

This item is the archived peer-reviewed author-version of:

A bioreactor and nutrient balancing approach for the conversion of solid organic fertilizers to liquid nitrate-rich fertilizers : mineralization and nitrification performance complemented with economic aspects

Reference:

Xie Yankai, Spiller Marc, Vlaeminck Siegfried.- A bioreactor and nutrient balancing approach for the conversion of solid organic fertilizers to liquid nitrate-rich fertilizers : mineralization and nitrification performance complemented with economic aspects
The science of the total environment - ISSN 1879-1026 - 806(2022), 150415
Full text (Publisher's DOI): <https://doi.org/10.1016/J.SCITOTENV.2021.150415>
To cite this reference: <https://hdl.handle.net/10067/1817870151162165141>

1 **A bioreactor and nutrient balancing approach for the conversion of solid**
2 **organic fertilizers to liquid nitrate-rich fertilizers: Mineralization and**
3 **nitrification performance complemented with economic aspects**

4 **Yankai Xie¹, Marc Spiller¹, Siegfried E. Vlaeminck¹, ***

5 ¹. Research Group of Sustainable Energy, Air and Water Technology, Department of
6 Bioscience Engineering, University of Antwerp, 2020 Antwerpen, Belgium

7 *Corresponding author.

8 E-mail: Siegfried.Vlaeminck@UAntwerpen.be

9 **Abstract**

10 Due to the high water- and nutrient-use efficiency, hydroponic cultivation is
11 increasingly vital in progressing to environment-friendly food production. To further
12 alleviate the environmental impacts of synthetic fertilizer production, the use of recovered
13 nutrients should be encouraged in horticulture and agriculture at large. Solid organic
14 fertilizers can largely contribute to this, yet their physical and chemical nature impedes
15 application in hydroponics. This study proposes a bioreactor for mineralization and
16 nitrification followed by a supplementation step for limiting macronutrients to produce
17 nitrate-based solutions from solid fertilizers, here based on a novel microbial fertilizer.
18 Batch tests showed that aerobic conversions at 35°C could realize a nitrate (NO₃⁻-N)
19 production efficiency above 90% and a maximum rate of 59 mg N L⁻¹ d⁻¹. In the
20 subsequent bioreactor test, nitrate production efficiencies were lower (44-51%), yet rates
21 were higher (175-212 mg N L⁻¹ d⁻¹). Calcium and magnesium hydroxide were compared
22 to control the bioreactor pH at 6.0±0.2, while also providing macronutrients for plant
23 production. A mass balance estimation to mimic the Hoagland nutrient solution showed
24 that 92.7% of the NO₃⁻-N in the Ca(OH)₂ scenario could be organically sourced, while

25 this was only 37.4% in the $\text{Mg}(\text{OH})_2$ scenario. Besides, carbon dioxide (CO_2) generated
26 in the bioreactor can be used for greenhouse CO_2 fertilization to save operational
27 expenditure (OPEX). An estimation of the total OPEX showed that the production of a
28 nutrient solution from solid organic fertilizers can be cost-competitive compared to using
29 commercially available liquid inorganic fertilizer solutions.

30 **Keywords:** bioconversion; fertigation; liquid fertilizer; nutrient balancing; resource
31 recovery.

32 **1. Introduction**

33 Agriculture and food production take a major share in the consumption of global
34 resources, with for instance about 90% of global freshwater (Huang et al., 2019) and >
35 80% of global ammonia produced through the Haber-Bosch process (Hollevoet et al.,
36 2020). However, during the artificial nitrogen fixation from N_2 to ammonia (NH_3) via the
37 Haber-Bosch process, greenhouse gas and NH_3 emissions, and $\text{PM}_{2.5}$ (particulate matter
38 with a diameter less than $2.5 \mu\text{m}$) formation can aggravate the global climate change and
39 the air pollution, and thus threaten human health (Griffis & Baker, 2020). Therefore,
40 efficient use of nutrients and water is essential to progress towards an environmentally
41 friendly agricultural system. Hydroponics in greenhouses are among the most water- and
42 nutrient-use efficient agricultural systems, with up to 90% water and 85% nutrient saving
43 (AlShrouf, 2017). However, current hydroponic systems rely entirely on inorganic
44 nitrogen supplied by the Harber-Bosch process, dosed as liquid fertilizers (Maucieri et al.,
45 2019; Trejo-Téllez & Gómez-Merino, 2012). An alternative strategy is to increase the
46 share of recovered nutrients in horticulture, and agriculture at large, to reduce the demand
47 for synthetic fertilizers. Use of recovered nitrogen is claimed to reduce environmental
48 impact and energy demand of N synthesis via the Haber-Bosch process. Therefore,

49 reusing nitrogen and other nutrients derived from by-products or residues from the agro-
50 food industry can further reduce the environmental impact of greenhouse hydroponic
51 systems, and other crop production systems relying on liquid fertilizers or fertigation.

52 Solid organic fertilizers are conventionally produced from plant- and animal-based
53 sidestreams from the agro-food industry, and the majority of their nitrogen content is
54 organically bound. Decreasing the consumption of animal products was suggested to have
55 a considerably beneficial impact on the environment (Lacour et al., 2018); thus, plant-
56 based fertilizers could be more environmentally friendly than animal-based fertilizers.
57 However, microbial fertilizers are considered a novel type of organic fertilizers, which
58 can be produced on effluents from the food and beverage industry, for instance on potato
59 industry wastewater (Spanoghe et al., 2020; Spiller et al., 2020). Resource recovery here
60 typically occurs through the production and drying of microbial biomass, which can be
61 based on several groups of microbes, such as microalgae, purple non-sulfur bacteria, and
62 a consortium of aerobic bacteria (CAB).

63 Given the physical (solid) and chemical (organic) nature of nitrogen in solid organic
64 fertilizers, these are not directly usable in hydroponics and fertigation applications, where
65 there is a demand for liquid and nitrate-based fertilizers. Indeed, the primary nitrogen
66 form utilized by plants is nitrate (NO_3^- -N) (Jones Jr, 2014; Ramírez et al., 2008). For
67 organic nitrogen to become available for crops, the biomass must undergo a
68 mineralization and nitrification process (Cabello et al., 2009). In the mineralization
69 process, microorganisms convert organic nitrogen (e.g., proteins) to an inorganic form
70 (i.e., ammoniacal nitrogen) through hydrolysis and ammonification (Khatoun et al., 2017;
71 Zaman et al., 1999). Parameters such as temperature and dissolved oxygen (DO) affect
72 the hydrolysis of organic nitrogen (Botheju, 2011; Crohn, 2004). The hydrolysis rate of
73 biowaste was shown to increase at higher temperatures (Veeken & Hamelers, 1999).

74 Some studies suggested limited aeration (i.e., micro-aeration) could significantly enhance
75 the hydrolysis of proteins (Botheju, 2011; Johansen & Bakke, 2006). However, micro-
76 aeration could induce denitrification risk, resulting in the loss of NO_3^- -N (Niu et al., 2016).
77 In the nitrogen mineralization process, hydrolysis is considered the rate-limiting step
78 (Katipoglu-Yazan et al., 2012). Therefore, it is indispensable to explore the optimal
79 hydrolysis conditions (e.g., temperature and DO) of organic fertilizers to promote their
80 mineralization performance.

81 Obtaining mineralization and nitrification within hydroponic systems poses several
82 challenges hindering the use of organic nitrogen. Besides the practical difficulty of dosing
83 solid fertilizer to the root zone over the full production season, the release pattern of the
84 macronutrients cannot really be controlled. The direct dosing of organic fertilizers in
85 hydroponic systems was proved to limit plant growth due to the deficiency of NO_3^- -N
86 (Garland et al., 1997; Ramírez et al., 2008). When using organic growing media for the
87 plants, pre-dosing of organic fertilizers is sometimes practiced, yet it is challenging to
88 control the bioconversion rates, even though a proper selection of the growing media
89 constituents influences these (Grunert et al., 2016; Jones Jr, 2014). Shinohara et al. (2011)
90 proposed directly dosing organic fertilizers into a hydroponic pot and introducing
91 inoculum with continuous aeration to bioconvert organic fertilizers. However, this
92 technique cannot easily manage the solution quality (e.g., composition and concentration)
93 in the rhizosphere immediately and flexibly, especially for large-scale hydroponic
94 cultivation (Jones Jr, 2014). Furthermore, direct dosing could stimulate heterotrophic
95 pathogens, increasing the competition for oxygen in the root environment and creating
96 food safety concerns (Garland et al., 1993; Trejo-Téllez & Gómez-Merino, 2012).
97 Disinfection of the fertigation water loop is typically in place, for instance using
98 ultraviolet radiation to control the proliferation of fungal and bacterial pathogens such as

99 *Fusarium spp.* and *Escherichia coli* (Jones Jr, 2014; Lee et al., 2015; Yaron & Römling,
100 2014).

101 This study proposes a novel pH-controlled bioreactor system for mineralization and
102 nitrification of solid organic fertilizers, followed by a chemical supplementation step to
103 achieve a well-balanced hydroponic nutrient solution (Fig. 1). This concept has the
104 advantage of accurately tuning the composition and concentration of a more sustainable
105 liquid nitrate-based fertilizer to match the plant needs (Fig. 1a), while avoiding the entry
106 of organic carbon, oxygen demand, and stimulation of microbial risks in the hydroponic
107 system. Besides, carbon dioxide (CO₂) generated during the organic fertilizer
108 mineralization can also be used for CO₂ fertilization in the greenhouse. The proposed
109 concept for the controlled mineralization and nitrification system comprises four parts: (i)
110 an influent tank to mix the solid organic fertilizer with water; (ii) a bioreactor for
111 mineralization and nitrification with pH control (base addition); (iii) a membrane module
112 (e.g., microfiltration) to remove the solids and yield a baseline nutrient solution (BNS);
113 and (iv) a nutrient balancing step to further tune the BNS macronutrient composition to
114 the plant's needs.

115 This research aims at a proof of concept for the bioreactor conversion performance and
116 at an economic estimation of the overall concept. The specific objectives are to (i)
117 optimize the mineralization performance by defining the best-operating conditions, (ii)
118 realize the liquid nitrate-rich fertilizer production from solid organic fertilizer in a
119 mineralization and nitrification bioreactor, where various pH control reagents were tested,
120 (iii) investigate the microbial community in the bioreactor, (iv) model the nutrient
121 balancing according to plant needs, and (v) assess the economic feasibility of the concept
122 by comparing the operational expenditure (OPEX) for producing the nutrient solution
123 from the bioreactor with that of inorganic fertilizer nutrient solutions.

124 **2. Material and methods**

125 The research was carried out in 4 steps. Initially, batch tests were implemented to
126 determine the optimal conditions (i.e., temperature and DO) for nitrogen mineralization
127 (section 2.2). Informed by the batch tests, the operating conditions were applied to a
128 mineralization and nitrification bioreactor, which is fully aerated as well as pH and
129 temperature-controlled (section 2.3). Furthermore, a nutrient balancing strategy for the
130 BNS was developed (section 2.5). Finally, the economics of the nutrient solution
131 produced from organic fertilizers was compared to commercially available inorganic
132 fertilizer nutrient solutions (section 2.5).

133 **2.1 Organic fertilizers**

134 BioAgenasol® profigreen (BAPN) (AGRANA group, Austria) and CAB were tested in
135 this study. BAPN represents a class of commercially available plant-based fertilizers and
136 is a by-product of bioethanol production. CAB represents a class of novel microbial
137 fertilizers obtained from the company Avecom (Wondelgem, Belgium). CAB was
138 produced through a patented aerobic fermentation process based on diluted process
139 streams of food processing companies. Based on the dry weight, the total nitrogen (TN)
140 content of BAPN and CAB are 4.8% and 8.2%, respectively. No inorganic nitrogen (i.e.,
141 $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$, and $\text{NO}_3^-\text{-N}$) could be detected in TN.

142 **2.2 Batch tests**

143 Batch tests were carried out in both anaerobic and aerobic conditions. Anaerobic
144 digestion sludge (ADS) and nitrifying activated sludge (AS) were used as inocula for the
145 anaerobic and aerobic tests, respectively. They were sampled from a municipal
146 wastewater treatment plant (Aquafin Antwerpen-Zuid, Belgium). The inoculum of 0.4 g
147 volatile suspended solids (VSS) L^{-1} was added to 2L of tap water and mixed continuously
148 by a magnetic stirrer. For anaerobic tests, the ADS inoculum was flushed with N_2 gas for

149 30 mins to eliminate the residual DO in tap water, and no aeration was provided during
150 the whole batch period, which resulted in a DO of around 0 mg O₂ L⁻¹ in the anaerobic
151 tests. Two different reaction temperatures of 20°C and 35°C were implemented in
152 anaerobic tests. To explore the effect of DO on nitrogen mineralization, the aerobic tests
153 were implemented in open glass flasks with the AS as an inoculum. The DO level in the
154 aerobic test was controlled at 3±0.5 mg O₂ L⁻¹ via air pumps (Tetra TEC APS 150).

155 An initial TN (i.e., organic nitrogen) concentration of 370 mg N L⁻¹ was set for all the
156 batch tests. This concentration was determined according to the weight proportion of TN
157 in BAPN and CAB in the dry biomass (section 2.1). The concentration of 370 mg N L⁻¹
158 was based firstly on the typical values in growing media (240-530 mg N L⁻¹) (Spanoghe
159 et al., 2020) and secondly on the TN requirements in hydroponic nutrient solutions (140-
160 300 mg N L⁻¹) (Jones Jr, 2014), while accounting for a potentially incomplete N
161 mineralization. The pH in the reactors was continuously monitored by a pH probe (SP11X,
162 Consort) and controlled at 7.0±0.2 via dosing hydrochloric acid (HCl) or potassium
163 hydroxide (KOH) by a multi-parameter controller (R3610, Consort). To monitor the
164 mineralization performance of each batch test, samples were taken periodically, filtered
165 via 0.2 µm syringe filters (CHROMAFIL Xtra PVDF), and stored at 4°C until analysis.

166 **2.3 Nitrate conversion in a controlled mineralization and nitrification bioreactor**

167 A controlled bioreactor system for converting solid organic fertilizers to liquid nitrate-
168 rich fertilizers is shown in Fig. 1a. The solid organic fertilizer was mixed in an influent
169 tank with water before a mineralization and nitrification bioreactor. The system was
170 equipped with timer-controlled influent and effluent pumps (Seko peristaltic pumps, PR7)
171 and overhead stirrers (ES Overhead Stirrer, Velp Scientifica). The bioreactor was
172 operated with a cycle time of 8 h, including 2 min continuous feeding and 2 min decanting.
173 Initially, the bioreactor was run with a 30 min settling phase (day 0-42) to retain as much

174 biomass as possible in the bioreactor. Following day 42 until the end of the experiments
175 on day 170, the bioreactor was run with continuous mixing without settling, which meant
176 the sludge retention time (SRT) was equal to the HRT.

177 Based on the batch test results, CAB was used in this step, and aerobic bioconversion
178 at 35°C was implemented in the bioreactor. The inoculum was taken from the aerobic
179 batch test and added into the 3 L bioreactor with an initial concentration of 0.4 g VSS L⁻¹.
180 The influent TN concentration increased from 500 to 3000 mg N L⁻¹ and the loading rate
181 from 100 to 400 mg N L⁻¹ d⁻¹, resulting in a volume exchange ratio of 4.4-6.67% and a
182 hydraulic retention time (HRT) of 5.5-7.5 days. The pH of the bioreactor was initially
183 controlled at 7.0±0.2 (day 0-133), and then at 6.0±0.2 (day 134-170), which was directly
184 consistent with the optimum pH range of most nutrient solutions (5.5-6.5) (Trejo-Téllez
185 & Gómez-Merino, 2012). To explore the optimal pH control reagent according to the
186 nutrient composition and concentration for plant needs, various pH control reagents
187 including KOH, sodium hydroxide (NaOH), calcium hydroxide (Ca(OH)₂), and
188 magnesium hydroxide (Mg(OH)₂) were tested. The operational parameters are detailed in
189 the top table of Fig. 3. To monitor the performance of the bioreactor, samples were taken
190 periodically from the influent and effluent.

191 **2.4 Physicochemical water, biomass, and microbiome analyses**

192 The NH₄⁺-N, NO₂⁻-N, NO₃⁻-N, and phosphate (PO₄³⁻) were measured with the San++
193 Automated Wet Chemistry Analyzer. The TN, total phosphorus (TP), total chemical
194 oxygen demand (TCOD), soluble chemical oxygen demand (SCOD) were
195 photometrically determined using NANOCOLOR test tubes (Macherey-Nagel,
196 Germany). The VSS was measured using standard methods (Clesceri et al., 1998).
197 Elements of potassium (K), calcium (Ca), magnesium (Mg), and sodium (Na) were
198 measured by inductively coupled plasma mass spectrometry (Thermo Scientific™ iCAP

199 Q, Thermo Fisher Scientific).

200 To understand the microbial community working in the bioreactor, biomass samples
201 were collected for microbiome analysis during the operation period. According to the
202 manufacturer's instructions, DNA was extracted using a PowerFecal® DNA isolation kit
203 (QIAGEN, Germany). The DNA extracts were sent to Novogene (UK) Co., Ltd for
204 microbial amplicon-based metagenomics sequencing (Illumina Novaseq6000 PE250,
205 Q30 \geq 75%). 16S rRNA genes of 16S V3-V4 were amplified used specific primers 341F
206 (CCTAYGGGRBGCASCAG) and 806R (GGACTACNNGGGTATCTAAT). All PCR
207 reactions were carried out with Phusion® High-Fidelity PCR Master Mix (New England
208 Biolabs). For each representative sequence, Mothur software was performed against the
209 SSUrRNA database of SILVA Database (see details <http://www.arb-silva.de/>) for species
210 annotation (Wang et al., 2007). The alpha diversity (Shannon's) and beta diversity (Bray-
211 Curtis dissimilarity) were analyzed in each sample and between different samples.

212 **2.5 Nutrient balancing strategy and economic assessment**

213 The effluent of the bioreactor was filtered through the 0.2 μm filters to simulate the
214 function of a membrane module. The filtrate was regarded as the BNS. Hoagland nutrient
215 solution (HNS) is considered one universal nutrient solution for plant growth in
216 hydroponics. The concentration of major elements in HNS is 210 mg $\text{NO}_3^- \text{-N L}^{-1}$, 31 mg
217 P L^{-1} , 235 mg K L^{-1} , 200mg Ca L^{-1} , and 48 mg Mg L^{-1} (Jones Jr, 2014). To model the
218 nutrient balancing according to the HNS recipe, a nutrient balancing strategy was
219 illustrated in Fig. 5a. Water of low salinity ($< 0.75 \text{ mS cm}^{-1}$) is recommended in the
220 dilution step (van Os et al., 2016). Commonly used chemical compounds for making
221 nutrient solutions such as calcium nitrate ($\text{Ca}(\text{NO}_3)_2$), magnesium nitrate ($\text{Mg}(\text{NO}_3)_2$),
222 magnesium sulfate (MgSO_4), calcium sulfate (CaSO_4), potassium sulfate (K_2SO_4) and
223 dipotassium phosphate (K_2HPO_4) were selected to supplement relevant nutrients to the

224 BNS (in Supplementary Material (SM), Table S1). The market reference prices of
225 chemicals were obtained from two large global suppliers (i.e., <https://www.alibaba.com/>
226 and <https://www.ec21.com/>). The selection of compounds followed two major principles:
227 firstly, the dosage of each compound should not exceed water solubility after the final
228 dilution to HNS; secondly, the cheaper compounds according to the indicative market
229 price should always be selected in priority, only using the relative expensive chemicals
230 once other nutrient elements in the cheaper chemicals reached the required level. Based
231 on the three types of pH control reagents used in phase VII, VIII, and IX, the nutrient
232 balancing strategy was applied to three organic scenarios (i.e., NaOH, Ca(OH)₂, and
233 Mg(OH)₂). Subsequently, the concentration of nonessential element sodium (Na) and the
234 electric conductivity (EC) in balanced organic scenarios were checked.

235 Following the strategy to prepare HNS from the BNS organic scenarios, two types of
236 commercially available inorganic fertilizers, YaraTera Kristalon Scarlet and Floraflex
237 B1TM/B2TM (composition and price in SM Table S2), were used to design the HNS as
238 commercial scenarios for the convenience of further cost comparison with organic
239 scenarios. Besides, HNS composed of individual chemical compounds was set as a
240 control scenario.

241 The OPEX was calculated based on the cost of CAB, commercial hydroponic fertilizers,
242 supplemented chemical compounds, the aeration for COD removal and nitrification, and
243 the saved cost of CO₂ production for CO₂ fertilization. The price for CAB was 1 € g⁻¹ dry
244 matter, communicated by the company Avecom. The O₂ consumption for complete
245 nitrification was assumed to be 4.57 g g⁻¹ NO₃⁻-N (Metcalf & Eddy, 1991). The average
246 O₂ transfer efficiency and electricity cost were taken as 2kg kWh⁻¹ and 0.117€ kWh⁻¹
247 (Eurostat, 2018; Roman & Mureşan, 2014).

248 **3. Results and discussion**

249 **3.1 Batch test screen for optimal mineralization conditions**

250 In the batch tests, the performance of inorganic nitrogen (i.e., $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$, and
251 $\text{NO}_3^-\text{-N}$) release was employed to indicate the mineralization and nitrification of BAPN
252 and CAB (Fig. 2). The first-order kinetics fitted the total inorganic nitrogen (TIN)
253 releasing performance in all the batch tests ($R^2 \geq 0.92$), and k is the kinetic rate constant.
254 In the anaerobic tests, only $\text{NH}_4^+\text{-N}$ was detected as TIN. The mineralization performance
255 of both BAPN and CAB at 35°C was better than at 20°C with higher rate constants and
256 efficiencies (Fig. 2a and 2b). In the anaerobic tests at 35°C , the TIN releasing efficiency
257 of CAB reached 43% in two days, which was much higher than BAPN with only 9%
258 released at day 2. Based on the better mineralization performance at 35°C , aerobic tests
259 were implemented at 35°C for BAPN and CAB. There was negligibly further
260 improvement of mineralization in the BAPN batch (Fig. 2c). By contrast, the aerobic
261 incubation further improved the mineralization efficiency of CAB to 91% and stabilized
262 in 9 days. Therefore, CAB was more promising than BAPN as organic fertilizer in the
263 bioconversion process. Fig. 2d showed the dynamic changes of $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$, and
264 $\text{NO}_3^-\text{-N}$ during the aerobic batch test of CAB. There was no $\text{NO}_2^-\text{-N}$ detected during the
265 whole batch period. $\text{NH}_4^+\text{-N}$ was seen in the first 5 days. Since day 6, only $\text{NO}_3^-\text{-N}$ could
266 be detected in the reactor, indicating the nitrification process could be completed under
267 aerobic mineralization. Moreover, the maximum and average nitrification rates of the
268 CAB aerobic test were calculated based on the increase in TIN concentrations over the
269 corresponding periods (the first 4 and 9 days), which were 59 and 38 $\text{mg N L}^{-1} \text{d}^{-1}$,
270 respectively.

271 The poor mineralization performance of BAPN could be attributed to some intrinsic
272 characteristics, such as the potential hydrolysis inhibition by tannins in plants (Campos

273 et al., 2014; Kraus et al., 2003) or by cellobiose from cellulose degradation (Duff &
274 Murray, 1996). Besides the improvement via increasing temperature to 35°C, sufficient
275 aeration could break the bottleneck of CAB hydrolysis. In our batch study, aerobic
276 mineralization at $3\pm 0.5 \text{ g O}_2 \text{ L}^{-1}$ substantially improved the $\text{NH}_4^+\text{-N}$ released and
277 completely converted the $\text{NH}_4^+\text{-N}$ to $\text{NO}_3^-\text{-N}$. Spanoghe et al. (2020) showed more than
278 70% of the organic nitrogen mineralized from CAB into ammonium and nitrate in 77
279 days. This prolonged mineralization process should be attributed to the poor incubation
280 conditions via blending CAB with a commercial organic growing medium and storing it
281 at a relative humidity of 75% and temperature of 16 °C.

282 **3.2 Nitrate production in the mineralization and nitrification bioreactor**

283 In the influent tank, TIN only accounted for $3\pm 1\%$ of TN (SM Fig. S1). The
284 performance of $\text{NO}_3^-\text{-N}$ production in the bioreactor was divided into nine phases (I to
285 IX, Fig. 3a). A new phase started when a parameter was changed to improve the
286 performance of $\text{NO}_3^-\text{-N}$ production or balance the nutrient quality in BNS. In the
287 bioreactor, the accumulation of $\text{NH}_4^+\text{-N}$ and $\text{NO}_2^-\text{-N}$ was detected only for short periods
288 at the beginning of phases I, III, and IV (Fig. 3a).

289 **3.2.1 Performance of nitrate production under various controlling parameters**

290 The bioreactor was started with an influent loading rate of $100 \text{ mg TN L}^{-1} \text{ d}^{-1}$ in phase
291 I. The concentration of $\text{NO}_3^-\text{-N}$ in the bioreactor gradually increased and stabilized at
292 $275\pm 8 \text{ mg N L}^{-1}$ with a conversion efficiency of $55\pm 2\%$ after three rounds of HRT (i.e.,
293 15 days) (Fig. 3a). Under the same operational condition, doubling the loading rate to 200
294 $\text{mg TN L}^{-1} \text{ d}^{-1}$ in phase II (via increasing the influent concentration) further increased the
295 $\text{NO}_3^-\text{-N}$ concentration. In this phase, the bioreactor obtained a stable $\text{NO}_3^-\text{-N}$ conversion
296 efficiency of $54\pm 2\%$ after three rounds of HRT again, with $540\pm 16 \text{ mg NO}_3^-\text{-N L}^{-1}$. Due
297 to the poor settleability of sludge with 6.5 g VSS L^{-1} in phase II, the 30 min settling was

298 discontinued at day 42. Afterward, the HRT was always equal to the SRT.

299 In phase III, the TN loading rate was further increased to 400 mg TN L⁻¹ d⁻¹. The
300 bioreactor overflowed twice due to a foaming issue on day 49 and 61, resulting in a
301 temporary TIN decrease in the bioreactor (Fig. 3a). To tackle this foaming problem,
302 Antifoam SE-15 (Sigma-Aldrich), as a typical active silicon antifoam, was applied to the
303 reactor with the dosage of 0.1% (v/v of influent) (Sathiyamoorthi et al., 2019). By the end
304 of this phase, nitrate concentrations stabilized at 1060±26 mg N L⁻¹ with its production
305 efficiency of 53±1% and rate of 212±6 mg N L⁻¹ d⁻¹. Since the potassium (K)
306 concentration of bioreactor effluent was 3428±40 mg L⁻¹, the K to NO₃⁻-N ratio reached
307 around 3.2. The influent K content of 1581±49 mg L⁻¹ could obtain a K to NO₃⁻-N ratio
308 of about 1.5 in the bioreactor, which was already 1.3 times higher than that in HNS. Thus,
309 to avoid the excessive K supplement to the BNS, KOH was replaced by NaOH as a pH
310 control reagent in phase V. In phase IV and V, prolonging the HRT to 7.5 days under the
311 loading of 400 mg TN L⁻¹ d⁻¹ could achieve 1301±45 mg NO₃⁻-N L⁻¹. Still, the TIN
312 conversion efficiency only reached 46% without further improvement. Starting at phase
313 VI, the HRT was decreased back to 5 days. Subsequently, the nitrate concentration was
314 stable at 874±23 mg N L⁻¹ with a conversion efficiency of 44±1% and conversion rate of
315 175±5 mg N L⁻¹ d⁻¹ in phase VI to IX.

316 Starting in phase VII, the pH of the bioreactor was lowered to 6.0±0.2 to meet the
317 optimum pH range of most nutrient solutions. Compared to Phase VI, decreasing pH to
318 6.0±0.2 had no negative impact on the TIN conversion. Due to the deficiency of elements
319 Ca (around 42 mg L⁻¹) and Mg (about 46 mg L⁻¹) in the influent and subsequently in the
320 BNS of phase VI and VII, Ca(OH)₂ and Mg(OH)₂ were used as pH control reagents in
321 phase VIII and IX, respectively. In phase VIII of day 152, the dosage of Ca(OH)₂ could
322 significantly increase the element Ca to 752 mg L⁻¹, but the PO₄³⁻ concentration dropped

323 to 19 mg P L⁻¹ (Fig. 3b). The P recovery efficiency in the effluent was less than 10%,
324 much lower than previous phases with around 50% recovery. Presumably, the loss of
325 PO₄³⁻ could be attributed to the chemical precipitation of calcium phosphates such as
326 dicalcium phosphate (CaHPO₄) and tricalcium phosphate (Ca₃(PO₄)₂) at high calcium
327 concentration (Carlsson, 1997). After replacing Ca(OH)₂ with Mg(OH)₂ as a pH control
328 reagent, the PO₄³⁻ concentration in effluent gradually increased to 106±5 mg P L⁻¹, and
329 its recovery efficiency reached around 50% again (Fig. 3b).

330 From phase I to III, stepwise increasing the TN loading rate could always obtain over
331 50% TIN production. The decreased NO₃⁻-N concentration (phase V) and the relatively
332 low TIN releasing efficiency (phase VI-IX) compared to phase III were probably related
333 to the influence of cations (e.g., K⁺ and Na⁺ from pH reagents) on microbial activity and
334 community. The nitrogen mineralization was suggested to be less tolerant to Na⁺ than K⁺
335 (Sindhu & Cornfield, 1967). Hence, the decreasing trend of NO₃⁻-N concentration in
336 phase V was probably due to the switching of pH reagent from KOH to NaOH.

337 Nevertheless, under the influent loading rate of 400 mg TN L⁻¹ d⁻¹ at 5-day HRT and
338 SRT, NO₃⁻-N production efficiency and rate could stabilize in a range of 44-53% and
339 175-212 mg N L⁻¹ d⁻¹, respectively. The NO₃⁻-N production rate in the bioreactor was
340 significantly improved over the long-term operation, compared to the CAB aerobic batch
341 test of 59 mg N L⁻¹ d⁻¹ (section 3.1). By contrast, mineralization rates of organic fertilizers
342 in soils or growing media were considerably slower (SM, Table S4). Laboratory
343 incubation of common organic fertilizers (e.g., thermally-dried biosolids, manures, and
344 composts) in soils only obtained the nitrogen mineralization rates in a range of 0.13-1.88
345 mg N L⁻¹ d⁻¹) (Lazicki et al., 2020; Masunga et al., 2016; Smith & Durham, 2002). In
346 most cases, even if under these low rates, complete nitrification could not be guaranteed.

347 Previous mineralization tests for CAB realized complete nitrification only at a rate of
348 $5.12 \text{ mg N L}^{-1} \text{ d}^{-1}$ in organic growing media (Spanoghe et al., 2020). These slow nitrogen
349 transformation rates should mainly be attributed to the relatively low incubation
350 temperature ($16\text{-}25^\circ\text{C}$) as well as water and oxygen availability (Geisseler et al., 2019;
351 Rigby et al., 2016). Similarly, the carbon mineralization rates in soil incubations were
352 only $36\text{-}158 \text{ mg CO}_2\text{-C L}^{-1} \text{ d}^{-1}$ (Guo et al., 2019; Ribeiro et al., 2010). Assuming the
353 microbial cell formula is $\text{C}_5\text{H}_7\text{O}_2\text{N}$, the corresponding carbon mineralization rates of CAB
354 were $750\text{-}909 \text{ mg CO}_2\text{-C L}^{-1} \text{ d}^{-1}$ during the NO_3^- -N production (DİNÇER & ARAL,
355 2020). Therefore, the proposed well-controlled bioreactor approach produced liquid
356 nitrate-rich fertilizer more rapidly than was realized in soils. Additionally, the
357 performance of SCOD removal was excellent over the whole operation period, especially
358 the phase V-IX with above 90% removal (SM Fig. S2), which could benefit the
359 hydroponic cultivation by decreasing the risk of the proliferation of heterotrophic
360 pathogens and biofilms (Lee et al., 2015; Seo et al., 2017).

361 **3.2.2 The microbial community in the bioreactor**

362 Three bioreactor samples from days 86, 133, and 161 (i.e., phase III, VI, and IX) were
363 analyzed to identify the active microbial community working on nitrogen mineralization
364 and nitrification (Fig. 4). The influent CAB sample was regarded as a control group. In
365 all samples, the most predominant genera were aerobic hydrolysis and fermentative
366 bacteria, including *Thermomonas*, *Permianibacter*, and *Acinetobacter*. The relative
367 abundance of these hydrolysis and fermentative bacteria stepwise decreased from 42% of
368 day 86 to 17% of day 161, mainly related to the constant operating conditions (e.g.,
369 substrate and DO). The Shannon index of each bioreactor sample decreased from day 86
370 to 161, indicating the number of genera decreased during the bioreactor operation. The
371 abundance of *Thermomonas* increased from 4% of day 86 to 14.7% of day 161, while

372 *Permianibacter* and *Acinetobacter* decreased from 28.8% and 9.6% of day 86 to 0.9%
373 and 1.3% of day 161, respectively (Fig. 4). Yang et al. (2011) revealed the Shannon index
374 representing species diversity was positively related to the number of utilizable carbon
375 sources. Hence, the observed decreasing species diversity could be related to the unitary
376 organic carbon source (i.e., CAB) provided throughout the 170-day bioreactor operation.
377 Furthermore, the Bray-Curtis dissimilarity between the first sample (day 86) and the
378 following two samples (day 133 and 161) increased from 0.39 to 0.69. Thus, the
379 community shift occurred during the experiment, indicating the inoculum structure may
380 not be as decisive as the operating conditions in shaping the community. The decrease in
381 diversity in bioreactors has been observed previously due to the acclimatization of
382 inoculum to stable and optimized operating conditions (Cabrol et al., 2012; Eichorst et
383 al., 2013). The decreased abundance of hydrolysis and fermentative bacteria resulted in a
384 slight decrease in nitrogen mineralization efficiency (phase VI-IX) compared to phase III.

385 The relative abundance of microorganisms correlated with nitrification was relatively
386 low in all bioreactor samples. The total abundance of ammonium oxidizing microbes,
387 including *Nitrosomonas*, *Nitrospira*, and *Candidatus Nitrocosmicus* (Lu et al., 2019),
388 was always less than 1% (Fig. 4). Only two types of nitrite-oxidizing bacteria (NOB), i.e.,
389 *Nitrospira* and *Nitrolancea*, were detected in this study. The relative abundance of NOB
390 in all three bioreactor samples was lower than 0.1%. Surprisingly, the extremely low
391 abundance of nitrifying microbes could still achieve complete nitrification in this
392 mixotrophy condition.

393 **3.3 Nutrient quality balancing and economic analysis**

394 The nutrient quality balancing was modeled in each BNS organic scenario (i.e., NaOH,
395 Ca(OH)₂, and Mg(OH)₂) according to the proposed balancing strategy (Fig. 5a).
396 Consequently, the sodium concentration of HNS reached 332, 74, and 28 mg Na L⁻¹ in

397 NaOH, Ca(OH)₂, and Mg(OH)₂ scenario, respectively, and the salinity of HNS was 4.4,
398 3.0, and 2.8 mS cm⁻¹. Since the allowable nonessential element sodium and the salinity
399 should not exceed 180 mg Na L⁻¹ and 4 mS cm⁻¹ (Jones Jr, 2014; Sonneveld & Voogt,
400 2009), the NaOH scenario was evidently out of the ranges.

401 The Sankey diagrams based on the nutrient mass flow in each section of the Ca(OH)₂
402 and Mg(OH)₂ organic scenarios are shown in Fig. 5b and 5c. In the bioreactor section,
403 the observed accumulative dosing of Ca(OH)₂ and Mg(OH)₂ until the sampling time
404 (around 14 days) introduced 1656 mg Ca and 552 mg Mg into 1 L bioreactor content,
405 respectively. As a result of the membrane filtration, nutrients are separated into liquid and
406 solid fractions. A share of 57.5% or 1150 mg TN was retained in the solid fraction (i.e.,
407 retentate), which can be applied to land as a fertilizer. According to the typical
408 microfiltration process, the volume ratio of permeate and primary flow was assumed to
409 be 0.98 (Metcalf & Eddy, 1991). After the membrane section, soluble nutrients could be
410 recovered in 0.98 L permeate as the BNS. In the nutrient balancing section, element K
411 was the limiting factor of Ca(OH)₂ scenario, and extra NO₃⁻-N (67 mg), P (117 mg), Ca
412 (136 mg), and Mg (164 mg) should be supplemented into the BNS tank (Fig. 5b).
413 Eventually, after 4.5 times of dilution by H₂O, 4.38 L HNS could be obtained in the
414 Ca(OH)₂ scenario, and the NO₃⁻-N sourced from CAB mineralization accounted for 92.7%
415 of NO₃⁻-N in HNS. While in the Mg(OH)₂ scenario, element Mg was the limiting factor,
416 and the additional NO₃⁻-N (1427 mg), P (231 mg), K (1524 mg), and Ca (2129 mg) were
417 supplemented (Fig. 5c). After 11.1 times dilution, 10.88L HNS could be obtained for the
418 Mg(OH)₂ scenario. Compared to the Ca(OH)₂ scenario, the proportion of organic-sourced
419 NO₃⁻-N (i.e., NO₃⁻-N_{OG}) in HNS of Mg(OH)₂ scenario was much lower (only 37.4%).

420 The OPEX is shown in Table 1. All the expenditure was expressed as euro per liter of
421 HNS. According to the Sankey diagram in Fig. 5, approximately 25 g CAB was needed

422 to obtain 1 L BNS. In the $\text{Ca}(\text{OH})_2$ organic scenario, there was roughly 0.19 g NO_3^- - NO_G
423 in HNS. Calculated from the stoichiometry of nitrification (i.e., 4.57g O_2 g^{-1} N), 0.9 g O_2
424 L^{-1} HNS was needed. Besides, the O_2 consumption for TCOD removal was around 14.9
425 g O_2 L^{-1} BNS (SM Fig. S2), namely 3.3 g O_2 L^{-1} HNS. Based on the average O_2 transfer
426 efficiency and electricity cost, the total aeration cost of 2.5×10^{-4} € L^{-1} HNS could be
427 calculated. According to the microbial cell formula of $\text{C}_3\text{H}_7\text{O}_2\text{N}$, the aerobic
428 mineralization of CAB was assumed to release CO_2 of 15.71 g g^{-1} NO_3^- - NO_G (DİNÇER
429 & ARAL, 2020). Therefore, around 3.1g CO_2 L^{-1} HNS could be produced in the $\text{Ca}(\text{OH})_2$
430 scenario. Since the market price of liquid CO_2 is in a range of 0.08-0.15 € kg^{-1} (Mikunda
431 et al., 2015), the HNS produced in the $\text{Ca}(\text{OH})_2$ scenario could save a maximum of 4.6
432 $\times 10^{-4}$ € L^{-1} for the CO_2 fertilization. As a result of the lower NO_3^- - NO_G concentration in
433 the $\text{Mg}(\text{OH})_2$ organic scenario (around 0.08 g L^{-1} HNS), the total aeration cost and CO_2
434 fertilization saving were only 1.0×10^{-4} and 1.8×10^{-4} € L^{-1} HNS, respectively.

435 Among the three cost categories, the cost of CAB dominated the OPEX of the organic
436 scenarios, while chemical costs were four to seven orders of magnitude lower than that
437 of fertilizers for either of the suppliers investigated (SM Table S3). The OPEX of
438 commercial scenarios only included the costs of fertilizer and supplemented compounds.
439 As a result, the OPEX of YaraTrea and FloraFlex was 1×10^{-3} and 34×10^{-3} € L^{-1} HNS,
440 respectively. The cost of the control scenario was the lowest (1.7 - 1.8×10^{-8} € L^{-1} HNS),
441 but individually purchasing every chemical compound is not practically feasible.

442 In Table 1, the OPEX of $\text{Ca}(\text{OH})_2$ scenario was 2.5-fold the $\text{Mg}(\text{OH})_2$ scenario, due to
443 its higher NO_3^- - NO_G proportion. Compared to the commercial scenarios, the OPEX of
444 $\text{Ca}(\text{OH})_2$ scenario was 5.3-fold of the YaraTera scenario, but only 0.16-fold the FloraFlex
445 scenario, indicating its potential to be cost-competitive in hydroponic fertilization.

446 **4. Conclusion**

447 This study delivered a proof of concept for an approach based mainly on a bioreactor
448 and a nutrient balancing step to produce a nitrate-rich liquid fertilizer from a solid organic
449 fertilizer. Batch tests showed that the best operating condition was aerobic conversion at
450 35°C. The nitrification performance of the microbial fertilizer could reach an efficiency
451 above 90% and a maximum rate of 59 mg N L⁻¹ d⁻¹, while completely oxidizing the
452 released NH₄⁺-N to NO₃⁻-N. Subsequently, 35°C was chosen to operate a bioreactor,
453 reaching a nitrate production rate of 212 mg N L⁻¹ d⁻¹, yet at a lower efficiency of 51%.
454 A nutrient balance estimation to simulate the Hoagland nutrient solution showed a higher
455 proportion of NO₃⁻-N (92.7%) in the Ca(OH)₂ scenario was organically sourced. The CO₂
456 generated in the bioreactor can be used for greenhouse CO₂ fertilization to cut the OPEX.
457 The Hoagland solution produced from the microbial fertilizer is potentially cost-
458 competitive compared to the commercially available inorganic fertilizer nutrient solution
459 (2.2×10⁻³ versus 34 ×10⁻³ € L⁻¹ in total OPEX).

460 **Supplementary Material**

461 Supplementary data of this work can be found in the online version of the paper.

462 **Acknowledgments**

463 This work was supported by the China Scholarship Council (File No.
464 CSC201706130131). The authors would like to thank: (i) company Avecom (Wondelgem,
465 Belgium) for the CAB supply, (ii) Veerle Van Malderen for her insightful discussions,
466 (iii) Tim Van Winckel for his help in cation measurement via ICPMS in the Water-Link
467 laboratory (Rumst, Belgium), and (iv) Dr. Wannes Van Beeck for his guidance on the
468 microbial DNA extraction.

469 **Reference**

- 470 AlShrouf, A. 2017. Hydroponics, aeroponic and aquaponic as compared with conventional
 471 farming. *American Scientific Research Journal for Engineering, Technology, and Sciences*
 472 (*ASRJETS*), **27**(1), 247-255.
- 473 Botheju, D. 2011. Oxygen Effects in Anaerobic Digestion – A Review. *The Open Waste*
 474 *Management Journal*, **4**(1), 1-19.
- 475 Cabello, P., Roldán, M.D., Castillo, F., Moreno-Vivián, C. 2009. Nitrogen Cycle. in: *Encyclopedia*
 476 *of Microbiology (Third Edition)*, (Ed.) M. Schaechter, Academic Press. Oxford, pp. 299-
 477 321.
- 478 Cabrol, L., Malhautier, L., Poly, F., Lepeuple, A.-S., Fanlo, J.-L. 2012. Bacterial dynamics in steady-
 479 state biofilters: beyond functional stability. *FEMS Microbiology Ecology*, **79**(1), 260-271.
- 480 Campos, C.R., Mesquita, V.A., Silva, C.F., Schwan, R.F. 2014. Efficiency of physicochemical and
 481 biological treatments of vinasse and their influence on indigenous microbiota for
 482 disposal into the environment. *Waste Management*, **34**(11), 2036-2046.
- 483 Carlsson, H. 1997. Calcium phosphate precipitation in biological phosphorus removal systems.
 484 *Water Research*, **31**(5), 1047-1055.
- 485 Clesceri, L., Greenberg, A., Eaton, A. 1998. Standard Methods for the Examination of Water and
 486 Wastewater, APHA, Washington, DC. *Standard methods for the examination of water*
 487 *and wastewater. 20th ed. APHA, Washington, DC., -*.
- 488 Crohn, D. 2004. Nitrogen mineralization and its importance in organic waste recycling.
 489 *Proceedings, National Alfalfa Symposium*. pp. 13-5.
- 490 DİNÇER, A.R., ARAL, İ.F. 2020. CARBON DIOXIDE EMISSION IN THRACE REGION AND
 491 NEIGHBORING COUNTRIES AS A RESULT OF AEROBIC STABILIZATION OF URBAN
 492 TREATMENT SLUDGES. *Sigma: Journal of Engineering & Natural Sciences/Mühendislik ve*
 493 *Fen Bilimleri Dergisi*, **38**(4).
- 494 Duff, S.J., Murray, W.D. 1996. Bioconversion of forest products industry waste cellulose to fuel
 495 ethanol: a review. *Bioresource technology*, **55**(1), 1-33.
- 496 Eichorst, S.A., Varanasi, P., Stavila, V., Zemla, M., Auer, M., Singh, S., Simmons, B.A., Singer, S.W.
 497 2013. Community dynamics of cellulose-adapted thermophilic bacterial consortia.
 498 *Environmental Microbiology*, **15**(9), 2573-2587.
- 499 Eurostat, S.E. 2018. Electricity price statistics. *Electricity prices for non-household consumers*.
 500 *ISSN*, 2443-8219.
- 501 Garland, J.L., Mackowiak, C.L., Sager, J.C. 1993. Hydroponic crop production using recycled
 502 nutrients from inedible crop residues. *SAE Transactions*, 1103-1110.
- 503 Garland, J.L., Mackowiak, C.L., Strayer, R.F., Finger, B.W. 1997. Integration of waste processing
 504 and biomass production systems as part of the KSC Breadboard project. **20**(10), 1821-
 505 1826.
- 506 Geisseler, D., Miller, K.S., Aegerter, B.J., Clark, N.E., Miyao, E.M. 2019. Estimation of Annual Soil
 507 Nitrogen Mineralization Rates using an Organic - Nitrogen Budget Approach. *Soil*
 508 *Science Society of America Journal*, **83**(4), 1227-1235.
- 509 Griffis, T.J., Baker, J.M. 2020. Nitrogen management and air quality in China. *Nature Food*, **1**(10),
 510 597-598.
- 511 Grunert, O., Reheul, D., Van Labeke, M.-C., Perneel, M., Hernandez-Sanabria, E., Vlaeminck, S.E.,
 512 Boon, N. 2016. Growing media constituents determine the microbial nitrogen
 513 conversions in organic growing media for horticulture. **9**(3), 389-399.
- 514 Guo, Z., Han, J., Li, J., Xu, Y., Wang, X. 2019. Effects of long-term fertilization on soil organic
 515 carbon mineralization and microbial community structure. *PLOS ONE*, **14**(1), e0211163.
- 516 Hollevoet, L., Jardali, F., Gorbanev, Y., Creel, J., Bogaerts, A., Martens, J.A. 2020. Towards Green
 517 Ammonia Synthesis through Plasma - Driven Nitrogen Oxidation and Catalytic
 518 Reduction. *Angewandte Chemie*.

- 519 Huang, Z., Hejazi, M., Tang, Q., Vernon, C.R., Liu, Y., Chen, M., Calvin, K. 2019. Global agricultural
520 green and blue water consumption under future climate and land use changes. *Journal*
521 *of Hydrology*, **574**, 242-256.
- 522 Johansen, J.-E., Bakke, R. 2006. Enhancing hydrolysis with microaeration. *Water science and*
523 *Technology*, **53**(8), 43-50.
- 524 Jones Jr, J.B. 2014. *Complete guide for growing plants hydroponically*. CRC Press.
- 525 Katipoglu-Yazan, T., Cokgor, E.U., Insel, G., Orhon, D. 2012. Is ammonification the rate limiting
526 step for nitrification kinetics? *Bioresource Technology*, **114**, 117-125.
- 527 Khatoon, H., Solanki, P., Narayan, M., Tewari, L., Rai, J., Hina Khatoon, C. 2017. Role of microbes
528 in organic carbon decomposition and maintenance of soil ecosystem. *International*
529 *Journal of Chemical Studies*, **5**(6), 1648-1656.
- 530 Kraus, T.E.C., Dahlgren, R.A., Zasoski, R.J. 2003. Tannins in nutrient dynamics of forest
531 ecosystems - a review. *Plant and Soil*, **256**(1), 41-66.
- 532 Lacour, C., Seconda, L., Allès, B., Hercberg, S., Langevin, B., Pointereau, P., Lairon, D., Baudry, J.,
533 Kesse-Guyot, E. 2018. Environmental Impacts of Plant-Based Diets: How Does Organic
534 Food Consumption Contribute to Environmental Sustainability? *Frontiers in Nutrition*, **5**.
- 535 Lazicki, P., Geisseler, D., Lloyd, M. 2020. Nitrogen mineralization from organic amendments is
536 variable but predictable. *Journal of Environmental Quality*, **49**(2), 483-495.
- 537 Lee, S., Ge, C., Bohrerova, Z., Grewal, P.S., Lee, J. 2015. Enhancing plant productivity while
538 suppressing biofilm growth in a windowfarm system using beneficial bacteria and
539 ultraviolet irradiation. *Canadian journal of microbiology*, **61**(7), 457-466.
- 540 Lu, S., Liu, X., Liu, C., Wang, X., Cheng, G. 2019. Review of ammonia-oxidizing bacteria and
541 archaea in freshwater ponds. *Reviews in Environmental Science and Bio/Technology*,
542 **18**(1), 1-10.
- 543 Masunga, R.H., Uzokwe, V.N., Mlay, P.D., Odeh, I., Singh, A., Buchan, D., De Neve, S. 2016.
544 Nitrogen mineralization dynamics of different valuable organic amendments commonly
545 used in agriculture. *Applied Soil Ecology*, **101**, 185-193.
- 546 Maucieri, C., Nicoletto, C., Van Os, E., Anseeuw, D., Van Havermaet, R., Junge, R. 2019.
547 Hydroponic technologies. *Aquaponics Food Production Systems*, 77.
- 548 Metcalf, Eddy, I. 1991. *Wastewater engineering: Treatment, disposal and reuse*, McGraw-Hill
549 New York,, USA.
- 550 Mikunda, T., Neele, F., Wilschut, F., Hanegraaf, M. 2015. *A secure and affordable CO2 supply for*
551 *the Dutch greenhouse sector*. TNO, Earth, Life & Social Sciences.
- 552 Niu, T., Zhou, Z., Shen, X., Qiao, W., Jiang, L.-M., Pan, W., Zhou, J. 2016. Effects of dissolved
553 oxygen on performance and microbial community structure in a micro-aerobic
554 hydrolysis sludge in situ reduction process. *Water research*, **90**, 369-377.
- 555 Ramírez, W.A., Domene, X., Andrés, P., Alcañiz, J.M. 2008. Phytotoxic effects of sewage sludge
556 extracts on the germination of three plant species. *Ecotoxicology*, **17**(8), 834-844.
- 557 Ribeiro, H.M., Fangueiro, D., Alves, F., Vasconcelos, E., Coutinho, J., Bol, R., Cabral, F. 2010.
558 Carbon-mineralization kinetics in an organically managed Cambic Arenosol amended
559 with organic fertilizers. *Journal of Plant Nutrition and Soil Science*, **173**(1), 39-45.
- 560 Rigby, H., Clarke, B.O., Pritchard, D.L., Meehan, B., Beshah, F., Smith, S.R., Porter, N.A. 2016. A
561 critical review of nitrogen mineralization in biosolids-amended soil, the associated
562 fertilizer value for crop production and potential for emissions to the environment.
563 *Science of The Total Environment*, **541**, 1310-1338.
- 564 Roman, M.-D., Mureşan, M.-V. 2014. Analysis of oxygen requirements and transfer efficiency in
565 a wastewater treatment plant. *Int. J. Latest Res. Sci. Technol*, **3**, 30-33.
- 566 Sathiyamoorthi, E., Kumar, P., Kim, B.S. 2019. Lipid production by *Cryptococcus albidus* using
567 biowastes hydrolysed by indigenous microbes. *Bioprocess and Biosystems Engineering*,
568 **42**(5), 687-696.
- 569 Seo, K.W., Choi, Y.-S., Gu, M.B., Kwon, E.E., Tsang, Y.F., Rinklebe, J., Park, C. 2017. Pilot-scale

570 investigation of sludge reduction in aerobic digestion system with endospore-forming
571 bacteria. *Chemosphere*, **186**, 202-208.

572 Shinohara, M., Aoyama, C., Fujiwara, K., Watanabe, A., Ohmori, H., Uehara, Y., Takano, M. 2011.
573 Microbial mineralization of organic nitrogen into nitrate to allow the use of organic
574 fertilizer in hydroponics. *Soil science and plant nutrition*, **57**(2), 190-203.

575 Sindhu, M.t., Cornfield, A. 1967. Comparative effects of varying levels of chlorides and sulphates
576 of sodium, potassium, calcium, and magnesium on ammonification and nitrification
577 during incubation of soil. *Plant and Soil*, **27**(3), 468-472.

578 Smith, S.R., Durham, E. 2002. Nitrogen Release and Fertiliser Value of Thermally-Dried Biosolids.
579 *Water and Environment Journal*, **16**(2), 121-126.

580 Sonneveld, C., Voogt, W. 2009. Nutrient management in substrate systems. in: *Plant nutrition of*
581 *greenhouse crops*, Springer, pp. 277-312.

582 Spanoghe, J., Grunert, O., Wambacq, E., Sakarika, M., Papini, G., Alloul, A., Spiller, M., Derycke,
583 V., Stragier, L., Verstraete, H., Fauconnier, K., Verstraete, W., Haesaert, G., Vlaeminck,
584 S.E. 2020. Storage, fertilization and cost properties highlight the potential of dried
585 microbial biomass as organic fertilizer. *Microbial Biotechnology*, **13**(5), 1377-1389.

586 Spiller, M., Muys, M., Papini, G., Sakarika, M., Buyle, M., Vlaeminck, S.E. 2020. Environmental
587 impact of microbial protein from potato wastewater as feed ingredient: Comparative
588 consequential life cycle assessment of three production systems and soybean meal.
589 *Water Research*, **171**, 115406.

590 Trejo-Téllez, L.I., Gómez-Merino, F.C. 2012. Nutrient solutions for hydroponic systems.
591 *Hydroponics-a standard methodology for plant biological researches*, 1-22.

592 van Os, E., Blok, C., Voogt, W., Waked, L. 2016. Water quality and salinity aspects in hydroponic
593 cultivation. WUR Glastuinbouw.

594 Veeken, A., Hamelers, B. 1999. Effect of temperature on hydrolysis rates of selected biowaste
595 components. *Bioresource Technology*, **69**(3), 249-254.

596 Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R. 2007. Naive Bayesian classifier for rapid
597 assignment of rRNA sequences into the new bacterial taxonomy. *Applied and*
598 *environmental microbiology*, **73**(16), 5261-5267.

599 Yang, C., Zhang, W., Liu, R., Li, Q., Li, B., Wang, S., Song, C., Qiao, C., Mulchandani, A. 2011.
600 Phylogenetic Diversity and Metabolic Potential of Activated Sludge Microbial
601 Communities in Full-Scale Wastewater Treatment Plants. *Environmental Science &*
602 *Technology*, **45**(17), 7408-7415.

603 Yaron, S., Römling, U. 2014. Biofilm formation by enteric pathogens and its role in plant
604 colonization and persistence. *Microbial Biotechnology*, **7**(6), 496-516.

605 Zaman, M., Di, H., Cameron, K. 1999. A field study of gross rates of N mineralization and
606 nitrification and their relationships to microbial biomass and enzyme activities in soils
607 treated with dairy effluent and ammonium fertilizer. *Soil Use and Management*, **15**(3),
608 188-194.

609

Figures and Tables

Figure 1. Concept of a novel hydroponic nutrient supply system: (a) mineralization and nitrification of organic fertilizer to provide the suitable nutrient solution for (b) hydroponic cultivation in a greenhouse.

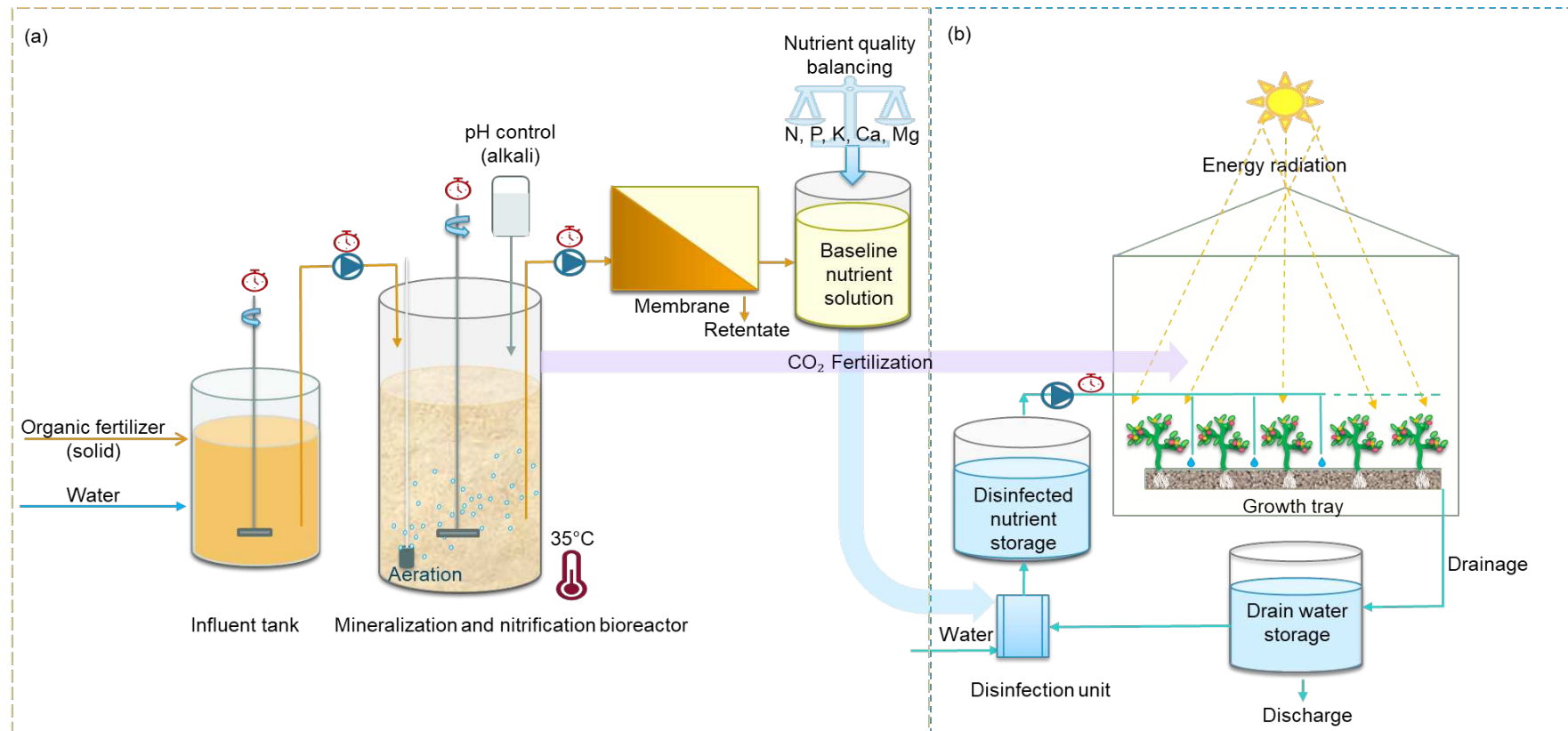


Figure 2. Performance evaluation of batch tests; anaerobic mineralization with ADS at 20°C and 35°C (a) BAPN and (b) CAB; (c) aerobic mineralization with AS at 35°C on BAPN and CAB; (d) dynamic changes of specific inorganic nitrogen during “AS-35°C-CAB” test. “k” is the kinetic rate constant. “EFF” means the mineralization efficiency.

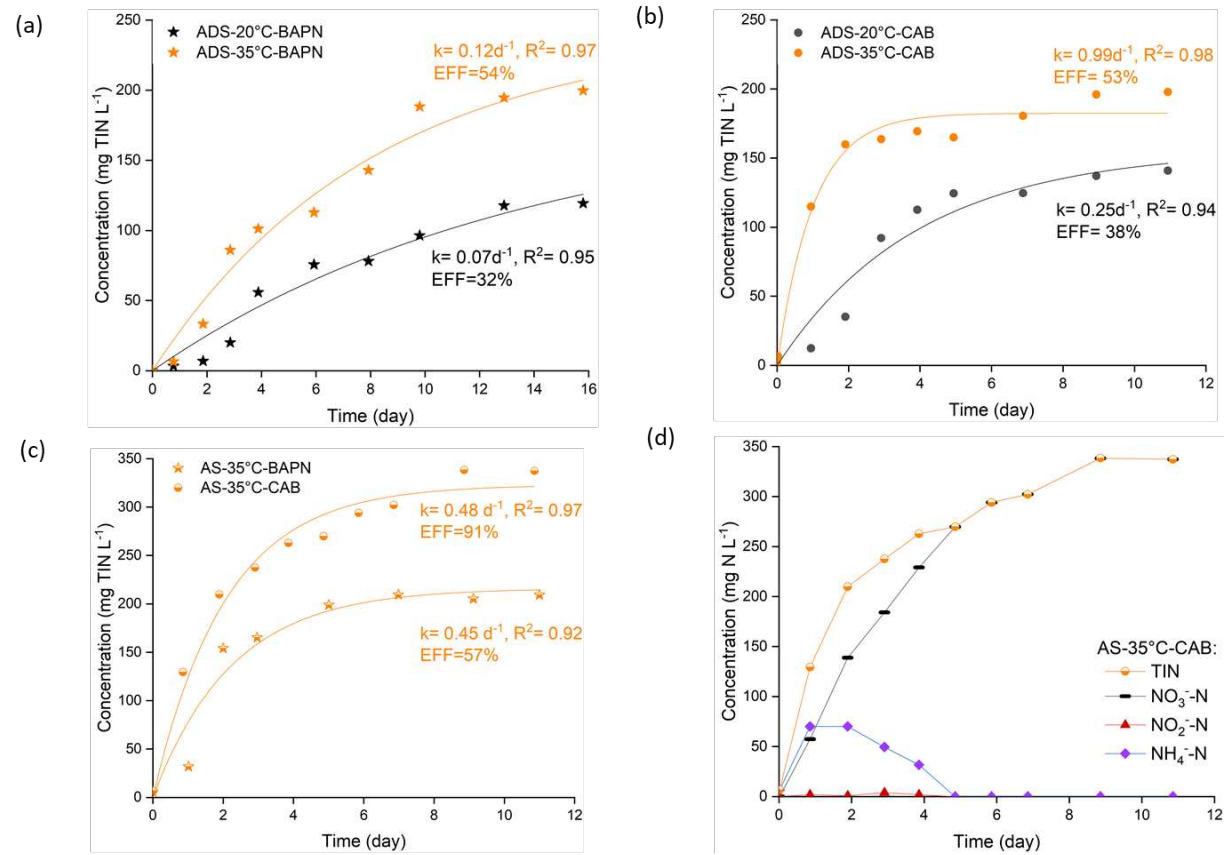


Figure 3. The profile of the inorganic nitrogen conversion (a) and phosphate recovery (b) in the effluent of mineralization and nitrification bioreactor from day 0 to day 170. The major variables are shown on top. TIN conversion efficiency and P recovery efficiency represent the concentration ratios of TIN and $\text{PO}_4^{3-}\text{-P}$ in the effluent to TN and TP in the influent, respectively.

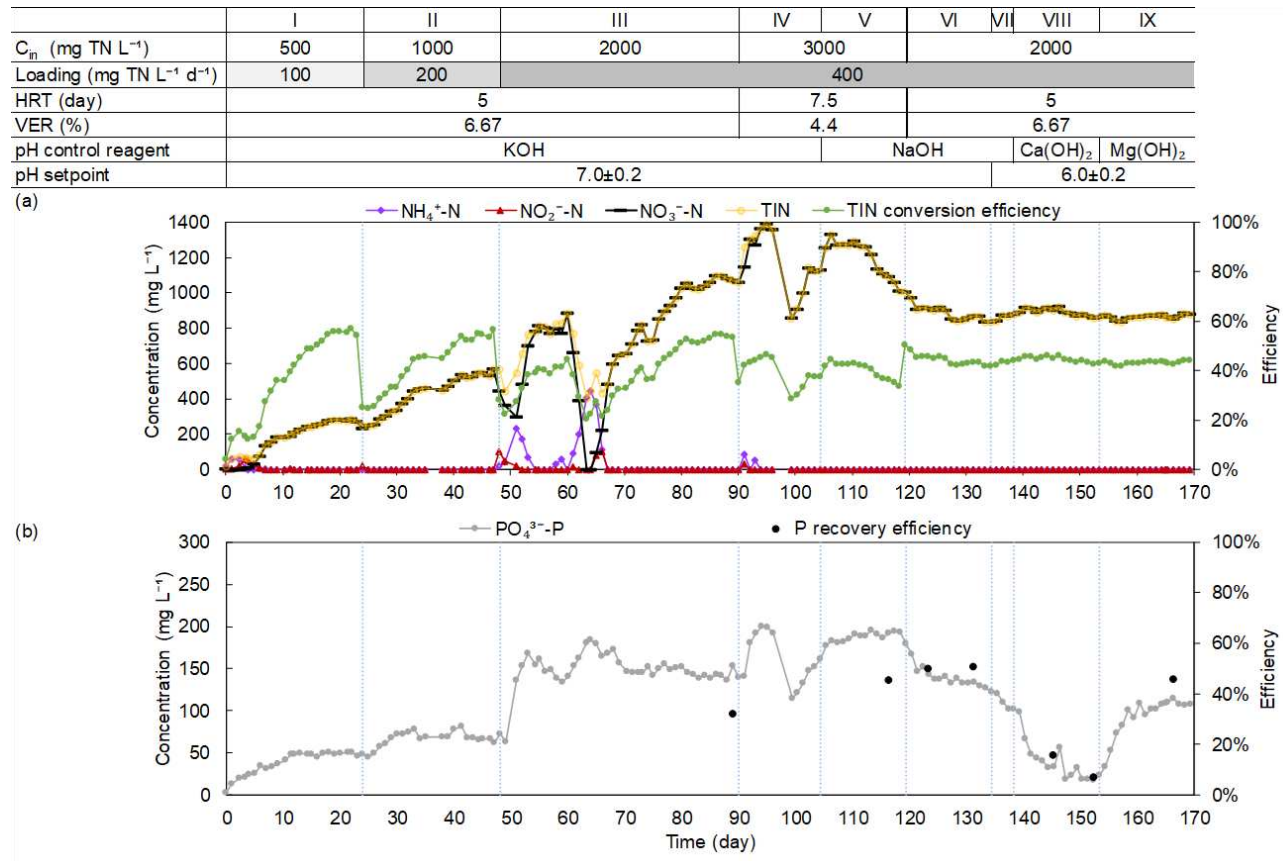


Figure 4. The relative abundance of microbes in the mineralization and nitrification bioreactor at genus levels. CAB means the influent composed of CAB. BC dissimilarity represents the Bray-Curtis dissimilarity between day 86 and each other sample.

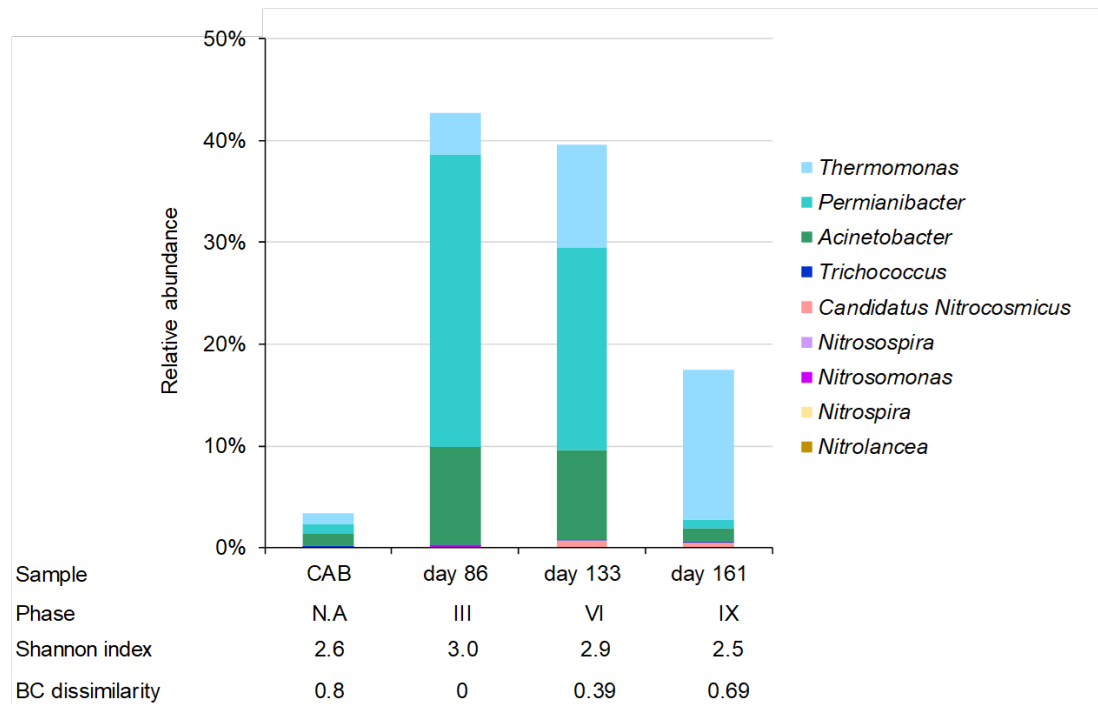
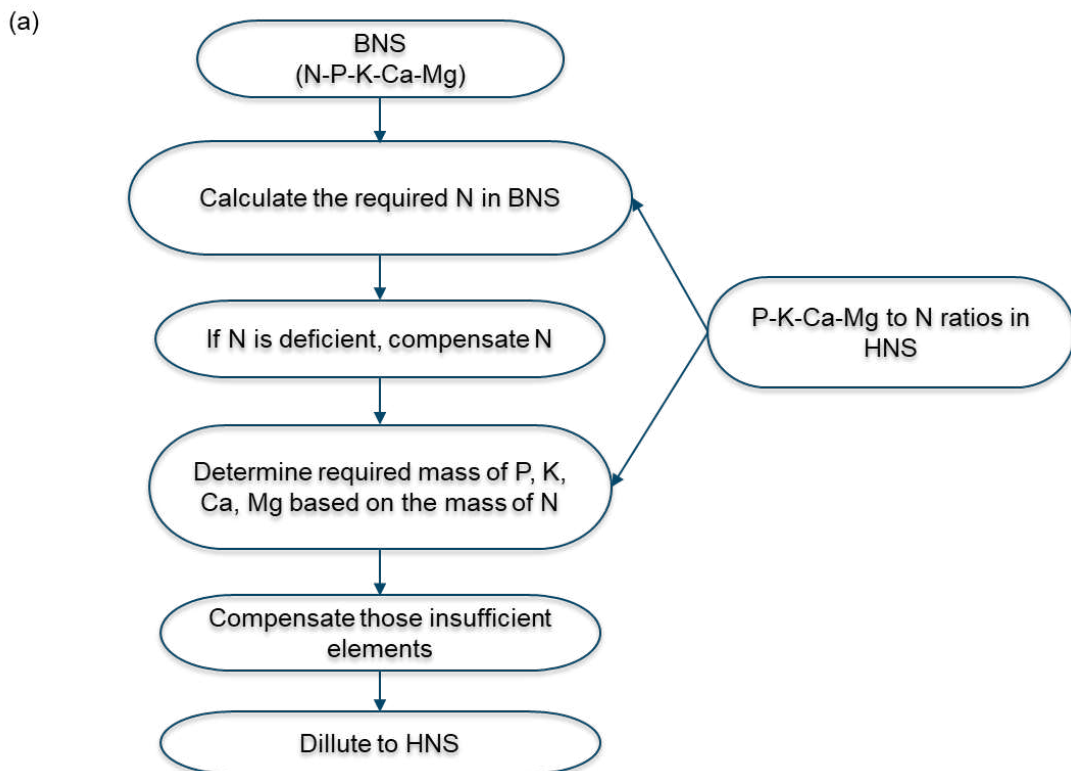
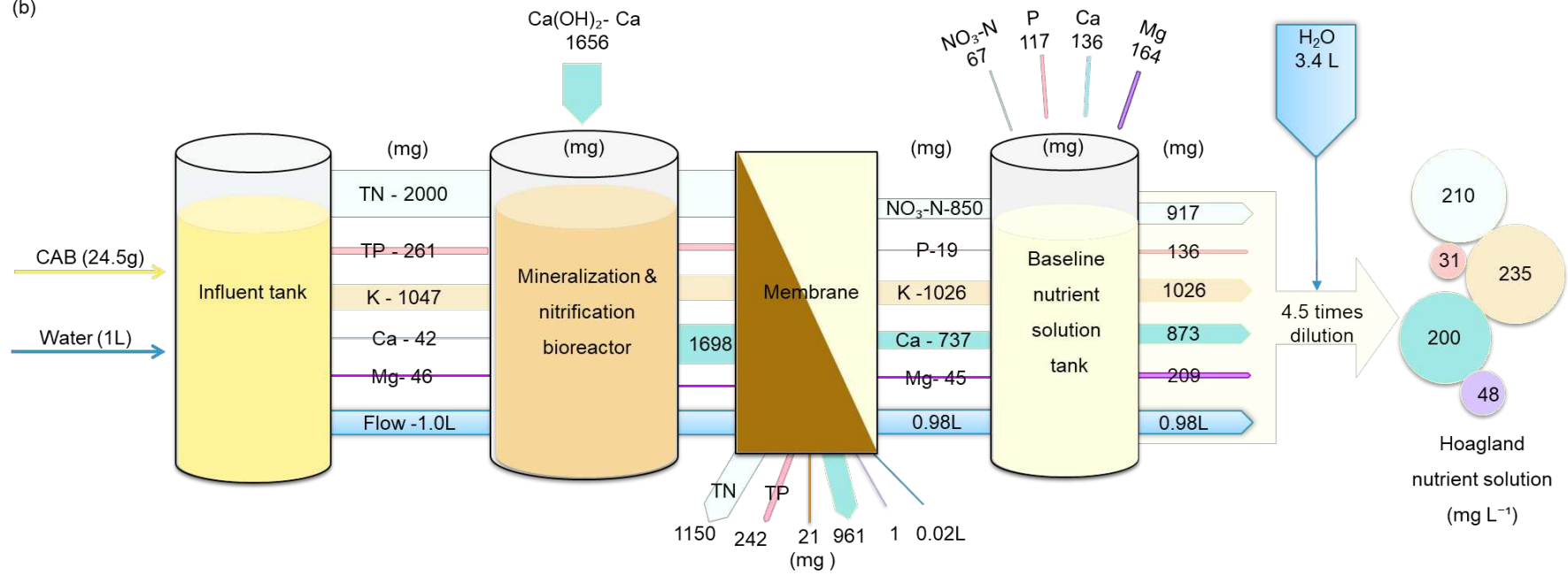


Figure 5. Nutrient quality balancing: (a) the methodology to carry out the mass balance of the baseline nutrient solution (BNS) according to the element composition and concentration in Hoagland nutrient solution (HNS); the Sankey diagrams based on the mass of nutrients in different sections: (b) the $\text{Ca}(\text{OH})_2$ organic scenario, (c) the $\text{Mg}(\text{OH})_2$ organic scenario. TN and TP mean the total nitrogen and total phosphorus, respectively.



(b)



(c)

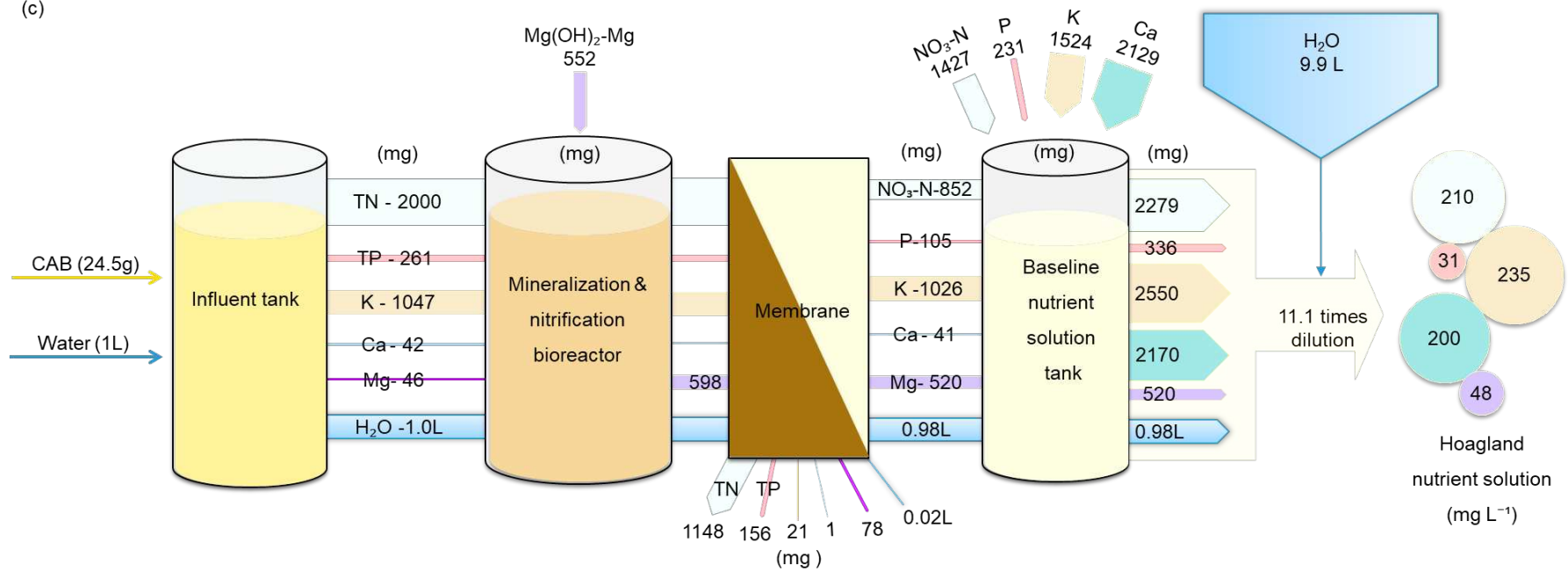


Table 1 Extrapolated operational expenditure (OPEX) categories for preparing Hoagland nutrient solution (HNS). “BNS” means the baseline nutrient solution. “N.A.” means the operation was not applicable. The costs of chemical compound supplementation were based on the market reference prices from two global suppliers (i.e., Alibaba/ EC21, details in SM, Table S3).

OPEX category		Ca(OH) ₂ organic scenario	Mg(OH) ₂ organic scenario	YaraTera	FloraFlex	Control scenario
CAB/ commercial fertilizer	g CAB L ⁻¹ BNS	25.0	25.0		N.A.	
	€ L ⁻¹ HNS	5.6 × 10 ⁻³	2.3 × 10 ⁻³	1.0 × 10 ⁻³	34 × 10 ⁻³	N.A.
Aeration	g NO ₃ ⁻ -N _{OG} L ⁻¹ HNS	0.19	0.08			
	g O ₂ L ⁻¹ HNS (nitrification)	0.9	0.4			
	g O ₂ L ⁻¹ HNS (COD removal)	3.3	1.3		N.A.	
	€ L ⁻¹ HNS	2.5 × 10 ⁻⁴	1.0 × 10 ⁻⁴			
CO ₂ production	g CO ₂ L ⁻¹ HNS	3.1	1.2			
	€ L ⁻¹ HNS (CO ₂ fertilization saving)	4.6 × 10 ⁻⁴	1.8 × 10 ⁻⁴			
Chemical compound supplementation	€ L ⁻¹ HNS	6.6 × 10 ⁻⁸ / 11 × 10 ⁻⁸	1.5 × 10 ⁻⁷ / 1.4 × 10 ⁻⁷	5.9 × 10 ⁻⁹ / 6.8 × 10 ⁻⁹	3.0 × 10 ⁻⁹ / 4.0 × 10 ⁻⁹	1.8 × 10 ⁻⁸ / 1.7 × 10 ⁻⁸
Total OPEX		5.4 × 10 ⁻³	2.2 × 10 ⁻³	1.0 × 10 ⁻³	3.4 × 10 ⁻³	1.8 × 10 ⁻⁸ / 1.7 × 10 ⁻⁸