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A short bibliographic review concerning biomethane production from wastewater sludge / Calabro, P.S., Elbeshbishy, E., Laqa Kakar, F., Zema, D.A.. - In: WASTE MANAGEMENT & RESEARCH. - ISSN 0734-242X. - (2024). [10.1177/0734242x241252906]

Availability:

This version is available at: <https://hdl.handle.net/20.500.12318/149387> since: 2024-11-25T08:35:47Z

Published

DOI: <http://doi.org/10.1177/0734242x241252906>

The final published version is available online at: <https://journals.sagepub.com/doi/10.1177/0734242x241252906>

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3 **Calabrò, P. S., Elbeshbishy, E., Laqa Kakar, F., & Zema, D. A. (2024). A short bibliographic**
4 **review concerning biomethane production from wastewater sludge. *Waste Management &***
5 ***Research*, 0734242X241252906.**

6

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which has been published in final doi

8

9

[10.1177/0734242X241252](https://doi.org/10.1177/0734242X241252906)

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(<https://journals.sagepub.com/doi/10.1177/0734242X241252906>)

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15 **SHORT REVIEW**

16

17 **A short bibliographic review concerning biomethane production from wastewater**
18 **sludge**

19

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31

32 **Abstract**

33

34 Biomethane production by anaerobic digestion (AD) of sludge from municipal wastewater
35 treatment is a viable practice to valorise the residues of these plants. However, although
36 the relevant literature is abundant, no comprehensive reviews have been recently
37 published on this topic. Detailed information concerning the factors influencing the AD
38 process and values of biomethane production from the sludge from municipal wastewater
39 treatment plants (MWWTPs) on the global scale may support technicians and researchers
40 in both the planning and the design steps of an AD process. This study proposes a

41 systematic review and a meta-analysis of the factors that noticeably influence biomethane
42 yield deriving from AD of sludge from MWWTP. The reported values were systematically
43 analysed compared to the main factors driving AD, including publication year, geographical
44 area of each study, type of digested sludge, treatment in the water line of the MWWTP,
45 possible sludge pre-treatments, type of digestion process, hydraulic retention time and
46 temperature regime of the AD process. A higher biomethane production was registered in
47 North American plants compared to countries in other continents. Older studies published
48 between 2001 and 2005 reported lower mean values compared to the more recent
49 experiments. A gradient of “primary sludge” > “mixed sludge” > “wastewater activated
50 sludge” was found for the mean biomethane yield in relation to the digested sludge type.
51 The mean biomethane yields for different types of sludge on a global scale are 0.425,
52 0.296, and 0.176 Nm³·kg_{VS}⁻¹, for primary sludge, mixed sludge, and waste activated
53 sludge, respectively. Overall, the study demonstrates: (i) the very large variability of
54 biomethane yields from AD of the residues from MWWTPs (mainly due to the different
55 characteristics of sludge); and (ii) the non-significance of some factors (i.e., treatment in
56 the water line, pre-treatments, type of process, hydraulic retention time and temperature
57 regime) on energy yields from the AD process.

58

59 **Highlights**

60

- 61 • Biomethane yields of AD of sludge from MWWTPs shows a large variability (0.03 to
62 0.72 Nm³·kg_{VS}⁻¹)
- 63 • Geographical area, date and sludge type influences these methane yields
- 64 • Studies between 2001 and 2005 report lower values compared to recent
65 experiments

66 • Higher yields are found for MWWTPs in North American plants compared to other
67 countries

68 • A gradient primary sludge > mixed sludge > activated sludge was found

69

70 **Keywords:** anaerobic digestion; biologic methane potential; mixed sludge; municipal
71 wastewater treatment plant.

72

73 **List of abbreviations**

74

75 MWWTPs: Municipal wastewater treatment plants

76 AD: Anaerobic digestion

77 VS: volatile solids

78 WAS: Waste Activated Sludge

79 PS: Primary Sludge

80 Mixed: Mixed Sludge (Primary + Mixed)

81 CAS: Conventional Activated Sludge

82 EA: Extended Aeration

83 AAS: Advanced Activated Sludge (e.g. Nitrogen and/or Phosphorus removal)

84 MBR: Membrane Bio-reactor

85 C/SC: Continuous/Semi-Continuous

86 CFC: Continuous Full scale

87 M: Mesophilic

88 T: Thermophilic

89 HRT: Hydraulic Retention Time

90

91 **1. Introduction**

92

93 Several management systems have been proposed to treat municipal and industrial
94 wastewater, such as intensive plants (using biological and physico-chemical processes),
95 natural systems, reuse in agriculture, and biorefineries (Kesari et al., 2021; Zema et al.,
96 2019). Some of these systems allow energy conversion (to biofuel, such as biomethane
97 and biodiesel) or the valorisation of by-products (fertilisers or conditioners) (Stavkova and
98 Maroušek, 2021). Among the intensive biological processes, anaerobic digestion is
99 becoming more and more widespread, especially when organic-rich wastewater is treated
100 (Folino et al., 2020). For other types of wastewater, such as municipal effluents, in view of
101 the circular economy perspectives and of the need to address the UN's Sustainable
102 Development Goals, increasing the recovery of materials and energy is imperative
103 (Kehrein et al., 2020). Municipal wastewater treatment plants (MWWTPs) produce a large
104 amount of sludge as a residue of treatment processes. The management of this residue
105 poses crucial issues either when further valorised or disposed of in landfills. For instance,
106 the treatment of excess sludge constitutes about 50% of the total operating cost of
107 MWWTPs (Domini et al., 2022). The MWWTPs will change greatly over the next few
108 years, due to the implementation of the "circular economy" concept, especially in those
109 countries where the production of fuels or fertilizers is not a legal requirement and an
110 established practice (Marami et al., 2022). This situation will progressively lead to a switch
111 from the common treatment of municipal wastewater by conventional MWWTPs to
112 biorefineries, where chemicals, fuels and fertilizers are produced (Güven et al., 2023).
113 Anaerobic digestion (AD) is a viable process for a sustainable conversion of organic
114 waste, such as agricultural and agro-industrial by-products, animal feedstock, and sludge
115 of various origins, into renewable energy (Metcalf, 2003). It has been applied for decades

116 in the conversion of wastewater sludge to biogas (a mix of methane, carbon dioxide,
117 nitrogen, hydrogen sulphide and other trace gases), in order to stabilize the generated
118 sludge and recover energy (Jenicek et al., 2012; Silvestre et al., 2015). In addition to the
119 production of renewable energy, AD yields digestate, which, if the required standards are
120 met, can be used as a soil conditioner/fertilizer, thus allowing the completion of the
121 procedure in the circular management of this residue (Awasthi et al., 2023; Rizzioli et al.,
122 2023). The technical and economic performance of AD processes greatly depends on
123 several factors, such as feedstock, technology, and operating parameters (Zema, 2017).
124 Therefore, the comparison of energy yields from different MWWTPs may be difficult and
125 often unreliable. For instance, a commercial WWTP treats a site-specific wastewater and
126 implements complex processes, in which multiple material and energy flows continuously
127 enter and exit. Therefore, they represent systems that may differ from each other.
128 However, these comparisons can give general but practical indications concerning the
129 influence of the aforementioned factors on the overall technical and economic
130 performance of AD.

131 Different types of sludges from MWWTPs can be used as substrates for AD. According to
132 the most common classification, the types of MWWTP sludge are: (i) Primary Sludge (PS,
133 i.e. the sludge produced by the primary settling of municipal wastewater); (ii) Secondary
134 sludge or Waste Activated Sludge (WAS, the latter being the most common term, hereafter
135 used in this paper) or excess sludge (i.e. the sludge extracted from aerobic tanks or
136 secondary settlers or return sludge line); and (iii) Mixed sludge (i.e., a mix of PS and
137 WAS). The sludge can be pre-treated before AD, typically through thickening, or
138 dewatering (these treatments do not theoretically affect chemical composition and thus
139 potential biomethane production of sludge) or, more rarely, by thermal treatments.

140 Furthermore, sludge characteristics and AD performance may be influenced by several
141 factors, the following being considered as the most important (Chudoba et al., 2011): (i)

142 wastewater treatment scheme (e.g., Conventional Activated Sludge process, – CAS;
143 Membrane Bioreactor – MBR; Advanced processes derived from CAS for nutrient removal
144 – AAS); (ii) specific characteristics of municipal wastewater; (iii) AD reactor configuration
145 (e.g. batch, continuous, semi-continuous); (iv) AD temperature (i.e., mesophilic,
146 thermophilic); (v) Hydraulic Retention Time (HRT). However, it is worth mentioning that,
147 when the environmental standard for discharged/reclaimed water is satisfied as a basic
148 requirement, the economic performance of the MWWTP is the main operating criterion
149 driving the choice of the best layout and the characteristics of MWWTPs (Akbari et al.,
150 2021; Pavolova et al., 2021). An optimisation of the technical operation parameters may
151 support the cheapest solutions for technicians and developers.

152 When AD integration in a MWWTP is planned, the most accurate estimate of methane
153 yield is essential to evaluate the feasibility of alternative schemes. The literature
154 concerning biomethane yields from sludge produced by wastewater treatment plants is
155 abundant, since more than a hundred studies have been published in the last 25-30 years
156 (Table 1.SM). However, at least to the authors' best knowledge, no articles have been
157 recently published with quantitative reports of biomethane yields from the AD of MWWTP
158 sludge under different conditions. The published reviews usually focus on co-digestion,
159 specific pre-treatment or other improvements to the traditional AD process (e.g., (Calabrò
160 et al., 2021; Nabaterega et al., 2021)). Due to the lack of recent and inclusive reviews,
161 there is the risk that researchers and practitioners rely on very old data, which report
162 biomethane yields in the range of 0.3 – 0.6 Nm³·kg_{VS}⁻¹ (Metcalf, 2003).

163 The availability of sophisticated analytical techniques, such as meta-analysis, allow us to
164 link the data reported in the literature to the important drivers of the AD process, such as
165 the specific characteristics of municipal wastewater, AD reactor configuration, temperature,
166 HRT and others.

167 To bridge this gap in our knowledge, this study proposes a systematic review with a meta-
168 analysis of the factors that significantly influence both sludge characteristics and
169 biomethane yield from AD processes. With this aim in mind, 1400 academic papers
170 published from 1995 onwards in the international peer-reviewed literature have been
171 analysed; the reported biomethane yields, together with the associated data concerning
172 publishing year, geographical area of plants, sludge type, layout of the wastewater line,
173 possible pre-treatments, processes, HRT and temperature of AD, have been included in a
174 database supporting the meta-analysis and statistically processed. We hypothesise that
175 many of the aforementioned factors play a clear role in the biomethane yields of AD
176 sludge, and the analysis should identify the most significant parameters. These insights
177 together with the values of biomethane production from the sludge of MWWTPs on a
178 global scale identified by this analysis can support technicians and researchers both in the
179 planning and in the design steps of an AD process.

180

181 **2. Materials and methods**

182

183 *2.1 Paper search and selection*

184

185 Comprehensive bibliographic research was carried out in late October 2023 on the Scopus
186 database, to find the academic papers, relevant to sludge AD, published in the last 30
187 years (1994-2023). The individual keywords or their combination were “wastewater sludge”
188 and “anaerobic digestion”, which were found as components in the “Title”, the “Abstract” or
189 the “Author-specified keywords” fields. We considered “research papers”, “reviews” and
190 “short communications”, and excluded technical reports, M.Sc. and Ph.D. theses (since
191 these documents did not undergo any peer review from independent referees). We also
192 excluded papers not published in English (since the reported data can not be checked by

193 most of the international scientific community). This bibliographic research produced 1400
194 papers, of which 1284 were discarded, since the related studies did not comply with at
195 least one of the following conditions: the selected paper (i) did not report the essential data
196 or show qualitative information, (ii) did not provide sufficient data about full-scale plant
197 schemes where the wastewater sludge was produced, (iii) was related to sludge, which
198 was not produced in full-scale MWWTPs, (iv) did not report biomethane yield in terms of
199 volume per volatile solids (VS) in the substrate fed to the plants (to avoid complex
200 conversion operations, as in the cases where the specific production was expressed as a
201 function of COD rather than VS, which could lead to biased data). This selection retained
202 116 papers that include 200 “experiments”, where each experiment identifies a complete
203 record of the required data.

204

205 *2.2 Data collection and database compilation*

206

207 All 116 papers with the 200 experiments were analysed, in order to compile a database in
208 an Excel[®] file (see Supplementary Material – Table 1.SM). This database consists of the
209 values of the specific biomethane production (volume per unit of VS weight fed to the
210 digester, which hereafter will be simply indicated as “biomethane yield”). Moreover, the
211 following features (hereafter referred to “AD plant driving factors” or, more simply, “driving
212 factors”) were also extracted from each paper and included in the Excel[®] database:

- 213 a) bibliographic information: author(s) and the publication date of the paper
- 214 b) the geographical area of each study (Africa, Eastern and Western-Central Asia,
215 Europe, USA-Canada, Oceania, and Latin America)
- 216 c) digested sludge (Waste Activated Sludge, Primary Sludge, and Mixed Sludge)

- 217 d) the layout of the wastewater line (hereafter simply “treatment”, Conventional
218 Activated Sludge, Extended Aeration, Advanced Activated Sludge (e.g. nitrogen
219 and/or phosphorus removal), Membrane Bio-Reactor, and Sequencing Bio-Reactor)
220 e) pre-treatments (Dewatering, Thickening or None).
221 f) AD reactor configuration (Batch, Continuous/Semi-Continuous on a laboratory
222 scale, and Continuous Full Scale)
223 g) Hydraulic Retention Time (HRT, days)
224 h) temperature (Mesophilic, 25 to 42 °C, or Thermophilic, > 53 °C).

225 Pre-treatments, such as thermal or chemical hydrolysis, intra-cellular disintegration
226 (Maroušek and Kwan, 2013) and steam explosion (Maroušek, 2013), were not considered
227 in this paper, since they completely alter the nature of the substrate and noticeably make
228 the sludge treatment line complicate. The publication year of the paper and the experiment
229 date were considered as the same.

230

231 *2.3 Data processing*

232

233 The biomethane yield reported in each experiment was associated with each driving factor,
234 in order to assess its variability under the effect of each factor. For these driving factors,
235 the relevant values were conventionally grouped into the following classes:

- 236 a) the publication year of the paper: < 2000; 2001-2005; 2006-2010; 2011-2015; 2016-
237 2020; > 2020
238 b) the geographical area: Latin America; USA-Canada; Europe; Oceania; Western and
239 Central Asia; Africa; Eastern Asia
240 c) the digested sludge: Waste Activated Sludge; Primary Sludge; Mixed Sludge
241 d) the pre-treatments before AD; Dewatering; Thickening; None

- 242 e) the layout of the wastewater line: Conventional Activated Sludge; Extended
243 Aeration; Advanced Activated Sludge; Membrane Bio-Reactor; Sequencing Bio-
244 Reactor
- 245 f) HRT (days): < 15; 15-30; 31-45; 46-60; > 60
- 246 g) AD reactor configuration: Batch; Continuous/Semi-Continuous; Continuous Full
247 Scale
- 248 h) temperature: Mesophilic; Thermophilic.

249

250 *2.4 Statistical analysis*

251

252 A one-way ANOVA was applied to the biomethane yield (response variable) and each
253 driving factor (explanatory variable), to evaluate the statistical influence of each driving
254 factor on the response variable. In order to satisfy the assumptions of the statistical tests
255 (equality of variance and normal distribution), the data were subjected to normality testing
256 or were square root-transformed whenever necessary. The pairwise comparison by
257 Tukey's test (at $p < 0.05$) was also used to evaluate the statistical significance of the
258 differences in the response variable. The statistical tests were carried out by XLSTAT
259 software, released in 2019, Addinsoft, Paris, France.

260

261 **3. Results and discussion**

262

263 The geographic distribution of available data is not uniform and follows the wastewater
264 treatment service coverage in different areas of the World (Ehalt Macedo et al., 2022).
265 These authors compiled a global database ("HydroWASTE") based on approximately
266 60000 wastewater treatment plants, which showed that effluents are discharged into 1.2
267 million km of the global river network. Pollution is detected in areas with high population

268 densities in Europe, the USA, China, India, and South Africa, while the quality of river
269 water complies with the common threshold for environmental concern only in 5% of the
270 surveyed plants. According to official data (UN Habitat and WHO, 2021), only about 56%
271 of all wastewater flows generated by households in 2020 were collected and safely
272 treated. Most of the data comes from Europe, the USA, and part of Asia (i.e., China,
273 Japan, South Korea, Iran and Turkey) and only an extremely limited amount of information
274 is available from Africa and Latin America.

275 Among the 116 reviewed papers, the highest biomethane yield ($0.715 \text{ Nm}^3 \cdot \text{kg}_{\text{VS}}^{-1}$) was
276 reported in the paper by Gavala et al., (2003). These authors worked in Denmark with a
277 continuous/semi-continuous plant that digested PS from a CAS under mesophilic
278 conditions and an HRT of 20 days without any pre-treatment. This yield is even more
279 appreciable, taking into account the presence of phthalic acid esters with high molecular
280 weight in the wastewater treated in that study. Phthalic acid esters are reported to be non-
281 biodegradable under methanogenic conditions. In contrast, the lowest value (0.030
282 $\text{Nm}^3 \cdot \text{kg}_{\text{VS}}^{-1}$) was found in Hao et al., (2017). This study was carried out in China in a
283 continuous/semi-continuous plant treating WAS coming from an AAS under mesophilic
284 conditions and an HRT of 32 days with thickening as the pre-treatment. The reason for this
285 low biomethane yield is not explained by the authors, who even used waste iron scraps,
286 an industrial residue, to potentially enhance the AD process (Zhang et al., 2020). The
287 significant difference between the extreme values in the biomethane yield (by a factor of
288 23) indicates the large variability in sludge AD.

289 According to one-way ANOVA, the biomethane yield was significantly different in the
290 studies published throughout the monitoring period (Table 2.SM). However, only the mean
291 yield reported in the studies published between 2001 and 2005 ($0.446 \text{ Nm}^3 \cdot \text{kg}_{\text{VS}}^{-1}$) was
292 significantly different from the values reported in the other studies (varying in the range
293 $0.109 \text{ Nm}^3 \cdot \text{kg}_{\text{VS}}^{-1}$, before 2000, to $0.267 \text{ Nm}^3 \cdot \text{kg}_{\text{VS}}^{-1}$, years 2006-2010). The other periods

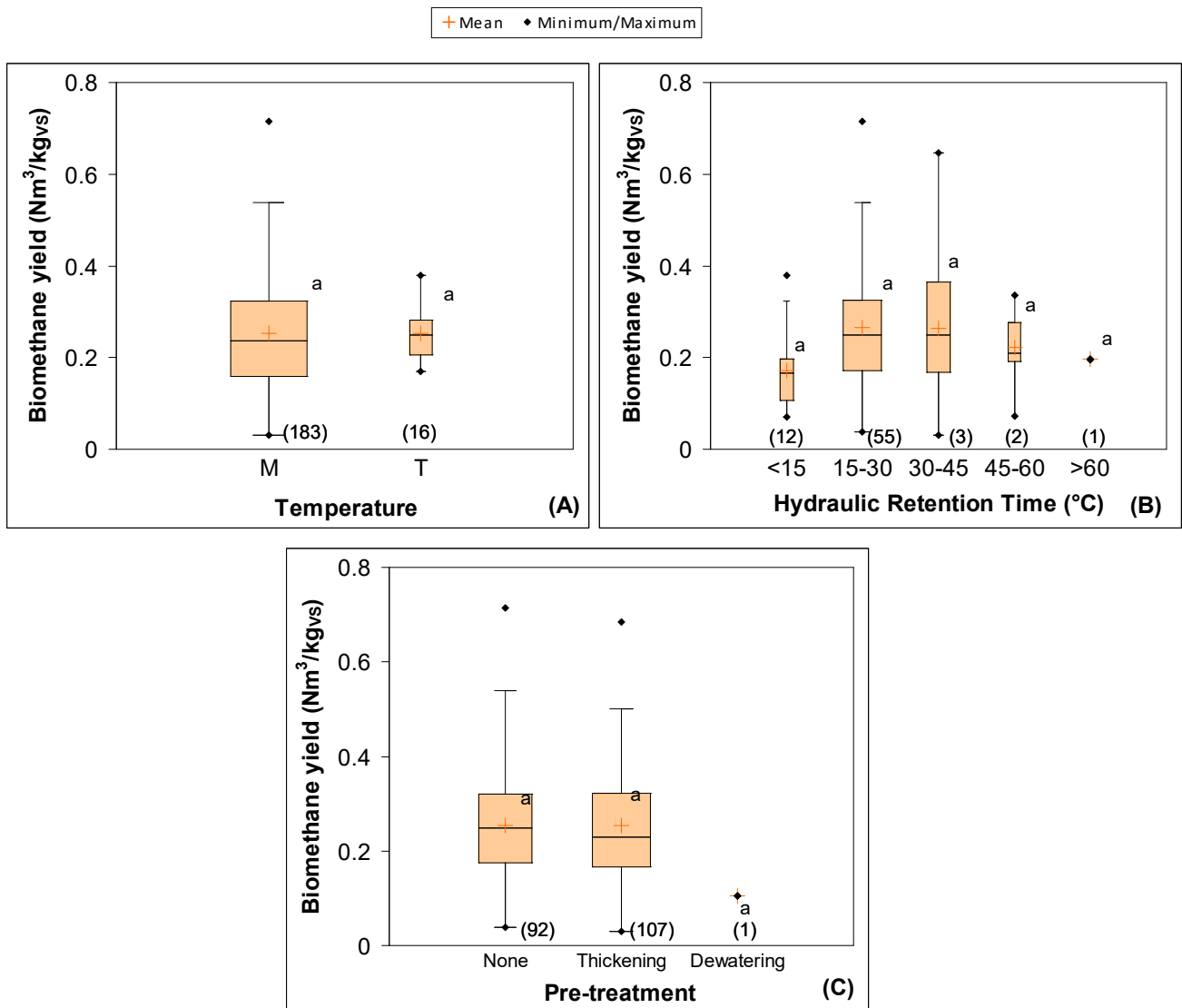
294 showed intermediate values, $0.446 \text{ Nm}^3 \cdot \text{kg}_{\text{VS}}^{-1}$ for the years 2001-2005, $0.243 \text{ Nm}^3 \cdot \text{kg}_{\text{VS}}^{-1}$
295 for the years 2011-2015, $0.262 \text{ Nm}^3 \cdot \text{kg}_{\text{VS}}^{-1}$ for the years 2016-2020, and $0.244 \text{ Nm}^3 \cdot \text{kg}_{\text{VS}}^{-1}$
296 after 2000 (Figure 1a). It is worth noting that after 2005 the mean biomethane yield is very
297 stable. A possible explanation is that, before 2005, the methods for assessing biogas
298 production (batch tests in particular) were scarcely standardized, and this could explain the
299 large variability in the experiments carried out before that date. The low number of
300 experiments (five before 2000 and once again five between 2001 and 2005) may have
301 also played a role in the variability between older and more recent data.

302 The differences in the mean biomethane yield in the different geographical areas of the
303 reviewed studies were also significant (Table 2.SM), but this conclusion may be affected
304 by the non-uniform distribution of studies on a global scale. Unfortunately, as
305 aforementioned, the published data was concentrated in Europe, the United States and
306 Canada, Western-Central and Eastern Asia (where the data was concentrated in Turkey,
307 Iran, China, Japan and South Korea) and, partially, in Oceania. Very few data were
308 available in studies from Africa and Latin America, and none from Southern and South-
309 Eastern Asia. The biomethane production in Eastern Asia ($0.148 \text{ Nm}^3 \cdot \text{kg}_{\text{VS}}^{-1}$), Africa (0.172
310 $\text{ Nm}^3 \cdot \text{kg}_{\text{VS}}^{-1}$), Oceania ($0.190 \text{ Nm}^3 \cdot \text{kg}_{\text{VS}}^{-1}$), Europe ($0.196 \text{ Nm}^3 \cdot \text{kg}_{\text{VS}}^{-1}$), and Western-Central
311 Asia ($0.246 \text{ Nm}^3 \cdot \text{kg}_{\text{VS}}^{-1}$) were quite similar to each other, and lower, although not
312 significantly, compared to the mean yields measured in the studies carried out in the
313 American continent (Figure 1b). The highest values were recorded in the USA-Canada
314 ($0.372 \text{ Nm}^3 \cdot \text{kg}_{\text{VS}}^{-1}$) and in Latin America ($0.396 \text{ Nm}^3 \cdot \text{kg}_{\text{VS}}^{-1}$). However, the result for this
315 latter area is most probably biased by the low number of experiments (only two) (Figure
316 1b).

317 Sewer conditions, people's diet, and climate can affect wastewater characteristics, and
318 these may be the additional reasons for the differences involving the geographic
319 conditions. For instance, an explanation for the higher yields recorded in North America

320 could be the diffusion of in-sink food waste disposers. Unlike Europe, where household
321 food waste is managed with municipal solid waste, in the USA and Canada, in-sink
322 grinders are often adopted, and food waste is disposed of with sewage (Bolzonella et al.,
323 2003; Marashlian and El-Fadel, 2005). This fact leads to an increase in suspended solids,
324 organic matter and nutrients in the MWWTP, to a more stringent need to adopt primary
325 treatment and to an increased biomethane yield, especially from PS (Guan et al., 2022;
326 Kim and Phae, 2023). This hypothesis appears confirmed by Van Drecht et al. (2009), who
327 worked with N and P produced by MWWTPs, and reported a projection for a significant
328 increase in the release in North-America due to increased loading and poor performance
329 of plants. This fact requires further research, since the combined treatment of household
330 organics and wastewater sludge has been the focus of many studies (Calabrò and Satira,
331 2020; Nghiem et al., 2017), and the inclusion of this option in the aforementioned switch
332 from conventional MWWTPs to modern wastewater biorefineries must be considered.

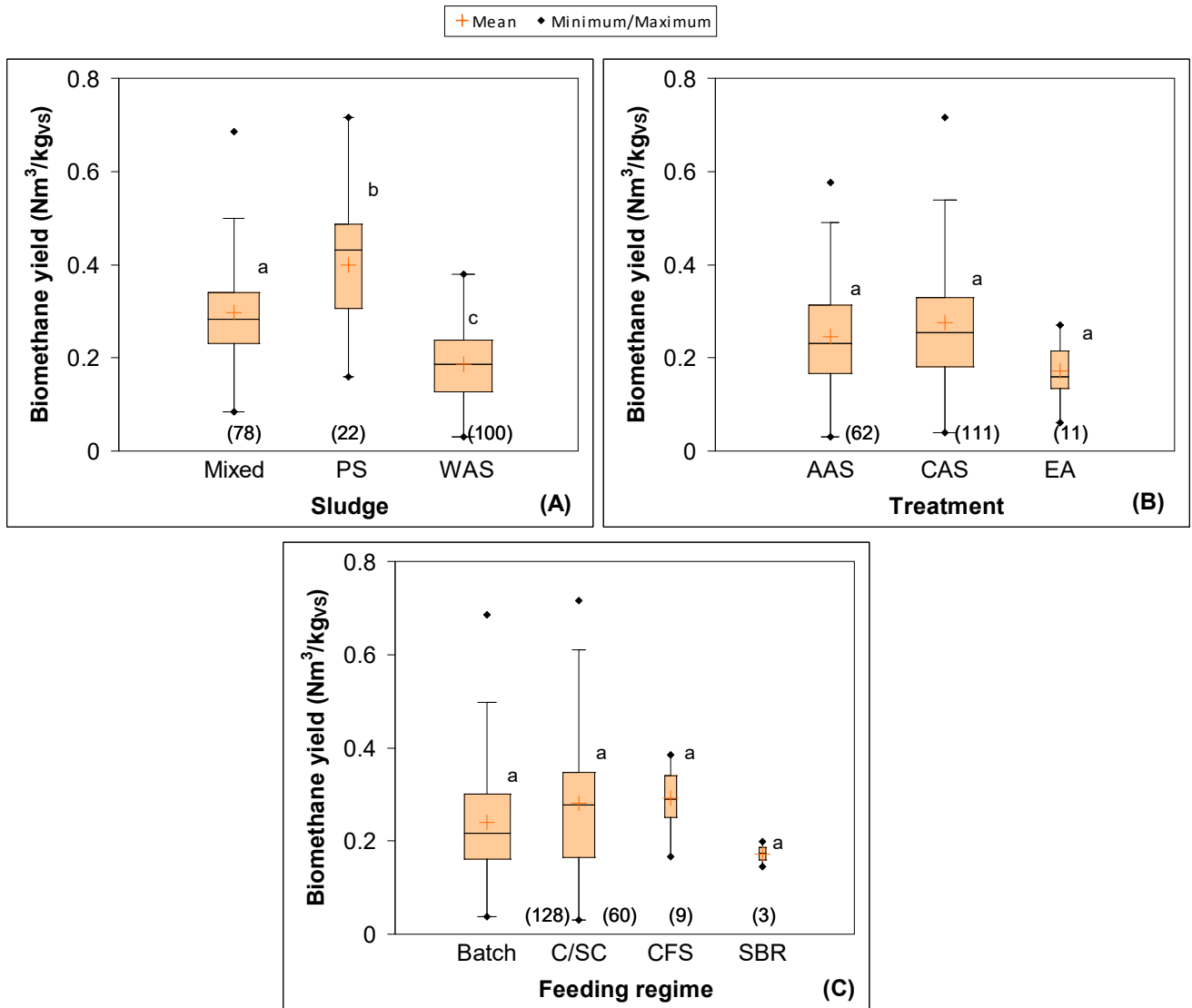
333



334

335 Figure 1 – Variability of biomethane yield (expressed as Nm^3 per unit weight of volatile
 336 solids, VS, fed to the reactor) reported in the reviewed studies involving publication year (a)
 337 and geographical area (b). Notes: Afr = Africa; W-C Asia = Western-Central; E Asia =
 338 Eastern Asia; Eur = Europe; Ocn = Oceania; USA-Can = USA and Canada; Lat Amer =
 339 Latin America). The number in brackets indicate the number of experiments in each class.
 340 Different letters indicate significant differences in the mean biomethane yield after Tukey's
 341 test ($p < 0.05$).

342



343

344 Figure 2 – Variability of biomethane yield expressed as Nm³ per unit weight of volatile
 345 solids, VS, fed to the reactor) per sludge type (a), treatment (b), AD reactor configuration
 346 (c) reported in the reviewed studies. *Notes: WAS = Waste Activated Sludge, PS = Primary
 347 Sludge; Mixed = Mixed Sludge (Primary + Mixed); CAS = Conventional Activated Sludge;
 348 EA = Extended Aeration; AAS = Advanced Activated Sludge (e.g., nitrogen and/or
 349 phosphorus removal); MBR = Membrane Bio-Reactor; SBR = Sequencing Bio-Reactor;
 350 C/SC = Continuous/Semi-Continuous; CFS = Continuous Full Scale. The number in
 351 brackets indicate the number of experiments in each class. Different letters indicate
 352 significant differences in the mean biomethane yield after Tukey's test (p < 0.05).*

353

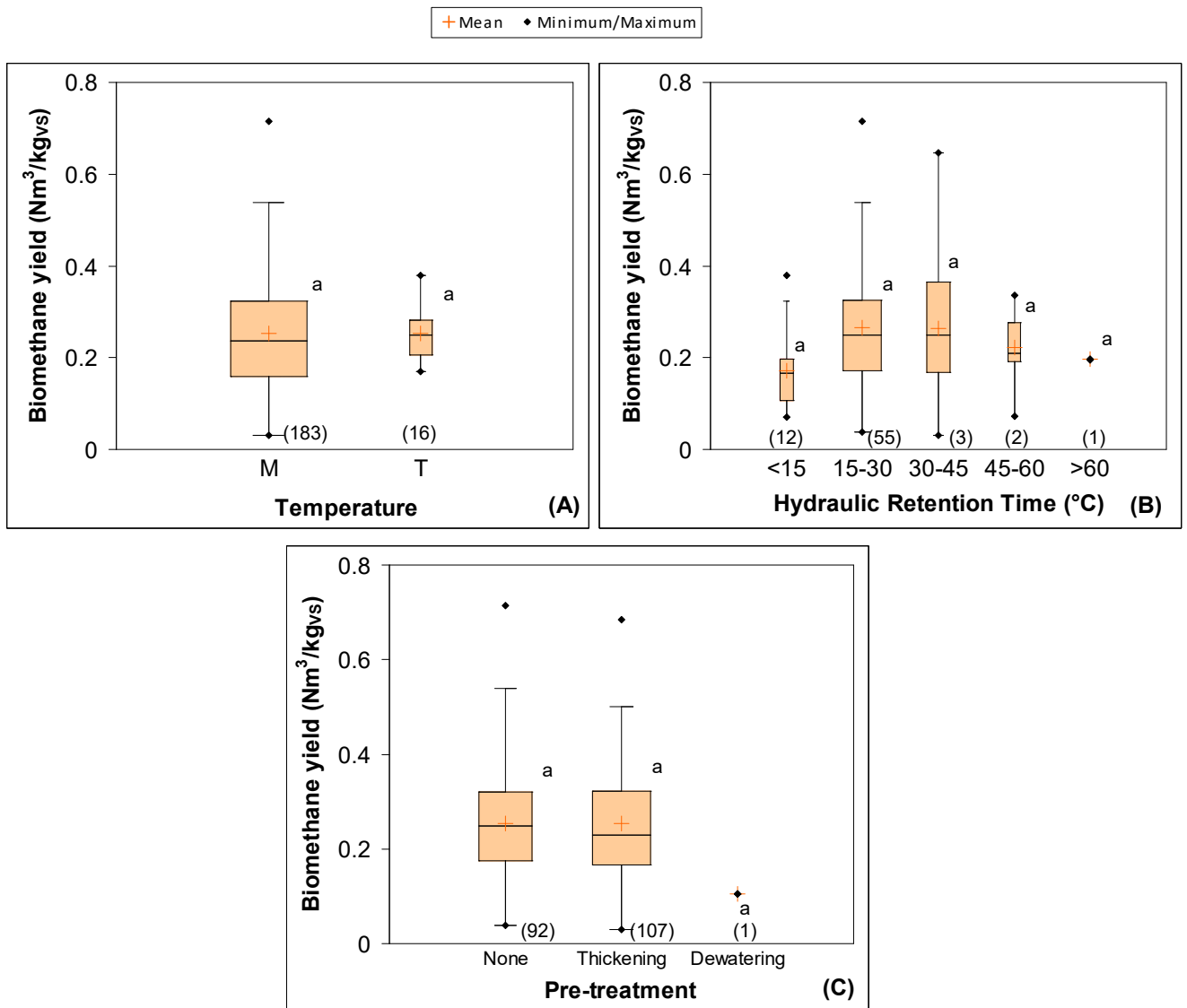
354 Biomethane production was significantly variable according to sludge type, and all
355 differences between the pairs of sludge types in the reviewed papers were significant
356 (Table 2.SM). To be more precise, PS and WAS gave the highest ($0.398 \text{ Nm}^3 \cdot \text{kg}_{\text{VS}}^{-1}$) and
357 lowest ($0.187 \text{ Nm}^3 \cdot \text{kg}_{\text{VS}}^{-1}$) mean yields, as expected. An intermediate value (0.296
358 $\text{Nm}^3 \cdot \text{kg}_{\text{VS}}^{-1}$) very close to the average value between these extremes was noticed for the
359 production from mixed sludge. The range of variability followed the gradient PS > Mixed >
360 WAS (Figure 2a). However, on a full scale the digestion of PS alone is usually not
361 practiced, since two separate digesters would be necessary. Therefore, only major
362 wastewater treatment plants carry out the separate digestion of PS and WAS. Moreover,
363 only WAS is produced in smaller and more advanced plants, especially when the primary
364 treatment is not installed (to enhance the removal of biological nutrients). Unfortunately, in
365 most of the examined studies, the details concerning the wastewater treatment were not
366 sufficient enough to understand whether the WAS used was produced in a plant with
367 primary treatment or not. This information would identify possible differences in WAS
368 between plants with and without primary treatment. It is worth noting that the values
369 reported in the reviewed studies are, even for PS, in the lower part of the range reported in
370 common textbooks ($0.3 - 0.6 \text{ Nm}^3 \cdot \text{kg}_{\text{VS}}^{-1}$) (Metcalf and Eddy, 2003). This is presumably
371 due to changes in wastewater and MWWTPs technologies, and to an improved separation
372 of municipal and industrial sewage.

373 In contrast to what was found for the sludge type, no difference in the different treatments
374 producing sludge in MWWTPs was revealed by the statistical analysis of the reviewed
375 studies (Table 2.SM). The mean biomethane yield was in the range of $0.171 \text{ Nm}^3 \cdot \text{kg}_{\text{VS}}^{-1}$
376 (EA) to $0.275 \text{ Nm}^3 \cdot \text{kg}_{\text{VS}}^{-1}$ (CAS). Moreover, biomethane yields from sludge produced in
377 EA and AAS plants showed a much larger variability than processes based on CAS
378 (Figure 2b). The aforementioned process gives a significantly higher methane yield than
379 EA, since waste sludge from EA is known to be more stabilized and thus less

380 biodegradable. However, the variability in methane yields from all processes is very high,
381 and this explains the lack of significant differences among the mean values. The mean
382 methane yield did not significantly vary with the AD process (Table 2.SM). The range of
383 variability was $0.172 \text{ Nm}^3 \cdot \text{kg}_{\text{VS}}^{-1}$ (SBR, but with a limited number of data) to 0.291
384 $\text{Nm}^3 \cdot \text{kg}_{\text{VS}}^{-1}$ (CFS plants) (Figure 2c), the latter plants showing an extreme variability. The
385 lack of significant differences in biomethane production between continuous/semi-
386 continuous (in a laboratory) and full-scale plants must also be highlighted. This suggests
387 using continuous/semi-continuous plants rather than batches in laboratory studies to
388 increase the statistical significance of data.

389 The ANOVA did not reveal significant differences in biomethane yield either for the
390 temperature regime (mesophilic or thermophilic) or the HRT (only continuous and semi-
391 continuous experiments either in a lab or full-scale were considered) of the AD process
392 (Table 2.SM). In actual processes, AD temperature was $36.1 \pm 1.9 \text{ }^\circ\text{C}$ and $54.9 \pm 0.3 \text{ }^\circ\text{C}$ for
393 mesophilic and thermophilic experiments, respectively. In spite of the similar mean value of
394 biomethane yield, mesophilic temperatures give a large variability compared to
395 thermophilic conditions (Figure 3a). Thus, this indicates that the adoption of a thermophilic
396 process is not always statistically advisable, due to its higher energy demand and complex
397 nature (Gebreeyessus and Jenicek, 2016). Therefore, the adoption of mesophilic vs.
398 thermophilic AD must be evaluated case-by-case. Moreover, if the aim of biogas upgrade
399 to biomethane is fuel injection into the energy grid (Nguyen et al., 2021), since heat is not
400 recovered as usually happens when biogas is used on-site to produce energy, the
401 feasibility of thermophilic process is very questionable.

402



403

404 Figure 3 – Variability of biomethane yield (expressed as Nm³ per unit weight of volatile
 405 solids, VS, fed to the reactor) per process temperature regime (a), hydraulic retention time
 406 (HRT, b), and pre-treatment (c) reported in the reviewed studies. *The number in brackets*
 407 *indicates the number of experiments in each class. Different letters indicate significant*
 408 *differences in the mean biomethane yield after Tukey's test (p < 0.05).*

409

410 The lack of statistical significance in the differences in biomethane production for
 411 experiments with different HRT could be due to the low number of experiments lasting
 412 over 30 days (only five). However, it is worth noting that the mean biomethane yield is

413 much higher at HRT between 15 and 30 days compared to the experiments where the
414 residence time is less than 15 days (Figure 3b).

415 No effect of the thickening process on biomethane yield was statistically detected in
416 comparison to the AD plants without pre-treatment (Table 2.SM and Figure 3c), while only
417 one experiment using dewatering as a pre-treatment was recorded. This makes a
418 statistical comparison with other plant layouts (absence of pre-treatments and thickening)
419 impossible.

420 Overall, Table 1 summarizes the average biomethane yield calculated on the basis of
421 data collected only in semi-continuous/continuous AD plants in the laboratory or full scale
422 plants for different sludge and geographical areas (the latter calculated for more than five
423 experiments). For mixed sludge, the highest values ($0.431 \text{ Nm}^3 \cdot \text{kg}_{\text{VS}}^{-1}$) are reported for
424 North American plants. In contrast, a much lower value (close to $0.3 \text{ Nm}^3 \cdot \text{kg}_{\text{VS}}^{-1}$) is
425 expected for European plants and at a global level. For WAS, the expected value at a
426 global level is $0.176 \text{ Nm}^3 \cdot \text{kg}_{\text{VS}}^{-1}$, while the production in Eastern Asia should be much
427 lower ($0.124 \text{ Nm}^3 \cdot \text{kg}_{\text{VS}}^{-1}$).

428

429 Table 1 – Main statistics of biomethane yield (expressed as Nm³ per unit weight of volatile
 430 solids, VS, fed to the reactor) for different types of sludge treated in semi-
 431 continuous/continuous and full-scale AD plants.

Sludge	Geographical area	Number of observations	Mean	Std. Dev.	Confidence interval	
PS	World	6	0.425	0.188	0.275	0.575
Mixed	World	48	0.296	0.112	0.264	0.328
	Europe	38	0.275	0.116	0.238	0.312
	USA-Canada	8	0.431	0.098	0.363	0.499
WAS	World	18	0.176	0.102	0.129	0.223
	Eastern Asia	6	0.124	0.099	0.045	0.203
	Europe	6	0.180	0.074	0.121	0.239

432

433 Notes: WAS = Waste Activated Sludge, PS = Primary Sludge; Mixed = Mixed Sludge (Primary +
 434 Mixed).

435

436 Overall, in the authors' opinion, the lack of data in more than 90% of papers (1287 out of
 437 approx.1400) resulting from the bibliographic research regarding the AD of MWWTPs
 438 implies the need for a standardisation of protocols for data collection in both lab- and full-
 439 scale plants, in order to allow a proper comparison of the technical and the energy
 440 performances of AD processes. In this sense, the scientific community should prepare a
 441 commonly-accepted minimum dataset to share with technicians (managing full-scale
 442 plants) and researchers (carrying out scientific investigations on a lab-scale).

443 Moreover, the concentration of studies regarding the biomethane yields of AD of sludge
 444 produced by plants treating only municipal wastewater suggests the need for further
 445 research exploring the advantages of the co-digestion of these effluents with other

446 domestic waste, such as the organic fraction of municipal solid waste or agri-food by-
447 products (in rural areas, where disposal of this waste poses environmental and economic
448 issue). Finally, according to Gebreeyessus and Jenicek (2016), the digestate quality is
449 neglected in most studies while it is the essential factor for the sustainability of the process
450 allowing its direct/indirect recovery as a fertilizer or soil improver. Of course, the soil's
451 suitability as receptor must be carefully evaluated on the environmental point of view, and
452 the compliance with national and local rules must be respected. This may lead not only to
453 an optimisation of the energy yields of AD, but also to the recovery of added-value
454 products from sludge (Maroušek et al., 2020).

455

456 **4. Conclusions**

457

458 In reply to the working hypothesis concerning the factors that greatly influence the
459 biomethane yields of anaerobic digestion of sludge from MWWTPs: the study has shown
460 that:

- 461 - The lowest and highest biomethane yields were 0.030 and $0.715 \text{ Nm}^3 \cdot \text{kg}_{\text{VS}}^{-1}$,
462 respectively, and this difference shows the large variability in sludge AD (mainly due
463 to the different characteristics of sludge).
- 464 - The statistical analysis demonstrated that, among the analysed driving factors, only
465 the geographical area, the experiment date, and the digested sludge type
466 significantly influenced the biomethane yield.
- 467 - Older studies (published between 2001 and 2005) report lower mean values
468 compared to more recent experiments.
- 469 - The higher yields found for Northern America can be justified by the widespread
470 adoption of in-sink food waste disposers.

- 471 - A gradient “primary sludge” > “mixed sludge” > “wastewater activated sludge” was
472 found for the mean biomethane yield in relation to the digested sludge type.
- 473 - The mean biomethane production was statistically similar in the different plant
474 layouts. and did not significantly change in the case of sludge pre-treatment.
- 475 - The energy potential did not considerably differ between continuous/semi-
476 continuous (on a laboratory scale) and full-scale plants.
- 477 - No significant difference in biomethane yield was observed at different hydraulic
478 retention times and temperatures.
- 479 - Finally, the mean biomethane yield with the confidence interval has also been
480 estimated for different types of sludge and geographical areas (0.425, 0.296, and
481 $0.176 \text{ Nm}^3 \cdot \text{kg}_{\text{VS}}^{-1}$, for PS, mixed sludge, and WAS, on a global scale) supporting
482 technicians and researchers in both the planning and the design steps of an AD
483 process.

484

485 **References**

486

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