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Glocal Assessment of Integrated Wastewater Treatment and Recovery Concepts Using Partial Nitrification/Anammox and Microalgae for Environmental Impacts

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Abstract

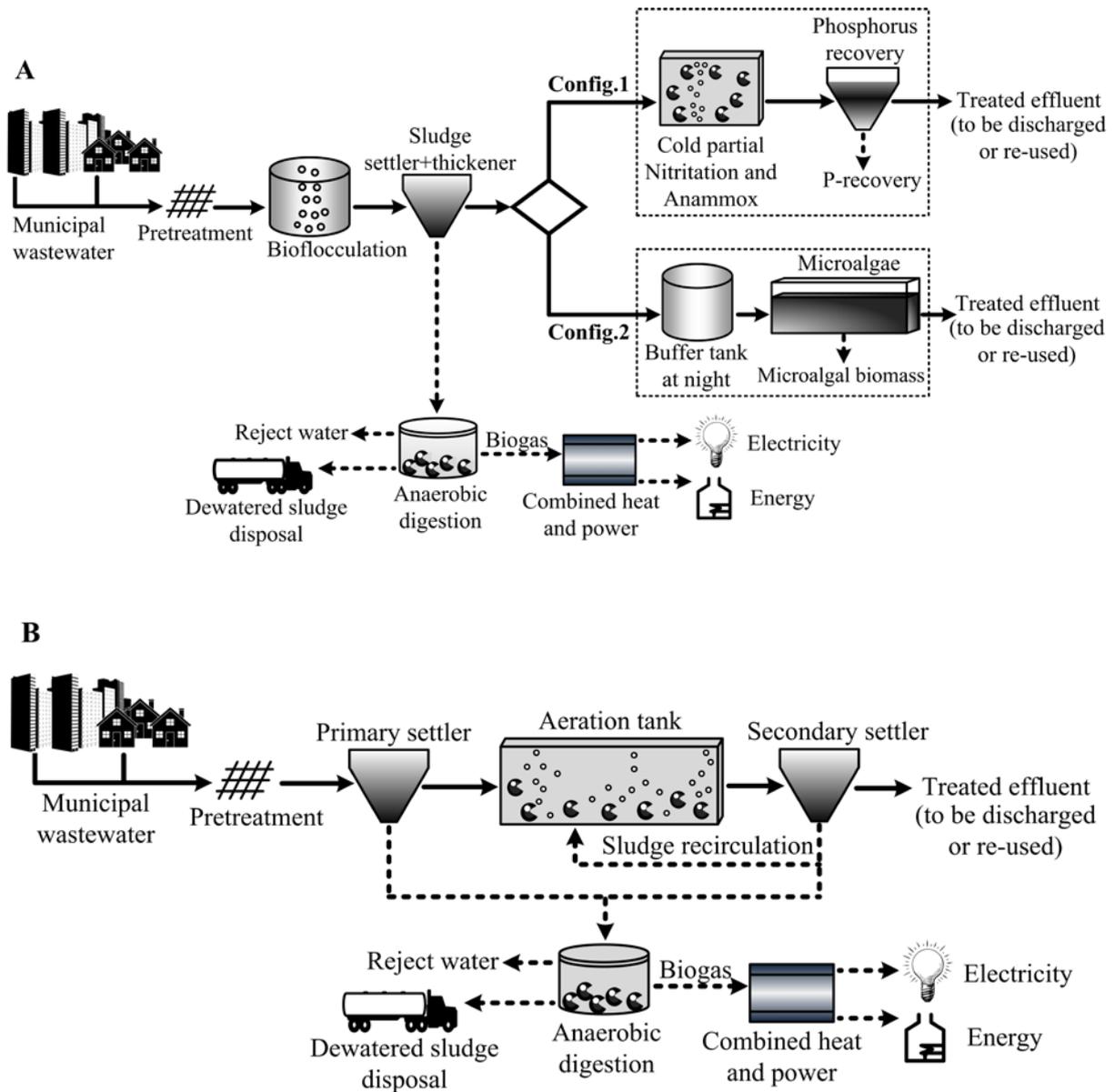
This study explored the feasibility and estimated the environmental impacts of two novel wastewater treatment configurations. Both include combined bioflocculation and anaerobic digestion but apply different nutrient removal technologies, i.e. partial nitrification/Anammox or microalgae treatment. The feasibility of such configurations was investigated for 16 locations worldwide with respect to environmental impacts, such as net energy yield, nutrient recovery and effluent quality, CO₂ emission, and area requirements. The results quantitatively support the applicability of partial nitrification/Anammox in tropical regions and some locations in temperate regions, whereas microalgae treatment is only applicable the whole year round in tropical regions that are close to the equator line. Microalgae treatment has an advantage over

25 the configuration with partial nitrification/Anammox with respect to aeration energy and nutrient
26 recovery, but not with area requirements. Differential sensitivity analysis points out the
27 dominant influence of microalgal biomass yield and wastewater nutrient concentrations on area
28 requirements and effluent quality. This study provides initial selection criteria for worldwide
29 feasibility and corresponding environmental impacts of these novel municipal wastewater
30 treatment plant configurations.

31 **Keywords:** Wastewater treatment; Wastewater management; Microalgae reactor; Energy
32 recovery; Nutrient recovery; Effluent quality

33 **1. Introduction**

34 Municipal wastewater is commonly treated by conventional activated sludge (CAS)
35 systems. However, these CAS systems cannot be considered sustainable because most of the
36 organic matter is aerobically mineralized and the treated water is not reused. Moreover, a cost-
37 effective technology that can recover valuable nutrients, such as nitrogen (N) and phosphorus
38 (P), from dilute wastewater streams still remains a technological challenge. Therefore, in recent
39 years new municipal wastewater treatment plants (WWTPs), which combine treatment with
40 recovery of these resources (Fernandez-Arevalo et al., 2017; Khiewwijit et al., 2015b; McCarty
41 et al., 2011) were proposed. In addition, a mathematical programming based optimization
42 framework/criteria was also developed to manage the complexity of the design problems for a
43 new WWTP (e.g. Bozkurt et al., 2015; Hauduc et al., 2015; Chhipi-Shrestha et al., 2017a, b).
44 Numerical simulation, based on literature information and experimental data, can be used to
45 assess the feasibility of such novel treatment and recovery concepts. Khiewwijit et al. (2015b)
46 used this approach to evaluate two novel WWTP configurations (Fig. 1A) that have the
47 potential to maximize energy and phosphorus recovery under Dutch conditions. They also
48 compared these configurations to the CAS system (Fig. 1B).



49

50

51 **Fig. 1:** (A) Two novel configurations for municipal wastewater treatment, suggested by Khiewwijit et
 52 al. (2015b), and (B) the CAS system. Solid lines indicate processes of the mainstream treatment and
 53 dashed lines indicate processes of the downstream solids treatment. ◊ is a decision block.

54

55 Given the composition of the wastewater, light intensities and temperatures at different
 56 locations around the world, each of these two configurations can be evaluated with respect to
 57 their impact on the environment. More specifically, the effluent of a WWTP with remaining
 58 N, P and chemical oxygen demand (COD) is an input to the receiving water body, e.g., a lake,

59 river or canal, thus affecting the water quality of the surrounding environment of the WWTP
60 (see, e.g., Wang et al., 2017). Furthermore, the CO₂ emission from the WWTP, as a result of
61 the oxidation of organic matter in the wastewater and other steps in the wastewater treatment,
62 contributes to greenhouse emissions (Bridle, 2007; Snip, 2010; Das, 2011; Gupta and Singh,
63 2012).

64 In this study, given the local conditions of a WWTP in terms of hydraulic and organic load,
65 light and temperature, we aim to find a configuration that not only reduces the negative
66 pollution effects of municipal WWTP's on the environment, but also recovers nutrients and
67 energy to a large extent. The calculated mass and energy flows can subsequently be used as
68 fluxes through the boundaries of a WWTP to evaluate the local effects of WWTP's on the
69 surrounding environment.

70 For instance, in Configuration 1 (Fig. 1A), the diluted organic matter in municipal
71 wastewater, after screening and grit removal, is concentrated by a bioflocculation process
72 (Faust et al., 2014). In experiments reported by Khiewwijit et al. (2015a), it was found that
73 bioflocculation in a high-loaded membrane bioreactor (HL-MBR) could concentrate 75.5% of
74 the sewage COD (chemical oxygen demand), whereas only 7.5% was mineralized into CO₂.
75 They also found that only a small fraction of the sewage NH₄-N and PO₄-P ended up in the
76 concentrate, and 90% of these compounds was conserved in the HL-MBR permeate. The
77 bioflocculated sewage organic matter is subsequently converted to methane in a mesophilic
78 anaerobic digester, followed by a combined heat and power (CHP) unit to convert the methane
79 to electricity and heat. The effluent of the bioflocculation process is subsequently treated by a
80 (cold) partial nitrification/Anammox process for N removal. The P can be recovered, for example
81 by struvite precipitation or by another low-cost technology (Desmidt et al., 2015). In the study
82 of Khiewwijit et al. (2015b) it was assumed that in the near future technologies which can
83 recover P from diluted wastewater streams will become available. It was also assumed that

84 such technologies can remove P down to levels that meet the discharge guidelines.
85 Bioflocculation, anaerobic sludge digestion and CHP processes are already applied in full-scale
86 municipal WWTPs. However, more research is still required for (cold) partial
87 nitrification/Anammox processes before it can be widely applied in practice.

88 In Configuration 2 (Fig. 1A), a similar approach with combined bioflocculation and
89 anaerobic digestion of the bioflocculated organic matter is applied. However, in this
90 configuration the nutrients N and P in the effluent of the bioflocculation process are assimilated
91 by microalgae. A buffer tank is required to store the bioflocculation effluent during the night
92 when there is no microalgae activity. Microalgae treatment of municipal wastewater has been
93 extensively studied because it reduces CO₂ emission and aeration energy otherwise needed for
94 nitrification. Furthermore, the microalgal biomass can be used as a low-cost application such
95 as a nutrient fertilizer and complex organic substrates, or as a source for bioethanol, methane,
96 biodiesel, and biohydrogen (Milledge and Heaven, 2014). Mahdy et al. (2015) showed the
97 potential to produce additional biogas when sludge and microalgal biomass are co-digested.

98 Khiewwijit et al. (2015b) evaluated the configurations of Fig. 1A with respect to a number
99 of key performance indicators (KPIs). It was found that Configuration 1 is the most promising
100 configuration for the Netherlands, because it can:

- 101 1) treat wastewater year round;
- 102 2) produce an effluent at a quality that meets the discharge guidelines;
- 103 3) reduce CO₂ emission by 35% compared to the CAS system;
- 104 4) achieve a net energy yield up to 0.24 kWh per m³ of wastewater compared to a negative
105 net energy yield of -0.08 kWh per m³ of wastewater for the CAS system;
- 106 5) recover 80% of the sewage P.

107 It was also demonstrated that Configuration 2 with microalgae treatment is not applicable
108 in the Netherlands, because of a limited light availability, low temperature and low irradiance
109 in the winter period. However, microalgae treatment still may be applicable in regions with a
110 tropical climate (Olguín et al., 2003). Hence, the question is: can these findings be extrapolated
111 on a global scale and how?

112 The objective of this study was, therefore, to quantitatively explore the feasibility of the
113 above-mentioned municipal wastewater treatment configurations, including combined
114 bioflocculation and anaerobic digestion with partial nitrification/Anammox or microalgae
115 treatment for different locations around the globe (global assessment). Combined
116 bioflocculation and anaerobic digestion, for energy saving and energy recovery, were already
117 analyzed in detail by Khiewwijit et al. (2015b). Therefore, the present analysis mainly focused
118 on nitrogen removal technologies, i.e. (cold) partial nitrification/Anammox in Configuration 1
119 and microalgae treatment in Configuration 2, and not on a comparison between different
120 models for microalgae systems.

121

122 **2. Materials and Methods**

123 ***2.1. Scenario-based analysis***

124 The Excel-based model described by Khiewwijit et al. (2015b) with conversion efficiencies
125 and design specifications for each of the processes in Configurations 1–2 and for the reference
126 CAS system, was used for the calculations of the mass and energy balances under steady-state
127 conditions. A more detailed of efficiency, conversion and design parameter values used for
128 each process in Configurations 1–2 and the reference CAS system can be found in the
129 supplementary material. It is important to note that Khiewwijit et al. (2015b) developed a

130 numerical Excel-based simulation tool by combining literature data and information from
131 recent experimental research. As reported in this previous study, Configuration 2 with
132 microalgae treatment was not feasible for a temperate climate country like the Netherlands.
133 Therefore, the feasibility of only Configuration 1 with (cold) partial nitrification/Anammox was
134 further explored in comparison with the reference CAS system with respect to the KPIs, i.e.
135 effluent quality, operation applicability, CO₂ emission, energy consumption/production, and
136 net energy yield, using the Netherlands as a case study.

137 In the current study, the feasibility of Configuration 1 with (cold) partial
138 nitrification/Anammox was further evaluated under different locations around the globe, as well
139 as the feasibility of using Configuration 2 with microalgae treatment. For all configurations, it
140 is presumed that at least 90% phosphate removal/recovery efficiency can be achieved. In
141 Configuration 1 it is expected that such a cost-effective P recovery technology from dilute
142 wastewater stream will become available in the near future (Desmidt et al., 2015). Therefore,
143 in the present study cost-effective P recovery technology was not further substantiated, but was
144 assumed to be already available. In Configurations 1–2, bioflocculation is an aerobic biological
145 process for concentrating the sewage colloidal and suspended organic matter with the aid of
146 extracellular polymeric substances produced by microorganisms. Aeration energy for the
147 bioflocculation process was therefore also considered in the calculation of total energy
148 consumption of Configuration 1 and 2. Besides, the biodegradability of organic matter from an
149 anaerobic digester treating waste sludge in the reference CAS system was assumed to be 50%
150 less than with bioflocculated concentrate (Bolzonella et al., 2005).

151 Initially, under average annual temperature and light intensity conditions in Thailand the
152 two configurations of Fig. 1A were compared to the CAS system (Fig. 1B) with respect to the
153 KPIs. Thailand was selected as an example of a region with tropical climates, thus having a
154 high potential for microalgae treatment. In Thailand winter and summer conditions with respect

155 to temperature and light intensity are similar (Table 1). Therefore, to calculate the heating
156 energy for anaerobic digestion at 35°C, the average annual temperature was used. For
157 calculation of the area requirement for microalgae treatment the average annual temperature
158 and annual light intensity were used. The target N concentration in the effluent was 2.2 mg
159 N_{total}/L , which obeys the maximum tolerable risk (MTR) guidelines used by the Dutch water
160 boards. The P concentration in the effluent should always be below 1 mg P_{total}/L (Khiewwijit
161 et al., 2015b).

162 Subsequently, a process-based model of a microalgae reactor for nutrient removal from
163 municipal wastewater in Configuration 2 was included to explore the effects of local conditions
164 of a WWTP with microalgae reactor on the surrounding environment. In this study, for 16
165 selected locations worldwide the area requirements for a microalgae reactor were estimated in
166 relation to seasonal changes of light intensity and temperature. The most promising wastewater
167 treatment configurations for each of these locations were identified. Wastewater characteristics
168 and required effluent quality were the same as used in the first step.

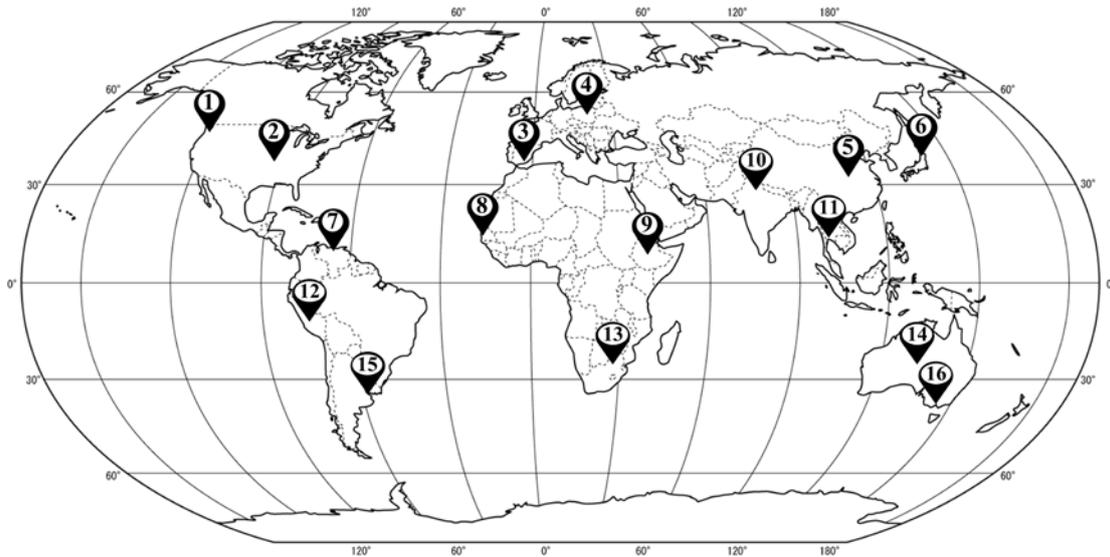
169 Finally, the effects of N and P sewage concentrations, microalgal biomass yield and
170 biomass maintenance coefficient on the area requirement of a microalgae reactor and on
171 effluent quality were examined in more detail for those locations where microalgae treatment
172 could possibly be applied with respect to temperature, light availability and light intensity. A
173 sensitivity analysis with respect to temperature and wastewater characteristics on cold partial
174 nitrification/Anammox process was already conducted by Khiewwijit et al. (2015b) and thus it
175 was excluded in this study. Minimum and maximum values for sewage NH_4-N of 20 and 35
176 mg N/L were used, respectively. For PO_4-P these values were 3 and 9 mg P/L, respectively
177 (von Sperling, 2007). In this study, a mass ratio of N and P in microalgal biomass of 5.38 g-
178 N/g-P was used (Tuantet, 2015).

179 **2.2. Characteristics of municipal wastewater**

180 The treatment configurations and the CAS system were evaluated for 100,000 inhabitants
181 (persons), which generated the average production of wastewater of 130 L/person/day, and
182 therefore a daily load of 13,000 m³ of wastewater was treated. Typical (average) concentrations
183 of organic matter, NH₄-N and PO₄-P in municipal wastewater were used: 600 mg COD/L, 25
184 mg N/L and 5 mg P/L (von Sperling, 2007). However, it is important to note that the wastewater
185 characteristics may vary from location to location, which caused by differences in separation
186 of storm water, precipitation and water scarcity. Khiewwijit et al. (2015b) found that a change
187 in total COD concentrations resulted in a significant different for the energy consumption and
188 energy production. Hence, a sensitivity analysis with respect to wastewater characteristics will
189 be further evaluated later in Section 2.7.

190 **2.3. Case study for different locations worldwide**

191 Fig. 2 shows 16 locations that were selected for the global assessment. To select these
192 locations, the globe was first divided into 36 regional groups with respect to degrees of
193 longitude and latitude, where the globe was longitude-wise divided into 6 sub-regions of 60
194 degrees each, and latitude-wise divided into 6 sub-regions of 30 degrees each. The final 16
195 regional groups were obtained after subtraction of 12 regions (polar zones), located above 60
196 degrees latitude North and South with an average yearly temperature below 0°C, and 8 regions
197 of which the surface is mainly covered by ocean from the 36 regions. A representative location,
198 i.e. a well-known city in each of the 16 regions, was then selected based on available datasets
199 given by PV Education (2015) and IET (2015).



200

201 **Fig. 2:** Map of the 16 selected locations used in this study; (1) USA, Washington, Seattle, (2) USA,
 202 Missouri, Kansas city, (3) Spain, Almeria, (4) Poland, Warsaw, (5) China, Xi'an, (6) Japan, Akita, (7)
 203 Venezuela, Caracas, (8) Senegal, Dakar, (9) Ethiopia, Addis Ababa, (10) India, New Delhi, (11)
 204 Thailand, Bangkok, (12) Peru, Huancayo, (13) South Africa, Pretoria, (14) Australia, Alice Springs,
 205 (15) Argentina, Buenos Aires, and (16) Australia, Melbourne.

206

207 **2.4. Photon flux density and temperature**

208 Table 1 shows average annual, summer and winter values for photon flux density (PFD)
 209 and temperature for each selected location. A regional dataset of surface solar radiation was
 210 taken from PV Education (2015) and IET (2015). The PFDs were then calculated following
 211 the steps in the study of Boelee et al. (2012), where it was assumed that 43% of the average
 212 photosynthetically active radiation (PAR), that is around 550 nm (400–700 nm), is utilized by
 213 microalgae. The temperatures at each location were taken from Weatherbase (2015). The 16
 214 locations were grouped into 3 different areas: (1) Northern hemisphere, i.e. locations above 30
 215 degrees Northern latitude; (2) nearby the equator line; and (3) Southern hemisphere, which are
 216 locations close to and above 30 degrees Southern latitude.

217 **Table 1**

218 Average annual, summer and winter values of photon flux density (PFD) and temperature for the
 219 selected locations.

Country/City	PFD ^{a,b} , mol/m ² /h (μmol/m ² /s)			Temperature ^c , °C		
	Annual	Summer	Winter	Annual	Summer	Winter
Northern Hemisphere						
1. USA, Washington, Seattle	0.99 (275)	1.71 (475)	0.33 (91)	11.4	17.9	5.6
2. USA, Missouri, Kansas city	1.28 (354)	1.87 (520)	0.69 (192)	12.5	24.6	-0.4
3. Spain, Almeria	1.45 (402)	2.10 (583)	0.86 (240)	18.7	24.9	13.1
4. Poland, Warsaw	0.79 (220)	1.49 (414)	0.19 (52)	7.8	16.7	-0.7
5. China, Xi'an	1.15 (320)	1.50 (417)	0.80 (222)	13.4	25.7	1.0
6. Japan, Akita	0.95 (264)	1.29 (358)	0.43 (119)	11.1	22.3	0.7
Nearby Equator line						
7. Venezuela, Caracas	1.31 (363)	1.40 (389)	1.22 (339)	22.8	23.0	21.7
8. Senegal, Dakar	1.73 (481)	1.71 (476)	1.58 (438)	24.0	26.3	21.7
9. Ethiopia, Addis Ababa	1.56 (432)	1.68 (465)	1.23 (342)	16.3	17.3	15.7
10. India, New Delhi	1.32 (368)	1.59 (443)	0.98 (272)	25.0	32.6	15.2
11. Thailand Bangkok	1.56 (434)	1.78 (494)	1.77 (491)	28.2	29.0	26.3
12. Peru, Huancayo	2.04 (567)	2.22 (618)	1.93 (535)	10.1	10.9	8.9
Southern Hemisphere						
13. South Africa, Pretoria	1.62 (450)	1.93 (537)	1.30 (361)	18.6	22.7	13.0
14. Australia, Alice Springs	1.86 (518)	2.26 (628)	1.40 (388)	20.3	27.3	12.3
15. Argentina, Buenos Aries	1.37 (381)	2.00 (556)	0.74 (207)	17.7	24.0	11.6
16. Australia, Melbourne	1.18 (329)	1.87 (520)	0.57 (158)	14.3	19.3	9.3

220 ^a Solar radiation on the horizontal surface in kWh/m²/day taken from PV Education (2015), excluding
 221 China, Xi'an.

222 ^b China, Xi'an, solar irradiation on the horizontal surface in Wh/m²/day taken from IET (2015).

223 ^c Temperatures taken from Weatherbase (2015).

224

225 **2.5. Area requirement for microalgae**

226 The influence of light intensity and temperature on microalgal growth has been widely
227 explored and modeled in various ways, for example, as reviewed by Béchet et al. (2013) and
228 reported in the studies of Béchet et al. (2017), Tuantet (2015) and Zijffers et al. (2010).
229 However, not only the factors of light intensity and temperature that have effects on the
230 microalgal growth, but also microalgae species, nutrient concentrations and operational
231 parameters, such as type of photobioreactor and light-path of photobioreactor, were
232 investigated. For instance, Tuantet (2015) developed a model to find out the biomass
233 productivity and area requirement with respect to light intensity for (1) a high concentration of
234 microalgal biomass cultivated on human urine, (2) a short light path photobioreactor (PBR) to
235 minimize the dark zone and (3) *Chlorella sorokiniana* as the main microalgae species.
236 Moreover, in this study of Tuantet (2015) the model to predict the biomass yield and biomass
237 maintenance coefficient at different dilution rates and nutrient concentrations was also
238 validated.

239 In the current study, the biomass productivity (P_{area} in g-dry weight/m²/h) and area
240 requirement (A in m²/person) for a microalgae treatment reactor were calculated using the
241 model and model parameters given in the studies of Tuantet (2015) and Zijffers et al. (2010),
242 as shown in Eq. (1) – Eq. (5) and in Table 2. This model was chosen because it is expected that
243 similar microalgae species and reactor design can be used for municipal wastewater treatment
244 (Abinandan and Shanthakumar, 2015). Whereas Tuantet (2015) showed that P was the major

245 factor limiting microalgae growth on human urine, in the current study N is the limiting
 246 nutrient, as will be shown later.

$$247 \quad C_{X,N} = \frac{N_{in} - N_{eff}}{F_N} \quad (1)$$

$$248 \quad r_{E,X} = \frac{PFD_{in}}{L * C_{X,N}} \quad (2)$$

$$249 \quad \mu_T = (r_{E,X} - m_{E,X}) * Y_{X,E} * f_T \quad (3)$$

$$250 \quad P_{area} = \mu_T * C_{X,N} * L \quad (4)$$

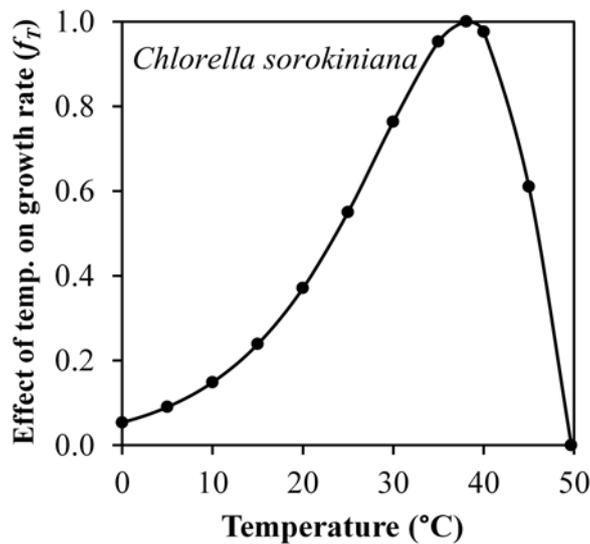
$$251 \quad A = \frac{F_w}{L * \mu_T} \quad (5)$$

252 with $C_{X,N}$ the biomass density assuming that the amount of N rather than P determines the
 253 biomass production (g-dw/m³), N_{in} and N_{eff} the concentrations of N in the influent and effluent,
 254 respectively (g N/m³), F_N the fraction of N in microalgal biomass of 0.078 g-N/g microalgal
 255 biomass, $r_{E,X}$ the specific light intensity (mol photons/g-dw/h), PFD_{in} the supplied photon flux
 256 density (mol photons/m²/h), L the light-path of photobioreactor (PBR) (m), μ_T the specific
 257 growth rate of microalgae (h⁻¹) with a temperature effect expressed by a function f_T (see below),
 258 $m_{E,X}$ the biomass maintenance coefficient (mol photons/g-dw/h), $Y_{X,E}$ the biomass yield on light
 259 energy (g-dw/mol photons), P_{area} the biomass productivity (g-dw/m²/h), A the area requirement
 260 (m²/person), and F_w the flow rate (m³/h/person).

261 However, not only irradiance, but also temperature affects microalgae growth, as presented
 262 in Eq. (3). Fig. 3 shows the effect of temperature on growth rate of *Chlorella sorokiniana*. The
 263 effect of temperature on growth rate was calculated using the temperature function suggested
 264 by Slegers et al. (2013):

265
$$f_T = \left(\frac{T_{let} - T_w}{T_{let} - T_{opt}} \right)^\beta \exp \left(-\beta \left(\frac{T_{let} - T_w}{T_{let} - T_{opt}} - 1 \right) \right) \quad (6)$$

266 with f_T the effect of temperature on growth rate (dimensionless), T_{let} the lethal temperature of
 267 specific microalgae species use ($^{\circ}\text{C}$), T_{opt} the optimal growth temperature of specific microalgae
 268 species ($^{\circ}\text{C}$), and β the curve modulating constant related to temperature coefficient Q_{10} , which
 269 is the proportional change in growth rate with a 10°C rise in temperature (dimensionless).



270

271 **Fig. 3:** Effect of temperature on growth rate of *Chlorella sorokiniana*.

272 If the temperature function $f_T = 0$, no growth is possible. If $f_T = 1$, growth is only influenced by light
 273 intensity, independent of temperature.

274

275 **2.6. Assumptions and parameter values**

276 The following assumptions were made: (1) wastewater temperature is equal to the air
 277 temperature; (2) the anaerobic digester is controlled at a (mesophilic) temperature of 35°C ; (3)
 278 photo-inhibition of the microalgae does not take place; (4) cold partial nitrification/Anammox
 279 can be applied if the temperature is above 10°C ; and (5) a change in temperature impacts both

280 the area requirement for cultivation of microalgae and the required heating energy for anaerobic
 281 digestion. Because information on the effect of temperature on the bioflocculation is still
 282 limited, further investigation is required. Lotti et al. (2014) showed that Anammox bacteria can
 283 be enriched at a temperature of 15°C. However, based on the work of Hendrickx et al. (2014),
 284 it is expected that in the near future it will be possible to apply partial nitrification/Anammox
 285 process at temperatures as low as 10°C. The system's and microalgae dependent parameters
 286 are given in Table 2.

287 **Table 2**

288 Parameters used in the calculations of area requirement for cultivation of microalgae.

Parameter	Unit	Type of parameters		Reference
		System parameter	<i>Chlorella</i> <i>sorokiniana</i>	
N_{eff}	g N/m ³	2.2	–	Boelee et al. (2012)
$Y_{X,E}$	g-dw/mol photons	–	0.933	Tuantet (2015)
$m_{E,X}$	mol photons/g-dw/h	–	0.0068	Tuantet (2015) and Zijffers et al. (2010)
L	m	0.01	–	Tuantet (2015)
T_{opt}	°C	–	38.1	Morita et al. (2000)
T_{let}	°C	–	49.7	Morita et al. (2000)
B	(-)	–	1.6	Vona et al. (2004)

289

290 **2.7. Sensitivity analysis**

291 Differential sensitivity analysis was conducted for the area requirement of microalgae
 292 reactor in Configuration 2 with respect to two uncertain factors: the microalgal biomass yield

293 on light energy ($Y_{X,E}$) and the microalgal biomass maintenance coefficient ($m_{E,X}$). The
 294 normalized sensitivity coefficients (dimensionless) indicate which of the two factors is most
 295 sensitive and this provides directions for future research. The normalized sensitivity coefficient
 296 for a particular independent factor was obtained by taking the partial derivatives of the
 297 dependent variable with respect to the independent factor and scaled by the nominal values of
 298 the dependent variable and independent factor. Analytical expressions for the normalized
 299 sensitivity coefficients of area (A) with respect to $Y_{X,E}$ and $m_{E,X}$ are given by (see Appendix for
 300 details):

$$301 \quad S_{A,Y_{X,E}} = \frac{-F \bar{Y}_{X,E}}{W} \quad (7)$$

$$L * \left(\frac{PFDF}{in N} - m_{E,X} \right) * f_T * \left(Y_{X,E} \right)^2 * \bar{A}$$

$$302 \quad S_{A,m_{E,X}} = \frac{F \bar{m}_{E,X}}{W} \quad (8)$$

$$L * \left(\frac{PFDF}{in N} - m_{E,X} \right)^2 * f_T * Y_{X,E} * \bar{A}$$

303 with $S_{A,Y_{X,E}}$ the nominalized sensitivity coefficient of area requirement on $Y_{X,E}$, $S_{A,m_{E,X}}$ the
 304 nominalized sensitivity coefficient of area requirement on $m_{E,X}$, $\bar{Y}_{X,E}$ the nominal value of $Y_{X,E}$,
 305 $\bar{m}_{E,X}$ the nominal value of $m_{E,X}$, and \bar{A} the area requirement related to the nominal values of
 306 each factor. Based on a theoretical maximum biomass yield and the performance of *Chlorella*
 307 *sorokiniana* under extreme conditions (Franco et al., 2012; Kliphuis et al., 2010), the area
 308 requirements were first calculated for the nominal value of $Y_{X,E}$ and then at values of $\pm 50\%$ with
 309 respect to the nominal value.

310 A one-at-a-time sensitivity analysis was used to quantify the changes in effluent quality
311 and area requirement by varying sewage N and P concentrations. As mentioned before, NH₄-
312 N concentrations varied from 20 mg N/L (N_{min}), 25 mg N/L (N_{typical}) to 35 mg N/L (N_{max}). PO₄-
313 P concentrations varied from 3 mg P/L (P_{min}), 5 mg P/L (P_{typical}) to 9 mg P/L (P_{max}).
314 Calculations of the microalgae reactor area requirement were performed based on average
315 annual light intensity and temperature conditions.

316

317 **3. Results and Discussion**

318 *3.1. Scenario-based analysis*

319 The study of Khiewwijit et al. (2015b) showed that year round wastewater treatment with
320 microalgae is not feasible in The Netherlands. Therefore, an initial quantitative scenario-based
321 analysis of the two new WWTP configurations and the CAS system was conducted for
322 Thailand, location 11 (Table 1) with relatively high PFD and annual temperature. Hence, it is
323 expected that in Thailand both partial nitrification/Anammox and microalgae treatment can be
324 applied throughout the entire year. Table 3 shows the KPIs for the three WWTP systems when
325 operated in Thailand.

326

327 **Table 3**

328 Numerical results based on the key performance indicators (KPIs) for Configurations 1 and 2 in
 329 comparison to the CAS system, using Thailand as a case study; (A) Total energy consumption, energy
 330 production and net energy yield, (B) Nutrient recovery and CO₂ emission.

A

Configuration	Total energy consumption/production/yield (kWh/m ³ of wastewater)				
	Total energy consumption	Aeration	Heating	Energy production ^a	Net energy yield ^b
Configuration 1	0.18	0.11	0.07	0.63	0.45
Configuration 2	0.15 ^c	0.03	0.07	0.63	0.48
CAS	0.36	0.29	0.07	0.20	-0.16

331 ^a This energy production includes both electricity and heat energy.

332 ^b This net energy yield is calculated based on energy consumption for aeration and heating. Energy
 333 needed for pumping, lighting and dewatering were not taken into account.

334 ^c This energy consumption includes also the impact of harvesting and separation of microalgal biomass
 335 on energy consumption. Assuming the harvesting step requires 0.196 kWh per kg of microalgae
 336 (Collet et al., 2011). In this study, the microalgal biomass productivity was calculated based on a
 337 fraction of N in microalgal biomass and this was 0.27 kg-dw/m³ of wastewater.

338

B

Configuration	Nutrient recovery (as 100% of initial amount)		CO ₂ emission/consumption (kg-CO ₂ /m ³ of wastewater)	
	Nitrogen	Phosphorus	CO ₂ emission	CO ₂ consumption
Configuration 1	0	72	0.38	0
Configuration 2	70	65	0.35	0.63 ^d

CAS	0	0	0.48	0
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339 ^d This is caused by CO₂ consumption by the microalgae.

340

341 ***3.1.1. Energy and nutrient recovery***

342 While in the CAS system the major fraction of sewage organic matter is aerobically
343 mineralized, in Configurations 1 and 2 most of this organic matter is distributed to the anaerobic
344 digester. This explains why in Configurations 1 and 2 significantly more methane is produced,
345 and thus more electricity and heat energy are generated than in the CAS system: 0.63 kWh per
346 m³ of wastewater compared to 0.20 kWh per m³ for the CAS system (Table 3A). Table 3A also
347 shows that for all configurations the same amount of energy was needed to heat up the
348 anaerobic digester. In this study, the concentration of solids going to the anaerobic digester for
349 both Configurations 1 and 2 was assumed to be the same, while the flow rate of thickener going
350 to the anaerobic digester was assumed to be the same for all configurations. Because during
351 bioflocculation oxidation of organic matter is minimized, the total aeration energy of
352 Configuration 1 (0.11 kWh/m³ of wastewater) and of Configuration 2 (0.03 kWh/m³ of
353 wastewater) was much lower than the aeration energy needed for the CAS system (0.29
354 kWh/m³ of wastewater). The higher aeration energy in Configuration 1 compared to
355 Configuration 2 can be explained by the oxygen that is needed for partial nitrification (Hao et al.,
356 2002).

357 When applied under Thai conditions, the net energy yield of Configuration 2 (0.48 kWh/m³
358 of wastewater) is slightly higher than for Configuration 1 (0.45 kWh/m³ of wastewater),
359 whereas a net energy deficit was found for the CAS system. It should be noted, however, that
360 in these results energy consumption for pumping, thickening and dewatering was not taken into
361 account, because they are only insignificant fractions of the total energy consumed during

362 wastewater treatment when compared to energy required for aeration, heating and microalgal
363 harvesting (Gu et al., 2017; Collet et al., 2011).

364 Table 3B shows that with Configuration 1 72% of the sewage P was recovered, while in
365 the CAS system all the P and N were wasted with the excess sludge or by N₂ emission,
366 respectively. This is because in the CAS system the sewage nutrients were removed by
367 chemical or biological P removal process and by subsequent biological nitrification and
368 denitrification for N removal (Khiewwijit et al., 2015b). In Configuration 2, 70% of the sewage
369 N and 65% of the sewage P was assimilated by microalgae. This implies that Configuration 2,
370 employing a microalgae reactor, presents a promising option for municipal wastewater
371 treatment with respect to amounts of nutrients that can be recovered.

372 ***3.1.2. CO₂ emission***

373 Table 3B shows that in Thailand CO₂ emission for the CAS system was 0.48 kg CO₂/m³ of
374 wastewater. In Configuration 1, CO₂ emission was 21% lower (0.38 kg CO₂/m³). In
375 Configuration 2, the CO₂ emission was 0.35 kg CO₂/m³ of wastewater, whereas in this
376 configuration the microalgae need 0.63 kg CO₂/m³ for growth.

377 ***3.1.3. Area requirement***

378 Based on the results in Table 3, Configuration 2 with microalgae treatment seems to be the
379 most promising design for future municipal WWTPs in Thailand and in other tropical regions.
380 However, the model calculations also show that a microalgae reactor requires an area of 2.2
381 m²/person. This is similar to the 2.1 m²/person found by Boelee et al. (2012) for a microalgae
382 biofilm reactor that was applied for nutrient removal after a high-rate activated sludge process
383 to remove organic pollutants. A typical CAS system requires only 0.2–0.4 m²/person (Boelee
384 et al., 2012), which was assumed to be the same as in Configuration 1 with (cold) partial

385 nitrification/Anammox. Thus, microalgae treatment may only be a viable option in areas where
386 sufficient land area is available. On larger scales, i.e. located in or nearby cities, land
387 availability and costs may be limiting factors. This implies that microalgae treatment only
388 would be attractive if high value products, such as carotenoids, aquaculture feed and dietary
389 supplements can be produced by the microalgae (Enzing et al., 2014). It is recognized,
390 however, that in this case contamination of the microalgal biomass with, for example
391 pathogens, heavy metals and organic micropollutants that are present in the wastewater could
392 present a serious problem.

393 ***3.2. Area requirement for different locations worldwide***

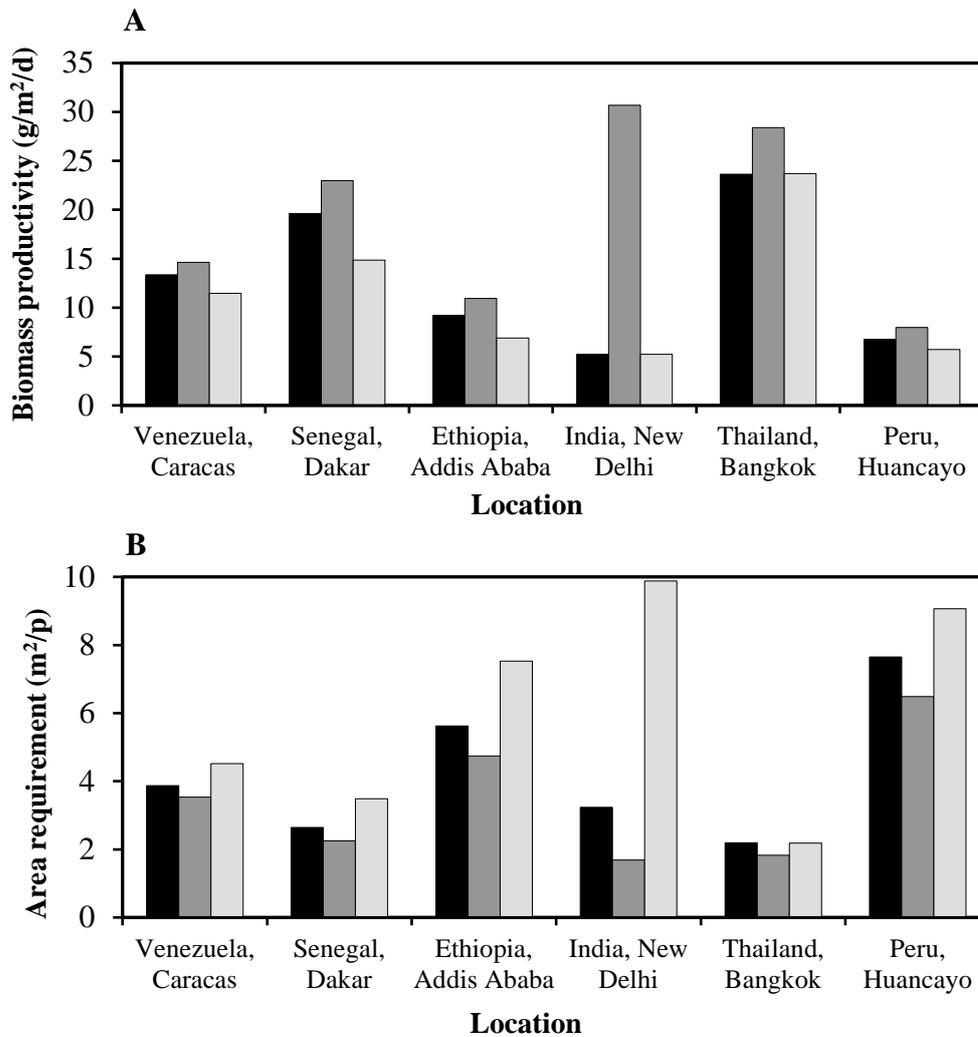
394 The productivity of microalgae is location specific, because it is largely determined by light
395 intensity and temperature (Slegers et al., 2013). To investigate this in more detail, microalgal
396 biomass productivity and area requirement were calculated for the 16 selected locations around
397 the globe, and under different seasonal conditions (Table 1).

398 In the Northern hemisphere, for example, Washington - Seattle, Missouri - Kansas city,
399 Spain - Almeria, Poland - Warsaw, China - Xi'an, and Japan - Akita, biomass productivities
400 were very different (0.2–25.5 g-dw/m²/d) between summer and winter. The area requirement
401 ranged between 2 and 6 m²/person for the summer period and between 14 and 273 m²/person
402 for the winter period. Although the model in this study allowed microalgae growth at
403 temperatures below 5°C, it still remains a challenge for a practical implementation at such low
404 temperatures. Nevertheless, because of the large area requirements in the winter periods it can
405 be concluded that Configuration 2 employing a microalgae reactor is not feasible for locations
406 in the Northern hemisphere. De Schampelaire and Verstraete (2009) showed that in temperate
407 climates algae cultivation is still possible if greenhouses are applied with extra light supplied

408 (2-HQI 400W lamps) and high temperatures of 20–40°C. However, the considerable amount
 409 of energy required for lighting and heating can be a severe bottleneck.

410 In contrast, microalgae treatment seems to be applicable for locations nearby the equator
 411 line. Fig. 4 shows biomass productivity and area requirements for the six locations nearby the
 412 equator line, which are Venezuela - Caracas, Senegal - Dakar, Ethiopia - Addis Ababa, India -
 413 New Delhi, Thailand - Bangkok, and Peru - Huancayo, as a function of average annual, summer
 414 and winter conditions for temperature and light intensity.

415



416

417 **Fig. 4:** Comparison of calculated (A) biomass productivity and (B) area requirements for
418 Configuration 2 with microalgae treatment for the six locations nearby the equator line, i.e.
419 Venezuela - Caracas, Senegal - Dakar, Ethiopia - Addis Ababa, India - New Delhi, Thailand -
420 Bangkok, and Peru - Huancayo; (black) annual, (grey) summer, and (light grey) winter.

421

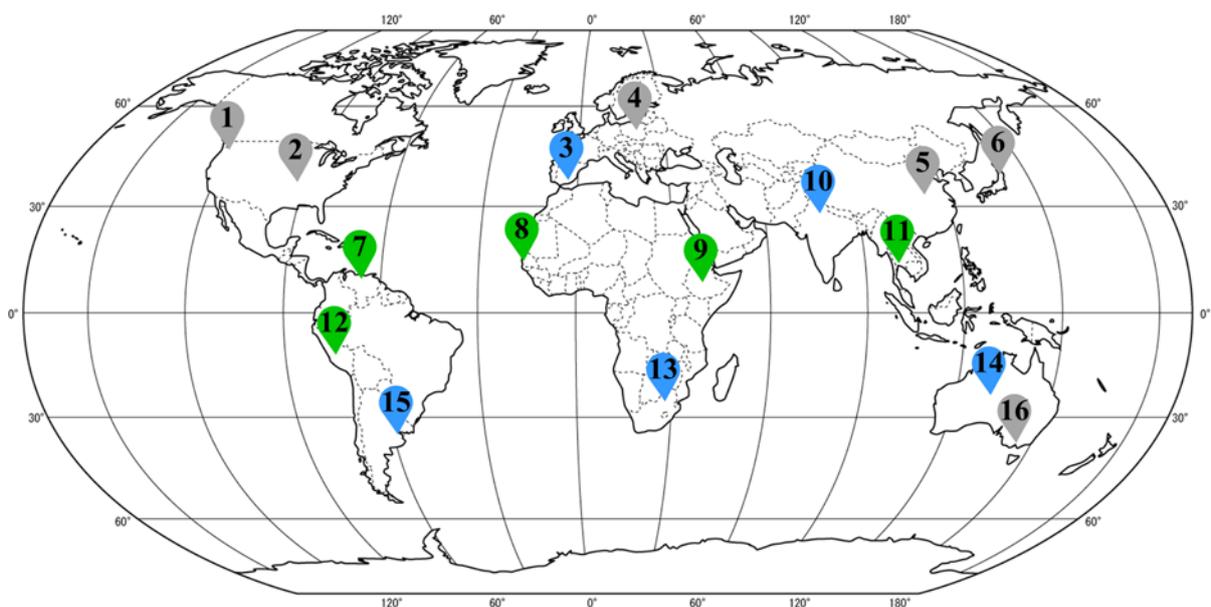
422 For the winter period, the lowest area requirement was found for Thailand - Bangkok with
423 2.2 m²/person, followed by Senegal - Dakar (3.5 m²/person), Venezuela - Caracas (4.5
424 m²/person), Ethiopia - Addis Ababa (7.5 m²/person), Peru - Huancayo (9.1 m²/person), and
425 India - New Delhi (9.9 m²/person). Thus, the area requirements for configuration based on
426 microalgae treatment always are much higher than for CAS systems (0.2–0.4 m²/person), but
427 are comparable to the area for other low-cost wastewater treatment systems such as vertical-
428 flow constructed wetlands of 1.2–5.0 m²/person and horizontal-flow constructed wetlands of
429 3.0–10.0 m²/person (Dotro et al., 2017). Interestingly, the results showed that in India the area
430 requirement in the winter period was almost 5 times higher than in the summer period (1.7
431 m²/person). Thus, when winter conditions are very different from summer conditions, for
432 instance more than a 10°C difference in temperature, microalgae treatment is only a promising
433 option for municipal wastewater for the summer period, while the CAS system or
434 Configuration 1 is needed for the winter period. This is probably not economically feasible and
435 therefore Configuration 1 with (cold) partial nitrification/Anammox is the best option to treat
436 municipal wastewater throughout the entire year.

437 With respect to the Southern hemisphere, microalgae treatment is only applicable for
438 tropical regions. The area requirements for the winter period for South Africa - Pretoria and
439 Australia - Alice Springs were 9.1 and 9.0 m²/person, respectively. Similar to India, on these
440 locations microalgae treatment only seems possible in the summer period, as the area

441 requirements were 2.6 m²/person for South Africa - Pretoria and 1.6 m²/person for Australia -
442 Alice Springs. In Argentina - Buenos Aires and Australia - Melbourne a microalgae treatment
443 is not realistic, because the area requirements were as high as 18 and 30 m²/person in the winter,
444 respectively.

445 Based on the results above, it was concluded that Configuration 2 with microalgae
446 treatment is only feasible for tropical locations, for example Venezuela - Caracas, Senegal -
447 Dakar, Ethiopia - Addis Ababa, Thailand - Bangkok and Peru - Huancayo, where light intensity
448 at the winter period is above 340 $\mu\text{mol photons/m}^2/\text{s}$ and differences in water temperature
449 between summer and winter are less than 5°C. Similarly, van Harmelen and Oonk (2006)
450 suggested that microalgae cultivation is only feasible for locations between 37 degrees latitude
451 North and South and with an average annual temperature of above 15°C. Also, in their study it
452 was concluded that large parts of Central and Southern America, Africa and Australia are not
453 feasible for microalgae because of a limited area. However, it is important to note that the
454 results obtained in this study are not entirely comparable to the previous study as a different
455 more detailed model, which includes the impact of rain and clouds, temperature and light
456 intensity, was used in the latter study. Configuration 1 with (cold) Anammox for N removal is
457 only feasible at locations where the winter water temperature is above 10°C (Hendrickx et al.,
458 2014). This concerns tropical regions and some locations in temperate regions, such as Spain -
459 Almeria, India - New Delhi, South Africa - Pretoria, Australia - Alice Springs, and Argentina
460 - Buenos Aires. However, a technological bottleneck may be partial nitrification at low
461 temperatures. This is because at temperatures below approximately 20°C, nitrite-oxidizing
462 bacteria (NOB) grow faster than ammonia-oxidizing bacteria (AOB) (Hao et al., 2002). Hence,
463 further investigation of the population dynamics of AOB for partial nitrification process remains
464 a challenge, in particular with respect to low temperatures (e.g. Giusti et al., 2011).

465 Fig. 5 summarizes the feasibility of applying Configuration 1 or 2 for different locations.
466 Configurations 1 and 2 are not feasible, for example in Washington - Seattle, Missouri - Kansas
467 city, Poland - Warsaw, China - Xi'an, Japan - Akita, and Australia - Melbourne. In these cases
468 CAS systems should be applied, because these work throughout the entire year. It should,
469 however, be realized that at very low water temperatures during the winter period also CAS
470 systems may not work efficiently because of a reduced nitrification efficiency (Kim et al.,
471 2008). However, it is important to note that a lack of experienced personal for the operation of
472 advanced treatment technologies, such as (cold) partial nitritation/Anammox in Configuration
473 1 and novel P recovery in Configuration 1-2, may be a bottleneck for developing countries.
474 Therefore, in developing countries, training programs should also be provided. Based on the
475 results obtained in this study, it is expected that Configuration 2 with microalgae treatment
476 requires a lower operational cost, in particular for the aeration energy, than Configuration 1
477 with (cold) partial nitritation/Anammox and the CAS system (Table 3A), whereas a higher
478 maintenance cost could be found in Configuration 2 due to the microalgal harvesting (Collet et
479 al., 2011). However, still a comparison of the overall economic feasibility of Configurations 1
480 Configuration 2 and the CAS system remains unclear and should be further investigated.



481

482 **Fig. 5:** Map of the 16 selected locations used in this study with the most promising candidate for
483 municipal wastewater treatment; (blue) Configuration 1 with (cold) partial nitrification/Anammox,
484 (green) Configuration 2 with microalgae, and (grey) the CAS system.

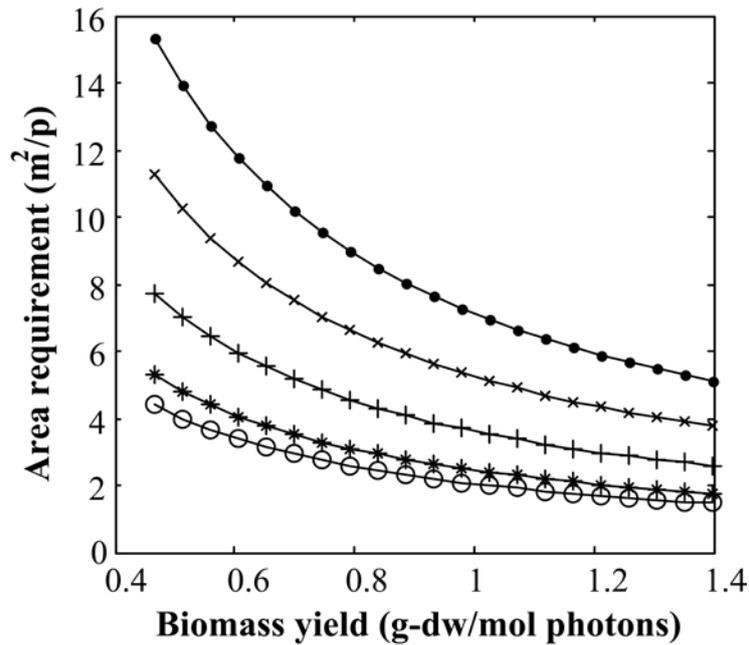
485

486 **3.3. Sensitivity analysis**

487 So far, for application of Configuration 2, the conclusion is that the area requirement of
488 microalgae reactor is the bottleneck. In order to estimate the sensitivity of the area requirement
489 with respect to wastewater composition, microalgal biomass yield and microalgal biomass
490 maintenance, a sensitivity analysis was performed for Configuration 2. This sensitivity analysis
491 was conducted only for Venezuela - Caracas, Senegal - Dakar, Ethiopia - Addis Ababa,
492 Thailand - Bangkok, and Peru - Huancayo, where microalgae treatment was previously shown
493 to be a promising treatment concept.

494 **3.3.1. Microalgal biomass yield and maintenance coefficient**

495 As shown in Fig. A.1–A.2 (see Appendix), the microalgal biomass yield has a major
496 impact, while microalgal biomass maintenance has only a minor effect on the area
497 requirements. Fig. 6 shows the effect of the yield on the area requirements for different
498 (average) annual temperatures and light intensities.



499

500 **Fig. 6:** Area requirements in relation to different microalgal biomass yield values for the five potential
 501 locations, that are applicable for Configuration 2 with microalgae treatment; (+) Venezuela - Caracas,
 502 (*) Senegal - Dakar, (x) Ethiopia - Addis Ababa, (○) Thailand - Bangkok, and (●) Peru - Huancayo.
 503 (Results represent the area requirement with respect to annual temperature, annual light intensity, and
 504 typical wastewater composition: 600 mg COD/L, 25 mg NH₄-N/L and 5 mg PO₄-P/L.)

505

506 Clearly, when more biomass can be grown per mole of photons, less area is needed.
 507 Kliphuis et al. (2010) reported a theoretical maximum yield on nitrate of 1.57 g-dw/mol
 508 photons. A similar value can be anticipated on ammonium as nitrogen source. In this study, a
 509 typical yield of 0.933 g-dw/mol photons was used. However, the yield depends on the
 510 microalgae species and/or reactor type (Boelee et al., 2014; Rigosi et al., 2011) and can cause
 511 huge differences in area requirements. For example, in Peru the area requirement would
 512 increase from 7.7 m²/person at a yield of 0.933 g-dw/mol photons to almost 16 m²/person at
 513 0.450 g-dw/mol photons. This demonstrates that interpretation of the model results should be
 514 done with great care, and more experimental data about the biomass yield is required before
 515 conclusions can be drawn about the applicability of microalgae treatment.

516 Unlike the effect of biomass yield, the microalgal maintenance coefficient did not give
517 significant differences in the area requirement (Fig. A.2). This can be explained by the low N
518 concentrations in municipal wastewater compared to other wastewater sources. The low
519 sewage N concentrations result in low biomass concentration (Eq. 1) and thus a large fraction
520 of the light that is available for the microalgae (Eq. 2). Under these conditions maintenance is
521 insignificant compared to the growth of the microalgae (Eq. 3). For example, after
522 bioflocculation a concentration of 21 mg N/L would be assimilated by the microalgae and this
523 would give a biomass concentration of only 0.3 g-dw/L. In contrast, other waste streams such
524 as urine have much higher N concentrations, causing biomass densities as high as 14.2 g-dw/L
525 (Tuantet, 2015) and under these conditions maintenance may become a significant factor.

526 ***3.3.2. Nitrogen and phosphorus concentrations in wastewater***

527 Table 4 shows effluent quality and area requirements for a range of different concentrations
528 of N and P (von Sperling, 2007). The numbers in boldface show that for some combinations of
529 wastewater N and P concentrations it is impossible to achieve the required effluent
530 concentrations of 2.2 mg N/L and 1 mg P/L, respectively with microalgae treatment and thus
531 additional treatment is required. If the concentration of P ($\text{PO}_4\text{-P}$) in wastewater would increase
532 from 5 to 9 mg P/L, N rather than P would become the limiting nutrient for microalgae growth.
533 This implies that P would end up in the effluent with 2.78 mg P/L when the concentration of
534 N ($\text{NH}_4\text{-N}$) in wastewater is at a maximum value of 35 mg N/L, and with 5.57 mg P/L when
535 the concentration of N is at a minimum value of 20 mg N/L. Because P in the effluent can no
536 longer meet the effluent guideline of 1 mg $\text{P}_{\text{total}}/\text{L}$, an additional post-treatment would be
537 needed, e.g. slow sand filtration with iron addition. In contrast, if the concentration of N ($\text{NH}_4\text{-}$
538 N) in wastewater would increase from 25 to 35 mg /L, while the concentration of P ($\text{PO}_4\text{-P}$)
539 would decrease from 5 to 3 mg P/L, P rather than N becomes the limiting nutrient. This implies

540 that N would end up as high as 20.78 mg N/L in the effluent. In this case additional N removal
 541 is required to reduce N to level that meet discharge guidelines, for example, through partial
 542 nitrification/Anammox process.

543 **Table 4**

544 Comparison of numerical results based on effluent quality (mg/L) and area requirement (m²/person) for
 545 microalgae cultivation with given annual PFD and annual temperature, as mentioned in Table 1, and
 546 based on a range of sewage concentrations of NH₄-N and PO₄-P. NH₄-N concentrations varied from 20
 547 mg N/L (N_{min}), 25 mg N/L (N_{typical}) and 35 mg N/L (N_{max}). PO₄-P concentrations varied from 3 mg P/L
 548 (P_{min}), 5 mg P/L (P_{typical}) and 9 mg P/L (P_{max}). Significant values are highlighted in bold.

Wastewater characteristic	N _{min} , P _{min}	N _{max} , P _{min}	N _{min} , P _{max}	N _{max} , P _{max}	N _{typical} , P _{typical}
Effluent quality					
NH ₄ -N (mg N/L)	5.64	20.78	2.20	2.20	2.20
PO ₄ -P (mg P/L)	0.15 ^a	0.15 ^a	5.57	2.78	0.60
Area requirement (m²/person)					
1. Venezuela, Caracas	2.3	2.3	2.9	5.8	3.9
2. Senegal, Dakar	1.6	1.6	2.0	3.9	2.6
3. Ethiopia, Addis Ababa	3.3	3.3	4.3	8.4	5.6
4. Thailand, Bangkok	1.3	1.3	1.7	3.3	2.2
5. Peru, Huancayo	4.5	4.5	5.8	11.4	7.7

549 ^a P becomes the limiting nutrient; therefore, the biomass density was calculated based on a fraction of
 550 P in microalgal biomass of 0.0145 g-P/g algal biomass (Tuantet, 2015) and P-target in effluent was
 551 0.15 mg P/L (Boelee et al., 2012).

553 Table 4 also shows that the composition of N (NH₄-N) and P (PO₄-P) in wastewater has a
 554 strong impact on the area requirement. The area requirement becomes about 40% lower when

555 the concentrations of both N and P changed from typical to minimum values and approximately
556 50% higher when concentrations change from typical values to maximum values. At a
557 minimum P concentration of 3 mg P/L, a higher N does not necessarily result in a higher area
558 requirement, because P is the limiting nutrient. However, at a maximum P concentration of 9
559 mg P/L and a maximum N of 35 mg N/L, the cultivation area was about 2 times the area needed
560 at a maximum P concentration and a minimum N concentration of 20 mg N/L. These results
561 show that, in addition to light intensity and temperature, the feasibility of microalgae treatment
562 is also strongly affected by the wastewater concentrations of N and P.

563

564 **4. Conclusions**

565 The feasibility of two novel municipal wastewater treatment configurations was
566 investigated for 16 locations around the globe with respect to their net energy yield, N and P
567 recovery, CO₂ emission and area requirements. The results were compared with the CAS
568 system. Both configurations are based on combined bioflocculation and anaerobic digestion
569 but with different nutrient removal technologies, i.e. partial nitrification/Anammox or microalgae
570 treatment. The results quantitatively support the pre-assumption that the applicability of the
571 two configurations is strongly location dependent. The configuration with (cold) partial
572 nitrification/Anammox is applicable in tropical regions and some locations in temperate regions,
573 such as Southern Europe and Southern part of South America. The configuration with
574 microalgae treatment is only applicable the whole year round in tropical regions that are close
575 to the equator line, such as Southeastern Asia and Northern part of South America. On the
576 locations with low sewage temperatures, for example in Northern America and Eastern Europe,
577 CAS systems are the only option.

578 Thus, to minimize the negative impacts of a wastewater treatment on its surrounding
579 environment, a deliberate decision (depending on local conditions) must be made with respect
580 to the technologies used in the treatment. This study provides numbers and a methodology to
581 support the decision-making process.

582

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703 **Supplementary material**

704 **Glocal Assessment of Integrated Wastewater Treatment and Recovery Concepts Using**
 705 **Partial Nitrification/Anammox and Microalgae for Environmental Impacts**

706 Rungnapha Khiewwijit^{1,2,3}, Huub Rijnaarts³, Hardy Temmink^{1,3}, Karel J. Keesman^{1,2,*}

707 **Appendix A—Analytical evaluation of normalized sensitivity coefficients**

708 Analytical evaluations of the normalized sensitivity coefficients of area requirement (A) with
 709 respect to biomass yield on light energy ($Y_{X,E}$) and biomass maintenance coefficient ($m_{E,X}$) were
 710 obtained from the partial derivatives of A with respect to $Y_{X,E}$ and $m_{E,X}$, respectively. That is,

$$\begin{aligned}
 711 \quad S_{A,Y_{X,E}} &= \left(\frac{\partial A}{\partial Y_{X,E}} \right) \left(\frac{\bar{Y}_{X,E}}{A} \right) \\
 712 &= \left(\frac{\partial A}{\partial \mu_T} \frac{\partial \mu_T}{\partial Y_{X,E}} \right) \left(\frac{\bar{Y}_{X,E}}{A} \right) \\
 713 &= \left(\frac{\partial}{\partial \mu_T} \left(\frac{F_W}{L^* \mu_T} \right) \frac{\partial}{\partial Y_{X,E}} \left[\left(r_{E,X} - m_{E,X} \right) * f_T * Y_{X,E} \right] \right) \left(\frac{\bar{Y}_{X,E}}{A} \right) \\
 714 &= \left(-\frac{F_W}{L^* \mu_T^2} \right) \left(\left(r_{E,X} - m_{E,X} \right) * f_T \right) \left(\frac{\bar{Y}_{X,E}}{A} \right) \quad \text{(A.1).}
 \end{aligned}$$

715 Substitute μ_T from Eq. (3) into Eq. (A.1), so that

$$716 \quad S_{A,Y_{X,E}} = \left(\frac{-F_W}{L} \right) \frac{1}{\left[\left(r_{E,X} - m_{E,X} \right) * Y_{X,E} * f_T \right]^2} \left(\left(r_{E,X} - m_{E,X} \right) * f_T \right) \left(\frac{\bar{Y}_{X,E}}{A} \right)$$

$$717 \quad = \left(\frac{-F_W}{L} \right) \frac{1}{\left(r_{E,X} - m_{E,X} \right)^* f_T^* \left(Y_{X,E} \right)^2} \left(\frac{\bar{Y}_{X,E}}{\bar{A}} \right) \quad (\text{A.2}).$$

718 Substitute $r_{E,X}$ from Eq. (2) and subsequently $C_{X,N}$ from Eq. (1) into Eq. (A.2), leading to

$$719 \quad S_{A,Y_{X,E}} = \frac{-F_W^* \bar{Y}_{X,E}}{L^* \left[\frac{PFD_{in}^* F_N}{L^* \left(N_{in} - N_{eff} \right)} - m_{E,X} \right]^* f_T^* \left(Y_{X,E} \right)^2 * \bar{A}} \quad (\text{A.3}).$$

720 Similarly,

$$721 \quad S_{A,m_{E,X}} = \left(\frac{\partial A}{\partial m_{E,X}} \right) \left(\frac{\bar{m}_{E,X}}{\bar{A}} \right)$$

$$722 \quad = \left(\frac{\partial A}{\partial \mu_T} \frac{\partial \mu_T}{\partial m_{E,X}} \right) \left(\frac{\bar{m}_{E,X}}{\bar{A}} \right)$$

$$723 \quad = \left(\frac{\partial}{\partial \mu_T} \left(\frac{F_W}{L^* \mu_T} \right) \frac{\partial}{\partial m_{E,X}} \left[\left(r_{E,X} - m_{E,X} \right)^* f_T^* Y_{X,E} \right] \right) \left(\frac{\bar{m}_{E,X}}{\bar{A}} \right)$$

$$724 \quad = \left(-\frac{F_W}{L^* \mu_T^2} \right) \left(-f_T^* Y_{X,E} \right) \left(\frac{\bar{m}_{E,X}}{\bar{A}} \right) \quad (\text{A.4}).$$

725 Substitute μ_T from Eq. (3) into Eq. (A.4), so that

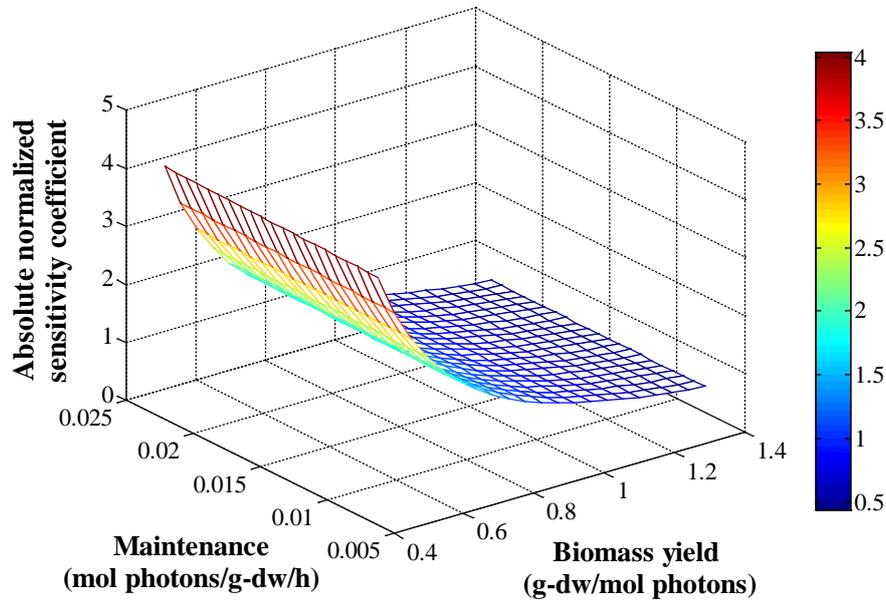
$$726 \quad S_{A,m_{E,X}} = \frac{F_W^* f_T^* Y_{X,E} * \bar{m}_{E,X}}{L^* \left[\left(r_{E,X} - m_{E,X} \right)^* f_T^* Y_{X,E} \right]^2 * \bar{A}}$$

$$727 \quad = \frac{F_W^* \bar{m}_{E,X}}{L^* \left(r_{E,X} - m_{E,X} \right)^2 * f_T^* Y_{X,E} * \bar{A}} \quad (\text{A.5}).$$

728 Substitute $r_{E,X}$ from Eq. (2) and subsequently $C_{X,N}$ from Eq. (1) into Eq. (A.5), leading to

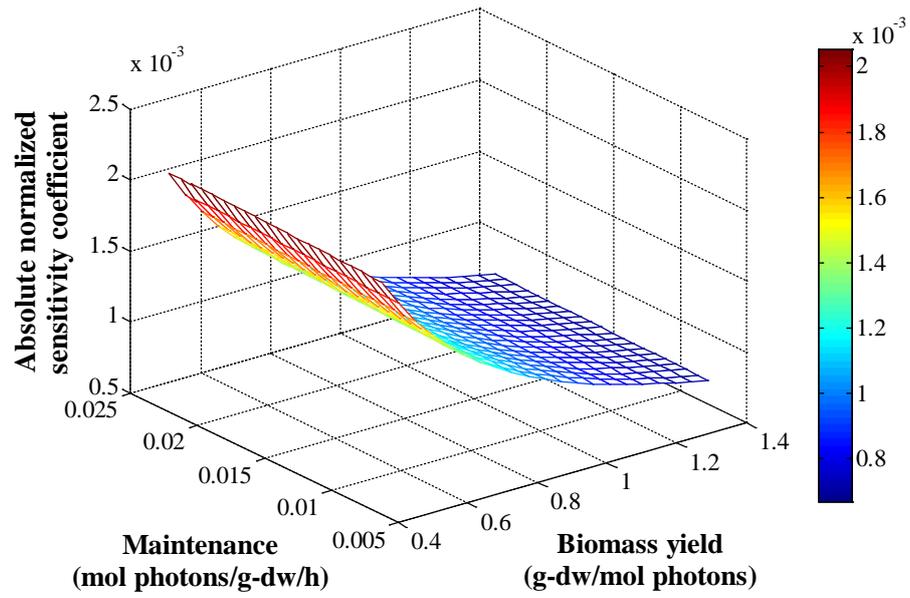
729
$$S_{A,m_{E,X}} = \frac{F \bar{m}_{E,X}}{W} \frac{1}{L^* \left(\frac{PFD_{in} * F}{L^* \left(\frac{N}{in} - N_{eff} \right)} - m_{E,X} \right)^2 * f_T * Y_{X,E} * \bar{A}} \quad (\text{A.6})$$

730 The absolute values of the normalized sensitivity coefficient of area requirement with respect
 731 to the microalgal biomass yield using Eq. (A.3) are shown in Fig. A.1 and to the microalgal
 732 biomass maintenance using Eq. (A.6) are shown in Fig. A.2.



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734 **Fig. A.1:** Absolute normalized sensitivity coefficient of area requirement with respect to biomass
 735 yield on light energy based on annual light intensity and annual temperature of Peru, Huancayo.



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Fig. A.2: Absolute normalized sensitivity coefficient of area requirement with respect to biomass

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maintenance based on annual light intensity and annual temperature of Peru, Huancayo.

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749 **Appendix B—Efficiency, conversion and design parameter values used in the**
750 **calculations, suggested by the study of Khiewwijit et al. (2015b)**

Process	Value	Unit
Bioflocculation		
• Total COD removal efficiency	80	% COD _{total}
• COD substrate need for biomass growth	40 ^a	% COD _{bs}
• O ₂ need	0.51	g O ₂ /g COD _{bs} _{removed}
• CO ₂ production	0.70	g CO ₂ /g COD _{bs} _{removed}
• Biomass yield	0.40	g VSS/g COD _{bs} _{removed}
• COD in biomass	1.42	g COD/g VSS
• N in biomass	0.124	g N/g VSS
• P in biomass	0.027	g P/g VSS
• Thickener capacity	50 ^a	g COD/L
Anaerobic sludge digestion		
• Total COD removal efficiency	70	% COD _b
• Methane production (digestion)	0.23	g CH ₄ /g COD _{removed}
• CO ₂ production	0.64	g CO ₂ /g COD _{removed}
• Biomass yield	0.058	g VSS/g COD _{removed}
• COD, N, P in biomass (see bioflocculation)		
Combined heat and power (CHP)		
• Electricity recovery	38	%
• Heat recovery	40	%
• Energy loss	22	%
• CO ₂ production	2.75	g CO ₂ /g CH ₄
• Enthalpy of combustion	13.9	kWh/kg CH ₄
Cold partial nitrification and Anammox		
• Overall N removal efficiency	90	% NH ₄ ⁺ -N
• O ₂ consumption	1.95	g O ₂ /g NH ₄ ⁺ -N _{removed}
• CO ₂ need	0.09	g CO ₂ /g NH ₄ ⁺ -N _{removed}
• N ₂ production	0.885	g N ₂ /g NH ₄ ⁺ -N _{removed}
• Nitrate production	0.11	g NO ₃ ⁻ /g NH ₄ ⁺ -N _{removed}
• Biomass yield (N-removal)	0.05	g VSS/g NH ₄ ⁺ -N _{removed}
• COD in biomass	1.42	g COD/g VSS
• N in biomass	0.09	g N/g VSS
• P in biomass	0.02	g P/g VSS
• COD removal efficiency (partial nitrification)	35	% of total COD
• COD removal efficiency (Anammox)	5	% of total COD
• Conversions for COD-removal, O ₂ need, CO ₂ production, and COD, N, P in biomass (see bioflocculation)		
P recovery/removal		
• Overall P recovery (removal) efficiency	90 ^a	% PO ₄ ³⁻ -P
Microalgae		
• N-target in effluent	2.2	mg NH ₄ ⁺ -N/L
• CO ₂ need	26.19	g CO ₂ / g NH ₄ ⁺ -N _{removed}
• O ₂ emissions	22.67	g O ₂ /g NH ₄ ⁺ -N _{removed}

• Biomass yield	12.82	g VSS/g NH ₄ ⁺ -N _{removed}
• COD in microalgal biomass	1.43	g COD/g VSS
• N in microalgal biomass	0.078	g N/g VSS
• P in microalgal biomass	0.014	g P/g VSS
• COD removal efficiency by heterotrophs	100 ^a	% COD _{bs}
• Conversions for COD-removal, O ₂ need, CO ₂ production, and concentration of COD, N, P in biomass (see bioflocculation)		
Reference CAS system		
• Total COD removal efficiency	85 ^a	%
• Total N removal efficiency	90 ^a	% NH ₄ ⁺ -N
• Total P removal efficiency	90 ^a	% PO ₄ ³⁻ -P
• O ₂ need (heterotrophs)	0.51	g O ₂ /g COD _{bremoved}
• O ₂ need (nitrification)	4.32	g O ₂ /g NH ₄ ⁺ -N _{removed}
• O ₂ need (biological P-removal)	0.49	g O ₂ /g COD _{bremoved}
• CO ₂ need (nitrification)	0.25	g CO ₂ /g NH ₄ ⁺ -N _{removed}
• COD need (denitrification)	3.92	g COD/g NO ₃ ⁻ -N
• COD need (biological P)	9.06	g COD/g PO ₄ ³⁻ -P _{removed}
• Biomass yield (COD-removal)	0.40	g VSS/g COD _{bremoved}
• Biomass yield (nitrification)	0.16	g VSS/g NH ₄ ⁺ -N _{removed}
• Biomass yield (denitrification)	0.30	g VSS/g COD _{used}
• Biomass yield (biological P)	0.37	g VSS/g COD _{used}
• COD, N, P in biomass (see bioflocculation)		
• N ₂ emissions (denitrification)	0.92	g N ₂ /g NO ₃ ⁻ -N
• CO ₂ emissions (heterotrophs)	0.70	g CO ₂ /g COD _{bremoved}
• CO ₂ emissions (biological P)	0.70	g CO ₂ /g COD _{bused}

751 ^a The values are based on design parameter.