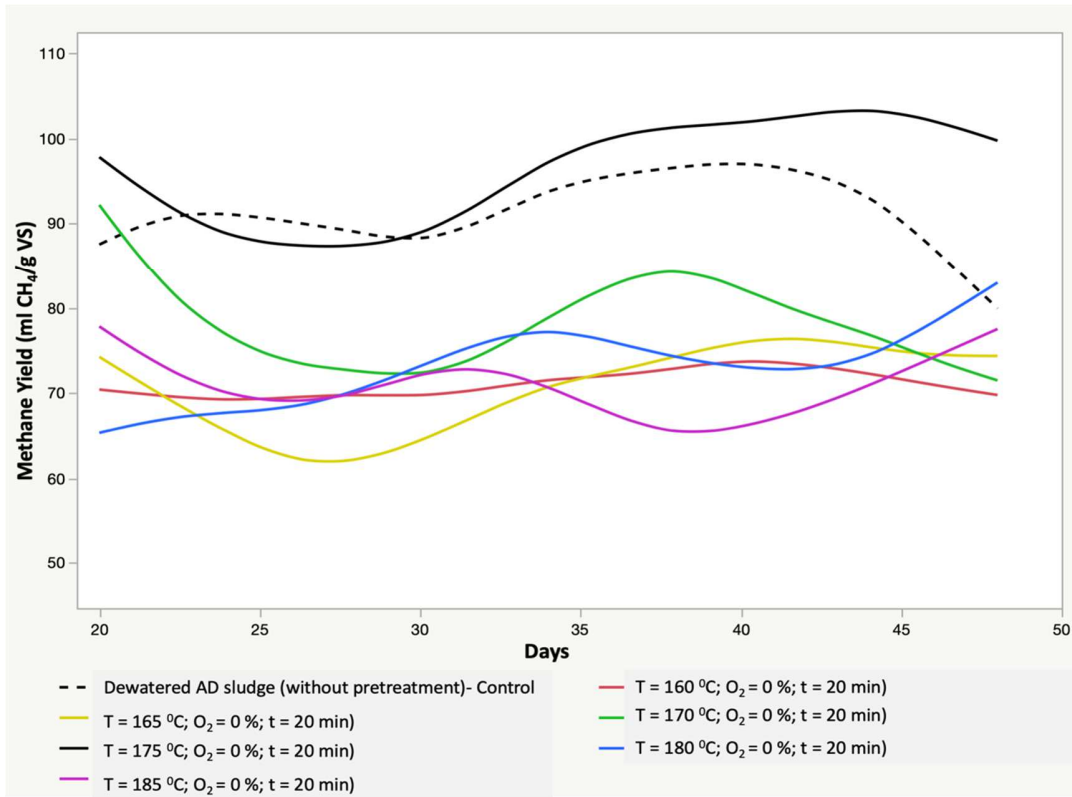
231 weighted in accordance with the relative size of each sample. In our study, Cohen's *d* was  
232 calculated to be 4.22; Glass's *delta* was calculated to be 4.41 and Hedges' *g* was 4.23.

233

### 234 **3.1.1 Effect of the AWOEx pretreatment on methane yield from DSS**

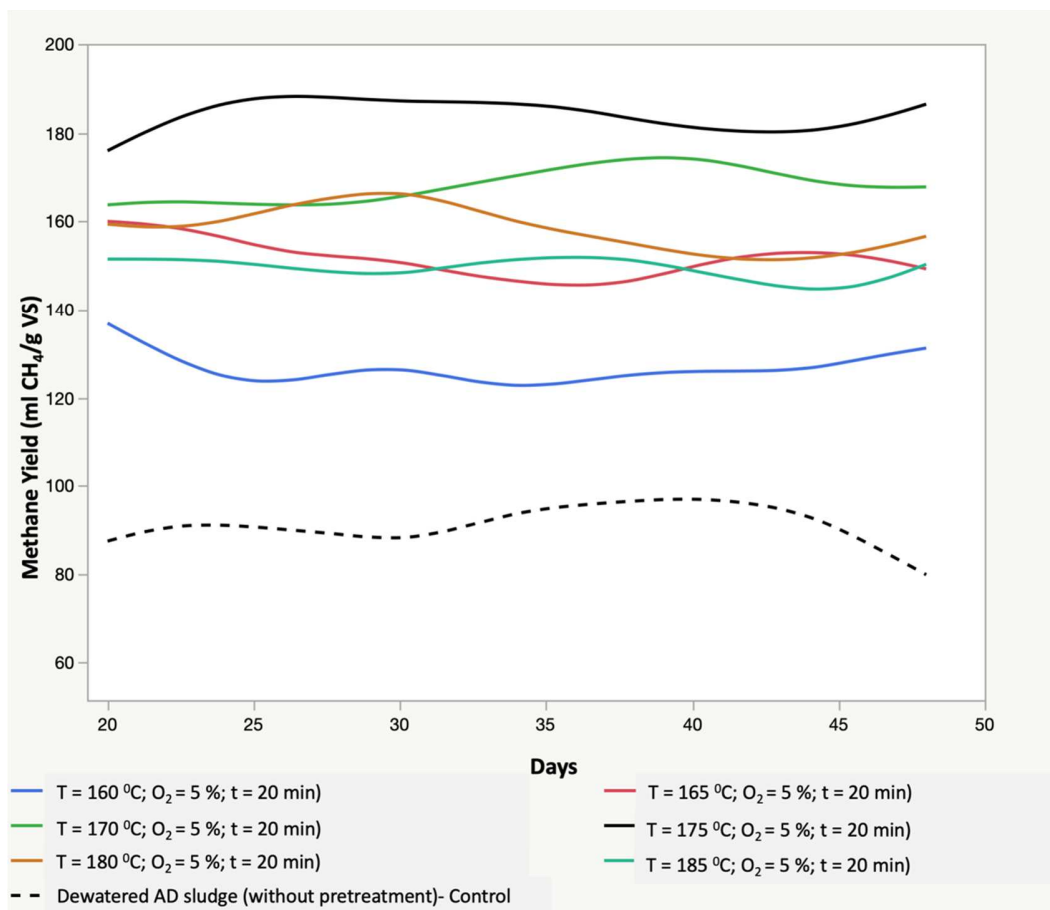
235 At the end of HRT 3, the average biogas aation of methane and carbon dioxide were in the  
236 range of  $62 \pm 1.5$  vol % and  $38 \pm 2.2$  vol %<sup>^</sup>, respectively, with traces of moisture, hydrogen  
237 and oxygen. For the control (DSS without pretreatment), methane and carbon dioxide content  
238 were in the range of 55-58 vol% and 40-42% **Figure 1** shows the methane yield from  
239 AWOEx pretreated DSS material at temperatures ranging from 160°C - 185 °C with 20 min  
240 residence time and without oxygen. **Figure 2** shows the trend in average methane yields from  
241 the semi-continuous bioreactors at 55 °C fed with DSS pretreated at temperatures ranging  
242 from 160°C to 185 °C with 20 min residence time and 5% oxygen. The control reactors were  
243 fed with DSS without AWOEx pretreatment to compare the effect of the different conditions  
244 during AWOEx pretreatment on methane production. It was found that the average methane  
245 yield of the control reactors was 92.92 mL/gVS. The average methane yields from 175 °C and  
246 170 °C were 183.91 and 170.93 mL/g VS, respectively. Since the residence time and oxygen  
247 dosage were kept constant for the optimally performing sets, it can be concluded that the

248 resulting methane yield was solely a function of pretreatment temperature.



250 **Figure 1.** Average methane yield of bioreactors fed with AWOEx pretreated DSS material pretreated without oxygen.

**Figure 2.** Average methane yield of bioreactors fed with AWOEx pretreated DSS material pretreated with 5% oxygen.



252 The average methane yield at 175°C was higher than 180°C, 170°C and 165 °C by 18.72%,  
 253 7.59%, and 23.77%, respectively. One of the possible reasons of lower methane yield at 180  
 254 °C might be due to the formation of inhibitory compounds at higher temperatures as  
 255 previously described during the pretreatment of sewage sludge (Hii et al., 2014; Suárez-  
 256 Iglesias et al., 2017). The highest average methane yields of 183.92 mL/gVS at 175°C and  
 257 170.93 mL/gVS at 170°C was for the same residence time and O<sub>2</sub> dosage but at different  
 258 temperatures, indicating that lower temperatures might have resulted in a lower hydrolysis of  
 259 the material than higher temperatures. The results for the control reactor are in accordance  
 260 with the study by Nordell et al., 2021, which found that only 46 mL/g VS of methane yield  
 261 was achieved during anaerobic digestion of digested sewage sludge under thermophilic  
 262 condition without any thermochemical pretreatment. Overall, AWOEx pretreatment of DSS  
 263 significantly increased the methane yield far over the control reactor based on the ANOVA  
 264 test (P = 0.0007). **Table 3** shows the difference of TS and VS % of all bioreactors for the feed  
 265 and the effluent, and it was found that the bioreactors with the highest methane yield, 175°C,  
 266 had the highest TS conversion of 72.82% out of all the groups. Highest single day average  
 267 methane yield was 191.4 ml/g VS and 181.3 ml/g VS observed at the pretreatment  
 268 temperature of 175 °C and 180 °C, respectively, followed by 180.8 ml/g VS at 170 °C.

269 **Table 3. TS and VS % of the thermophilic bioreactors at different AWOEx**  
 270 **pretreatment condition after 1,2 and 3 retention times (HRTs).**

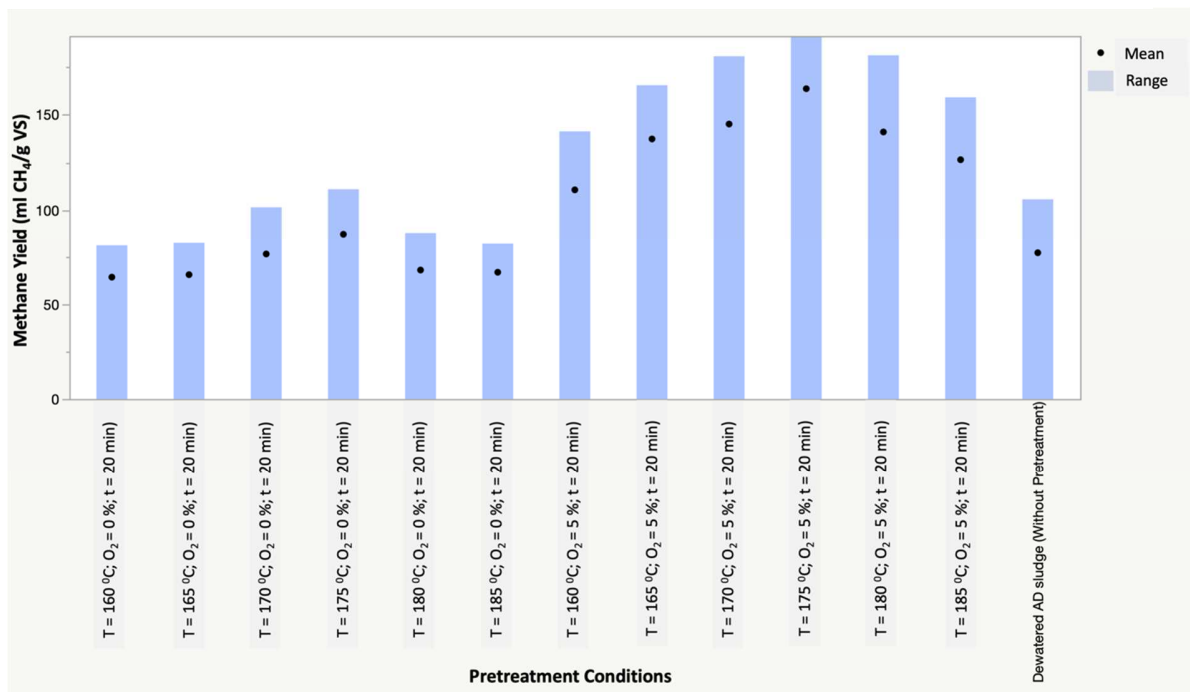
Run	Conditions	TS (%) [in]	TS (%) [out] [HRT 1]	TS (%) [out] [HRT 2]	TS (%) [out] [HRT 3]	VS (%) [in]	VS (%) [out] [HRT 1]	VS (%) [out] [HRT 2]	VS (%) [out] [HRT 3]
-----	------------	----------------	----------------------------	----------------------------	----------------------------	----------------	----------------------------	----------------------------	----------------------------

---

<b>1</b>	T =160 °C; t= 20 min; O <sub>2</sub> = 0 %	9.17	8.22	6.56	6.55	74.33	70.77	69.49	67.12
<b>2</b>	T =165 °C; t= 20 min; O <sub>2</sub> = 0 %	9.12	8.25	7.44	7.22	74.66	72.21	71.84	69.45
<b>3</b>	T =170 °C; t= 20 min; O <sub>2</sub> = 0 %	9.08	8.87	7.57	7.15	76.89	75.19	74.91	71.35
<b>4</b>	T =175 °C; t= 20 min; O <sub>2</sub> = 0 %	9.17	7.03	7.12	6.97	74.26	71.78	70.73	69.92
<b>5</b>	T =180 °C; t= 20 min; O <sub>2</sub> = 0 %	9.07	6.93	5.22	4.97	73.69	72.29	70.71	71.34
<b>6</b>	T =185 °C; t= 20 min; O <sub>2</sub> = 0 %	9.32	5.89	4.49	4.33	74.26	73.91	73.95	72.90
<b>7</b>	T =160 °C; t= 20 min; O <sub>2</sub> = 5 %	9.22	7.34	5.30	3.26	73.80	70.45	69.55	68.92
<b>8</b>	T =165 °C; t= 20 min; O <sub>2</sub> = 5 %	9.17	7.17	5.49	5.01	78.57	75.88	74.34	72.92
<b>9</b>	T =170 °C; t= 20 min; O <sub>2</sub> = 5 %	9.24	8.6	5.12	4.72	73.04	72.35	73.92	70.23
<b>10</b>	T =175 °C; t= 20 min; O <sub>2</sub> = 5 %	9.12	5.54	4.01	2.48	74.14	72.62	66.41	65.72

---

**Figure 3.** Comparing the effect of the two tested pretreatment variables on methane yield: DSS pretreated with and without oxygen at temperatures ranging from 160-185 °C. Range of the methane yield is plotted with mean marked for each pretreatment condition (bar).



273 Based on our results, optimal temperature range for pretreating DSS will be in the range of  
274 170-180 °C with oxygen addition. **Figure 3** shows the mean methane yield plotted for every  
275 pretreatment condition where oxygen addition resulted in an increase of the methane yield by  
276 92.65% over the pretreatment groups without oxygen addition at 175 °C, the temperature with  
277 the maximum average methane yield. The increasing trend of methane yield with an increase  
278 in temperature for 160 to 175 °C and decreasing trend from 175 to 185 °C, further supports  
279 that 175 °C is the optimal temperature for pretreating DSS.

### 280 **3.2 Influence of the different operational conditions of AWOEx pretreatment of DSS on** 281 **methane yield**

282 Oxygen addition had significant ( $P = 0.0003$ ) and higher effect on methane yield next to the  
283 temperature ( $P = 0.0573$ ) as seen in **Figure 4**. This is in accordance with a previous study on  
284 AWOEX pretreatment of sawdust (Biswas et al., 2020; Higgins et al., 2017). A previous  
285 study reported that AWOEx pretreatment increased the formation of small molecular weight  
286 organics, which is easily biodegradable during AD (Hii et al., 2014; Suárez-Iglesias et al.,  
287 2017). Further, the radicals produced during AWOEx pretreatment are powerful in oxidizing  
288 recalcitrant compounds such as lignin, which increased the biodegradability of DSS (Khan &  
289 Ahring, 2020). Anaerobic digestion breaks down long-chain molecules into shorter chains  
290 (Abelleira-Pereira et al., 2015; Hii et al., 2014; Liang et al., 2020; Lin et al., 2022; M'Arimi et  
291 al., 2020; Suárez-Iglesias et al., 2017). Furthermore, higher  $O_2$  pressure during pretreatment  
292 rapidly depolymerizes crosslinked bonds of complex compounds into simpler chain. The  
293 breakdown of bonds is somewhat constrained in the absence of oxygen. According to our  
294 findings, high temperatures enhance the dissolution of DSS, and adding  $O_2$  helps in oxidizing  
295 organic compounds in DSS into digestible molecules.

296 **3.3 Composition analysis of DSS before and after AWOEx pretreatment-AD**

297 AWOEx treatment affected the composition of DSS significantly. At the optimal  
298 pretreatment temperature of 175°C, the conversion of cellulose was 74.4%, hemicellulose was  
299 66.8%, and lignin was 68.8% of the material. However, for the control set (dewatered DSS)  
300 only 28.6% of the cellulose, 51.2% of the hemicellulose and 47.2% of the lignin was  
301 converted during secondary thermophilic AD. **Table 4** shows the composition analysis of the  
302 influent and effluent of the bioreactors between 160 to 185 °C compared to the control  
303 bioreactor.

304 **Table 4. Characteristics of influents and effluents of the thermophilic semi-continuous bioreactors.**

Sr. No	Thermophilic Reactor (Inlet material)	Protein (%) in	Protein (%) out	Lignin (%) in	Lignin (%) out	Cellulose (%) in	Cellulose (%) out	Hemicellulose (%) in	Hemicellulose (%) out	Crude fat (%) in	Crude fat (%) out
1	T =160 °C; t= 20 min; O <sub>2</sub> = 0 %	2.81	2.29	1.15	0.6	4.91	3.54	3.52	1.61	0.73	0.35
2	T =165 °C; t= 20 min; O <sub>2</sub> = 0 %	2.89	2.53	1.03	0.47	5.15	3.51	3.67	1.73	0.71	0.47
3	T =170 °C; t= 20 min; O <sub>2</sub> = 0 %	2.22	1.99	0.98	0.45	5.28	3.61	3.85	1.78	0.72	0.33
4	T =175 °C; t= 20 min; O <sub>2</sub> = 0 %	2.17	1.86	0.92	0.55	5.45	3.52	3.98	1.8	0.70	0.40
5	T =180 °C; t= 20 min; O <sub>2</sub> = 0 %	2.02	1.76	0.88	0.51	5.7	3.44	4.01	1.89	0.75	0.44
6	T =185 °C; t= 20 min; O <sub>2</sub> = 0 %	2.91	2.81	0.90	0.52	5.85	3.39	4.16	1.87	0.73	0.31
7	T =160 °C; t= 20 min; O <sub>2</sub> = 5 %	2.72	2.51	0.88	0.51	4.65	1.8	2.99	1.5	0.77	0.28
8	T =165 °C; t= 20 min; O <sub>2</sub> = 5 %	2.84	2.31	0.82	0.46	4.78	1.79	3.15	1.52	0.73	0.25
9	T =170 °C; t= 20 min; O <sub>2</sub> = 5 %	1.75	1.51	0.80	0.34	4.93	1.51	3.20	1.31	0.73	0.19
10	<b>T =175 °C; t= 20 min; O<sub>2</sub> = 5 %</b>	<b>1.68</b>	<b>1.39</b>	<b>0.76</b>	<b>0.29</b>	<b>4.99</b>	<b>1.28</b>	<b>3.46</b>	<b>1.15</b>	<b>0.75</b>	<b>0.16</b>
11	T =180 °C; t= 20 min; O <sub>2</sub> = 5 %	1.86	1.34	0.74	0.41	5.10	1.67	3.50	1.78	0.76	0.21
12	T =185 °C; t= 20 min; O <sub>2</sub> = 5 %	2.78	2.22	0.70	0.36	5.35	2.24	3.67	2.18	0.71	0.19
13	Control (Dewatered AD Sludge without Pretreatment)	3.01	2.67	1.76	1.47	4.45	3.54	3.16	1.69	1.3	0.83

305 The average protein content decreased with 17.3% and 28.1% in the bioreactors at 175°C  
306 and 180°C, respectively, compared to 11.3% protein conversion in the control reactor Thermal  
307 hydrolysis do not only lead to conversion of lipids, proteins and polysaccharides but  
308 simultaneously brings about hydrolysis of these compounds into lower molecular weight  
309 compounds by cleaving the peptide, ester and glycosidic bonds. The crude fat content in the  
310 effluent of our bioreactors fed with material pretreated at 175°C and 180°C decreased by  
311 78.66% and 72.36%, respectively, compared to 36.15 % reduction in the control bioreactor  
312 set. In the effluent sets of the bioreactors 175°C and 180°C without oxygen, the reduction in  
313 crude fat content was 42.85% and 41.33% showing that oxygen triggers an aggressive  
314 breakdown of crude fats in DSS. This result indicate that highly unsaturated fatty acids are  
315 more susceptible to oxidative reactions. Polymers in the material need to be hydrolyzed in  
316 order to allow for methane production by anaerobic digestion. Grease and fat hydrolysis into  
317 long chain fatty acids, which can be further degraded by AD possess a large biogas potential  
318 of importance for the methane yield. (Wett et al., 2006).

319 A recent study reported a hydrolysis of ca. 30% of the total proteins and 25% of the total  
320 polysaccharide content into simpler, smaller organics following thermic pretreatment (Yang et  
321 al., 2019). When thermal hydrolysis temperatures were increased from 120 to 170 °C, (Chen  
322 et al., 2019) reported a 15% increase in amino acid hydrolysis as compared to a 9.7% increase  
323 in proteins hydrolyzed to VFA. Another study reported a 60% increase in the amount of VFA  
324 produced from protein hydrolysis when the temperature was increased from 160 to 200  
325 °C (Wilson & Novak, 2009). Another study reported that during thermal hydrolysis at 160 °C  
326 for 50 minutes, around 12% of particulate protein was transformed into VFA (Han et al.,

327 2017). It has been estimated that 13 to 23% of the total VFA generated in wastewater sludge  
328 may be generated from the hydrolysis of protein (Wilson & Novak, 2009).  
329 Studies also indicate that lipid hydrolysis at ca. 170 °C primarily contained fractions of  
330 aldehydes(80%) and alcohol(11%) (Wilson & Novak, 2009). During thermal hydrolysis,  
331 long-chain fatty acids (LCFA) increase in concentration, inhibiting bacteria and methanogens,  
332 although the inhibitory concentration depends on the LCFA composition and microbial  
333 species composition. In contrast to saturated fatty acids, unsaturated LCFAs were more  
334 inhibitive to methanogens at threshold concentrations of 10 to 30 mg/L (Lalman & Bagley,  
335 2000).

336 The presence of lipids and proteins in wastewater sludge may generate compounds which  
337 are inhibitory to methanogenesis such as ammonia (Liu & Sung, 2002; Tanaka et al., 1997)  
338 and hydrophobic fatty acids (Angelidaki & Ahring, 1992; Chowdhury et al., 2007) through the  
339 thermal hydrolysis of proteins and lipids, respectively. Under the optimal conditions leading  
340 to the highest methane yield, the biodegradation of the organic content of the DSS  
341 significantly improved. A previous study also reported that AWOEX pretreatment showed  
342 enhanced solubilization of carbohydrates after AWOEx pretreatment, which resulted in a  
343 higher methane yield during AD of pretreated manure fibers (Khan & Ahring, 2020). In this  
344 study, we further saw improved conversion of other components such as proteins and lipids.  
345 As a result of the hydrolysis reaction of acetyl groups in hemicellulose producing acetic acid,  
346 the pretreated DSS after AWOEx at 175°C contained higher concentrations of VFAs (1650  
347 mg/L) (Jönsson & Martín, 2016). Acetic acid dominated the organic acids in all bioreactors,  
348 and the bioreactor that contained the highest concentration of VFA also contained the highest  
349 concentration of acetic acid (900 mg/L) (**Table 5**). The concentration of VFA in the effluents

350 of all bioreactors were, however, low (less than 20 mg/L), indicating that all bioreactors are  
351 functioning well and all digestible materials are being fully converted to methane.

352 **Table 5. VFAs analysis of influents and effluents of the thermophilic semi-continuous**  
353 **bioreactors**

VFAs (mg/L)	Inlet stream			
	175 °C; 20 min; 5	180 °C; 15 min;	175 °C; 20 min;	180 °C; 15 min;

354

355

	<b>%</b>	<b>5 %</b>	<b>0%</b>	<b>0 %</b>
Acetic acid	900	860	675	830
Propionic acid	240	330	220	210
Isobutyric acid	190	125	175	110
Butyric acid	200	150	100	120
Isovaleric acid	120	95	90	100
<b>VFAs (mg/L)</b>		<b>Outlet stream</b>		
Acetic acid	15	8.0	10.5	8.0
Propionic acid	2.0	1.25	2.50	1.50
Isobutyric acid	1.40	0.95	1.30	1.40
Butyric acid	1.50	1.30	1.20	1.52
Isovaleric acid	1.00	1.80	1.30	1.10

356

357 One of the major findings of our study was the degradation of crude fat and protein in the  
358 optimally performing set of reactors at 175 °C by ca.80% and 20%, respectively. It is obvious  
359 that this increased conversion is a major reason for the higher methane yield found after  
360 AWOEx. Overall, our results show that AWOEx pretreatment of DSS results in significant  
361 higher methane production compared to standard one-step AD of sewage sludge. This higher  
362 energy recovery is of major importance for valorizing sewage sludge at WWTPs. The study  
363 further demonstrated that both elevated temperature and oxygen are important for improving  
364 the carbon conversion of sewage sludge into methane. Compared to conventional AD with ca.  
365 50% VS conversion, incorporating a secondary AWOEx pretreatment process resulted in 41%  
366 extra conversion of organics in sewage sludge with over 100% net improved methane

367 production. This far higher methane production as well as the lower residual amount residing  
368 needing final disposal could potentially be game-changing for exploitation of the energy  
369 potential of sewage sludge in the future.

#### 370 **4. Conclusions**

371 This study demonstrates the efficiency of AWOEx for enhancing methane yield of AD of  
372 DSS demonstrating a 2-fold increase in methane yield compared to untreated DSS far higher  
373 than previously reported in other studies. The optimal pretreatment condition was found to be  
374 a temperature of 175 °C with oxygen (5% of VS) added resulting in a methane yield of 183.91  
375  $\pm$  4.93 mL/g VS of previous digested sewage sludge. Oxygen addition was the most critical  
376 parameter in improving the methane yield, followed by temperature. Pretreatment with a  
377 secondary AD step in tandem improved the CCE significantly leaving only small amounts of  
378 organics (15% of the input VS) behind. From this study we also demonstrated that AWOEx  
379 not only had a major effect on the digestibility of lignocellulosic polymers such as  
380 carbohydrates, which was present in low amounts in the DSS but also on the lipids and protein  
381 fraction, which was present in large concentrations in the sewage sludge.

#### 382 **Declaration of competing interest**

383 The authors declare that they have no known competing financial interests or personal  
384 relationships that could have appeared to influence the work reported in this paper.

#### 385 **Acknowledgments**

386 This research was funded by the DOE BETO under the award DOE EERE BETO DE-  
387 EE0008933. The authors thank Muhammad Usman for technical assistance and the PNNL-  
388 WSU Distinguished Graduate Research Program for support to Anthony Giduthuri.

389 **References**

390

391 Abelleira-Pereira, J.M., Pérez-Elvira, S.I., Sánchez-Oneto, J., de la Cruz, R., Portela, J.R.,  
392 Nebot, E. 2015. Enhancement of methane production in mesophilic anaerobic  
393 digestion of secondary sewage sludge by advanced thermal hydrolysis pretreatment.  
394 *Water Research*, **71**, 330-340.

395 Abu-Orf, M., Goss, T. 2012. Comparing Thermal hydrolysis processes (CAMBI™ and  
396 EXELYS™) for solids pretreatment prior to anaerobic digestion. *Digestion*, **16**, 8-12.

397 Ahring, B.K., Munck, J. 2013. Method for treating biomass and organic waste with the  
398 purpose of generating desired biologically based products, Google Patents.

399 Amin, M., Rizky Distiawan, M., Herlina, M., Cholifatun, R., Rohim, F., Anggraeni, C.,  
400 Alamsjah, M., Wahju, T., Sri, S. 2021. Optimization of production of enzymatic  
401 protein hydrolysate-based flavor from sea grape (*Caulerpa racemosa*). *Iranian Journal*  
402 *of Fisheries Sciences*, **20**(4), 1097-1113.

403 Angelidaki, I., Ahring, B.K. 1992. Effects of free long-chain fatty acids on thermophilic  
404 anaerobic digestion. *Applied Microbiology and Biotechnology*, **37**(6), 808-812.

405 Biswas, R., Teller, P.J., Ahring, B.K. 2015. Pretreatment of forest residues of Douglas fir by  
406 wet explosion for enhanced enzymatic saccharification. *Bioresource Technology*, **192**,  
407 46-53.

408 Biswas, R., Teller, P.J., Khan, M.U., Ahring, B.K. 2020. Sugar production from hybrid poplar  
409 sawdust: optimization of enzymatic hydrolysis and wet explosion pretreatment.  
410 *Molecules*, **25**(15), 3396.

411 Biswas, R., Uellendahl, H., Ahring, B.K. 2014. Wet explosion pretreatment of sugarcane  
412 bagasse for enhanced enzymatic hydrolysis. *Biomass and Bioenergy*, **61**, 104-113.

413 Bligh, E.G., Dyer, W.J. 1959. A rapid method of total lipid extraction and purification. *Can J*  
414 *Biochem Physiol*, **37**(8), 911-7.

415 Boguniewicz-Zablocka, J., Klosok-Bazan, I., Capodaglio, A.G. 2021. Sustainable  
416 management of biological solids in small treatment plants: overview of strategies and  
417 reuse options for a solar drying facility in Poland. *Environ Sci Pollut Res Int*, **28**(19),  
418 24680-24693.

419 Bora, R.R., Richardson, R.E., You, F. 2020. Resource recovery and waste-to-energy from  
420 wastewater sludge via thermochemical conversion technologies in support of circular  
421 economy: a comprehensive review. *BMC Chemical Engineering*, **2**(1), 8.

422 Callegari, A., Capodaglio, A.G. 2018. Properties and Beneficial Uses of (Bio)Chars, with  
423 Special Attention to Products from Sewage Sludge Pyrolysis. *Resources*, **7**(1), 20.

- 424 Capodaglio, A.G., Olsson, G. 2020. Energy Issues in Sustainable Urban Wastewater  
425 Management: Use, Demand Reduction and Recovery in the Urban Water Cycle.  
426 *Sustainability*, **12**(1), 266.
- 427 Chen, S., Dong, B., Dai, X., Wang, H., Li, N., Yang, D. 2019. Effects of thermal hydrolysis  
428 on the metabolism of amino acids in sewage sludge in anaerobic digestion. *Waste*  
429 *Management*, **88**, 309-318.
- 430 Chowdhury, N., Lalman, J.A., Seth, R., Ndegwa, P. 2007. Biohydrogen Production by  
431 Mesophilic Anaerobic Fermentation of Glucose in the Presence of Linoleic Acid.  
432 *Journal of Environmental Engineering*, **133**(12), 1145-1152.
- 433 Di Capua, F., Spasiano, D., Giordano, A., Adani, F., Fratino, U., Pirozzi, F., Esposito, G.  
434 2020. High-solid anaerobic digestion of sewage sludge: challenges and opportunities.  
435 *Applied Energy*, **278**, 115608.
- 436 Eliche-Quesada, D., Martínez-García, C., Martínez-Cartas, M.L., Cotes-Palomino, M.T.,  
437 Pérez-Villarejo, L., Cruz-Pérez, N., Corpas-Iglesias, F.A. 2011. The use of different  
438 forms of waste in the manufacture of ceramic bricks. *Applied Clay Science*, **52**(3),  
439 270-276.
- 440 González López, C.V., García, M.d.C.C., Fernández, F.G.A., Bustos, C.S., Chisti, Y., Sevilla,  
441 J.M.F. 2010. Protein measurements of microalgal and cyanobacterial biomass.  
442 *Bioresource Technology*, **101**(19), 7587-7591.
- 443 Gupta, A., Kumar, M., Srivastava, S. 2021. Recent Advances in Wastewater Sludge  
444 Valorization. in: *Bio-valorization of Waste: Trends and Perspectives*, (Eds.) S. Shah,  
445 V. Venkatramanan, R. Prasad, Springer Singapore. Singapore, pp. 225-247.
- 446 Hames, B., Ruiz, R., Scarlata, C., Sluiter, A., Sluiter, J., Templeton, D. 2008. Preparation of  
447 samples for compositional analysis. in: *Laboratory Analytical Procedure (LAP)*.  
448 *National Renewable Energy Laboratory*.
- 449 Han, Y., Zhuo, Y., Peng, D., Yao, Q., Li, H., Qu, Q. 2017. Influence of thermal hydrolysis  
450 pretreatment on organic transformation characteristics of high solid anaerobic  
451 digestion. *Bioresource Technology*, **244**, 836-843.
- 452 Higgins, M.J., Beightol, S., Mandahar, U., Suzuki, R., Xiao, S., Lu, H.-W., Le, T., Mah, J.,  
453 Pathak, B., DeClippeleir, H. 2017. Pretreatment of a primary and secondary sludge  
454 blend at different thermal hydrolysis temperatures: Impacts on anaerobic digestion,  
455 dewatering and filtrate characteristics. *Water Research*, **122**, 557-569.
- 456 Hii, K., Baroutian, S., Parthasarathy, R., Gapes, D.J., Eshtiaghi, N. 2014. A review of wet air  
457 oxidation and thermal hydrolysis technologies in sludge treatment. *Bioresource*  
458 *Technology*, **155**, 289-299.

- 459 Jönsson, L.J., Martín, C. 2016. Pretreatment of lignocellulose: Formation of inhibitory by-  
460 products and strategies for minimizing their effects. *Bioresource Technology*, **199**,  
461 103-112.
- 462 Khan, M.U., Ahring, B.K. 2020. Anaerobic digestion of biorefinery lignin: Effect of different  
463 wet explosion pretreatment conditions. *Bioresource Technology*, **298**, 122537.
- 464 Korpinen, R., Kallioinen, M., Hemming, J., Pranovich, A., Mänttari, M., Willför, S. 2014.  
465 Comparative evaluation of various lignin determination methods on hemicellulose-rich  
466 fractions of spruce and birch obtained by pressurized hot-water extraction (PHWE)  
467 and subsequent ultrafiltration (UF). *Holzforschung*, **68**(8), 971-979.
- 468 Lalman, J.A., Bagley, D.M. 2000. Anaerobic degradation and inhibitory effects of linoleic  
469 acid. *Water Research*, **34**(17), 4220-4228.
- 470 Li, C., Wang, X., Zhang, G., Yu, G., Lin, J., Wang, Y. 2017. Hydrothermal and alkaline  
471 hydrothermal pretreatments plus anaerobic digestion of sewage sludge for dewatering  
472 and biogas production: bench-scale research and pilot-scale verification. *Water*  
473 *Research*, **117**, 49-57.
- 474 Liang, J., Zhang, L., Ye, M., Guan, Z., Huang, J., Liu, J., Li, L., Huang, S., Sun, S. 2020.  
475 Evaluation of the dewaterability, heavy metal toxicity and phytotoxicity of sewage  
476 sludge in different advanced oxidation processes. *Journal of Cleaner Production*, **265**,  
477 121839.
- 478 Lin, W., Liu, X., Ding, A., Ngo, H.H., Zhang, R., Nan, J., Ma, J., Li, G. 2022. Advanced  
479 oxidation processes (AOPs)-based sludge conditioning for enhanced sludge  
480 dewatering and micropollutants removal: A critical review. *Journal of Water Process*  
481 *Engineering*, **45**, 102468.
- 482 Liu, T., Sung, S. 2002. Ammonia inhibition on thermophilic aceticlastic methanogens. *Water*  
483 *Sci Technol*, **45**(10), 113-20.
- 484 M'Arimi, M., Mecha, C., Kiprof, A., Ramkat, R. 2020. Recent trends in applications of  
485 advanced oxidation processes (AOPs) in bioenergy production. *Renewable and*  
486 *Sustainable Energy Reviews*, **121**, 109669.
- 487 Mainardis, M., Buttazzoni, M., Gievers, F., Vance, C., Magnolo, F., Murphy, F., Goi, D.  
488 2021. Life cycle assessment of sewage sludge pretreatment for biogas production:  
489 From laboratory tests to full-scale applicability. *Journal of Cleaner Production*, **322**,  
490 129056.
- 491 Miklos, D.B., Remy, C., Jekel, M., Linden, K.G., Drewes, J.E., Hübner, U. 2018. Evaluation  
492 of advanced oxidation processes for water and wastewater treatment—A critical review.  
493 *Water Research*, **139**, 118-131.

- 494 Ngo, P.L., Udugama, I.A., Gernaey, K.V., Young, B.R., Baroutian, S. 2021. Mechanisms,  
495 status, and challenges of thermal hydrolysis and advanced thermal hydrolysis  
496 processes in sewage sludge treatment. *Chemosphere*, **281**, 130890.
- 497 Nguyen, V.K., Chaudhary, D.K., Dahal, R.H., Trinh, N.H., Kim, J., Chang, S.W., Hong, Y.,  
498 La, D.D., Nguyen, X.C., Ngo, H.H. 2021. Review on pretreatment techniques to  
499 improve anaerobic digestion of sewage sludge. *Fuel*, **285**, 119105.
- 500 Nordell, E., Moestedt, J., Österman, J., Yekta, S.S., Björn, A., Sun, L., Schnürer, A. 2021.  
501 Post-treatment of dewatered digested sewage sludge by thermophilic high-solid  
502 digestion for pasteurization with positive energy output. *Waste Management*, **119**, 11-  
503 21.
- 504 Pilli, S., Pandey, A., Katiyar, A., Pandey, K., Tyagi, R. 2020. Pre-treatment Technologies to  
505 Enhance Anaerobic Digestion. in: *Sustainable Sewage Sludge Management and*  
506 *Resource Efficiency*, (Ed.) B.K. Taşeli, IntechOpen.
- 507 Seiple, T.E., Coleman, A.M., Skaggs, R.L. 2017. Municipal wastewater sludge as a  
508 sustainable bioresource in the United States. *Journal of Environmental Management*,  
509 **197**, 673-680.
- 510 Seiple, T.E., Skaggs, R.L., Fillmore, L., Coleman, A.M. 2020. Municipal wastewater sludge  
511 as a renewable, cost-effective feedstock for transportation biofuels using hydrothermal  
512 liquefaction. *Journal of Environmental Management*, **270**, 110852.
- 513 Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Crocker, D. 2008.  
514 Determination of structural carbohydrates and lignin in biomass, in: Laboratory  
515 Analytical Procedure (LAP). *National Renewable Energy Laboratory*.
- 516 Smol, M., Kulczycka, J., Henclik, A., Gorazda, K., Wzorek, Z. 2015. The possible use of  
517 sewage sludge ash (SSA) in the construction industry as a way towards a circular  
518 economy. *Journal of Cleaner Production*, **95**, 45-54.
- 519 Suárez-Iglesias, O., Urrea, J.L., Oulego, P., Collado, S., Díaz, M. 2017. Valuable compounds  
520 from sewage sludge by thermal hydrolysis and wet oxidation. A review. *Science of the*  
521 *Total Environment*, **584**, 921-934.
- 522 Sudalyandi, K., Jeyakumar, R. 2022. Hydrolysis and Assessment. in: *Biofuel Production*  
523 *Using Anaerobic Digestion*, Springer, pp. 53-84.
- 524 Svensson, K., Kjølraug, O., Higgins, M.J., Linjordet, R., Horn, S.J. 2018. Post-anaerobic  
525 digestion thermal hydrolysis of sewage sludge and food waste: Effect on methane  
526 yields, dewaterability and solids reduction. *Water Research*, **132**, 158-166.
- 527 Tanaka, S., Kobayashi, T., Kamiyama, K.-i., Signey Bildan, M.L.N. 1997. Effects of  
528 thermochemical pretreatment on the anaerobic digestion of waste activated sludge.  
529 *Water Science and Technology*, **35**(8), 209-215.

530 Usman, M., Hao, S., Chen, H., Ren, S., Tsang, D.C., Sompong, O., Luo, G., Zhang, S. 2019.  
531 Molecular and microbial insights towards understanding the anaerobic digestion of the  
532 wastewater from hydrothermal liquefaction of sewage sludge facilitated by granular  
533 activated carbon (GAC). *Environment International*, **133**, 105257.

534 Van Wychen, S., Ramirez, K., Laurens, L.M. 2016. Determination of total lipids as fatty acid  
535 methyl esters (FAME) by in situ transesterification, in: Laboratory Analytical  
536 Procedure (LAP). *National Renewable Energy Laboratory*.

537 Venkatesan, A.K., Done, H.Y., Halden, R.U. 2015. United States National Sewage Sludge  
538 Repository at Arizona State University--a new resource and research tool for  
539 environmental scientists, engineers, and epidemiologists. *Environmental science and  
540 pollution research international*, **22**(3), 1577-1586.

541 Wang, Z., Liu, T., Duan, H., Song, Y., Lu, X., Hu, S., Yuan, Z., Batstone, D., Zheng, M.  
542 2021. Post-treatment options for anaerobically digested sludge: Current status and  
543 future prospect. *Water Research*, **205**, 117665.

544 Wett, B., Eladawy, A., Ogurek, M. 2006. Description of nitrogen incorporation and release in  
545 ADM1. *Water Sci Technol*, **54**(4), 67-76.

546 Wilson, C.A., Novak, J.T. 2009. Hydrolysis of macromolecular components of primary and  
547 secondary wastewater sludge by thermal hydrolytic pretreatment. *Water Research*,  
548 **43**(18), 4489-4498.

549 Wu, B., Dai, X., Chai, X. 2020. Critical review on dewatering of sewage sludge: Influential  
550 mechanism, conditioning technologies and implications to sludge re-utilizations.  
551 *Water Research*, **180**, 115912.

552 Yadav, M., Balan, V., Varjani, S., Tyagi, V.K., Chaudhary, G., Pareek, N., Vivekanand, V.  
553 2022. Multidisciplinary Pretreatment Approaches to Improve the Bio-methane  
554 Production from Lignocellulosic Biomass. *BioEnergy Research*.

555 Yan, W., Xu, H., Lu, D., Zhou, Y. 2022. Effects of sludge thermal hydrolysis pretreatment on  
556 anaerobic digestion and downstream processes: mechanism, challenges and solutions.  
557 *Bioresource Technology*, **344**, 126248.

558 Yang, D., Dai, X., Song, L., Dai, L., Dong, B. 2019. Effects of stepwise thermal hydrolysis  
559 and solid-liquid separation on three different sludge organic matter solubilization and  
560 biodegradability. *Bioresource Technology*, **290**, 121753.

561

562

563