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

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

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Abstract

Due to the high water- and nutrient-use efficiency, hydroponic cultivation is increasingly vital in progressing to environment-friendly food production. To further alleviate the environmental impacts of synthetic fertilizer production, the use of recovered nutrients should be encouraged in horticulture and agriculture at large. Solid organic fertilizers can largely contribute to this, yet their physical and chemical nature impedes application in hydroponics. This study proposes a bioreactor for mineralization and nitrification followed by a supplementation step for limiting macronutrients to produce nitrate-based solutions from solid fertilizers, here based on a novel microbial fertilizer. Batch tests showed that aerobic conversions at 35°C could realize a nitrate (NO_3^--N) production efficiency above 90% and a maximum rate of 59 mg N L^{-1} d^{-1}. In the subsequent bioreactor test, nitrate production efficiencies were lower (44-51%), yet rates were higher (175-212 mg N L^{-1} d^{-1}). Calcium and magnesium hydroxide were compared to control the bioreactor pH at 6.0±0.2, while also providing macronutrients for plant production. A mass balance estimation to mimic the Hoagland nutrient solution showed that 92.7% of the NO_3^--N in the $Ca(OH)_2$ scenario could be organically sourced, while

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

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

1. Introduction

Agriculture and food production take a major share in the consumption of global resources, with for instance about 90% of global freshwater (Huang et al., 2019) and > 80% of global ammonia produced through the Haber-Bosch process (Hollevoet et al., 2020). However, during the artificial nitrogen fixation from N_2 to ammonia (NH_3) via the Haber-Bosch process, greenhouse gas and NH_3 emissions, and PM2.5 (particulate matter with a diameter less than 2.5 μm) formation can aggravate the global climate change and the air pollution, and thus threaten human health (Griffis & Baker, 2020). Therefore, efficient use of nutrients and water is essential to progress towards an environmentally friendly agricultural system. Hydroponics in greenhouses are among the most water- and nutrient-use efficient agricultural systems, with up to 90% water and 85% nutrient saving (AlShrouf, 2017). However, current hydroponic systems rely entirely on inorganic nitrogen supplied by the Harber-Bosch process, dosed as liquid fertilizers (Maucieri et al., 2019; Trejo-Téllez & Gómez-Merino, 2012). An alternative strategy is to increase the share of recovered nutrients in horticulture, and agriculture at large, to reduce the demand for synthetic fertilizers. Use of recovered nitrogen is claimed to reduce environmental 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 (Khatoon 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).

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

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

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

This study proposes a novel pH-controlled bioreactor system for mineralization and nitrification of solid organic fertilizers, followed by a chemical supplementation step to achieve a well-balanced hydroponic nutrient solution (Fig. 1). This concept has the advantage of accurately tuning the composition and concentration of a more sustainable liquid nitrate-based fertilizer to match the plant needs (Fig. 1a), while avoiding the entry of organic carbon, oxygen demand, and stimulation of microbial risks in the hydroponic system. Besides, carbon dioxide (CO_2) generated during the organic fertilizer mineralization can also be used for CO_2 fertilization in the greenhouse. The proposed concept for the controlled mineralization and nitrification system comprises four parts: (i) an influent tank to mix the solid organic fertilizer with water; (ii) a bioreactor for mineralization and nitrification with pH control (base addition); (iii) a membrane module (e.g., microfiltration) to remove the solids and yield a baseline nutrient solution (BNS); and (iv) a nutrient balancing step to further tune the BNS macronutrient composition to the plant's needs.

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

2. Material and methods

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

2.1 Organic fertilizers

BioAgenasol® profigreen (BAPN) (AGRANA group, Austria) and CAB were tested in this study. BAPN represents a class of commercially available plant-based fertilizers and is a by-product of bioethanol production. CAB represents a class of novel microbial fertilizers obtained from the company Avecom (Wondelgem, Belgium). CAB was produced through a patented aerobic fermentation process based on diluted process streams of food processing companies. Based on the dry weight, the total nitrogen (TN) content of BAPN and CAB are 4.8% and 8.2%, respectively. No inorganic nitrogen (i.e., NH_4^+-N, NO_2^--N, and NO_3^--N) could be detected in TN.

2.2 Batch tests

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

30 mins to eliminate the residual DO in tap water, and no aeration was provided during the whole batch period, which resulted in a DO of around 0 mg O_2 L^{-1} in the anaerobic tests. Two different reaction temperatures of 20°C and 35°C were implemented in anaerobic tests. To explore the effect of DO on nitrogen mineralization, the aerobic tests were implemented in open glass flasks with the AS as an inoculum. The DO level in the aerobic test was controlled at 3±0.5 mg O_2 L^{-1} via air pumps (Tetra TEC APS 150).

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

2.3 Nitrate conversion in a controlled mineralization and nitrification bioreactor

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

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

Based on the batch test results, CAB was used in this step, and aerobic bioconversion at 35°C was implemented in the bioreactor. The inoculum was taken from the aerobic batch test and added into the 3 L bioreactor with an initial concentration of 0.4 g VSS L^{-1}. The influent TN concentration increased from 500 to 3000 mg N L^{-1} and the loading rate from 100 to 400 mg N L^{-1} d^{-1}, resulting in a volume exchange ratio of 4.4-6.67% and a hydraulic retention time (HRT) of 5.5-7.5 days. The pH of the bioreactor was initially controlled at 7.0±0.2 (day 0-133), and then at 6.0±0.2 (day 134-170), which was directly consistent with the optimum pH range of most nutrient solutions (5.5-6.5) (Trejo-Téllez & Gómez-Merino, 2012). To explore the optimal pH control reagent according to the nutrient composition and concentration for plant needs, various pH control reagents including KOH, sodium hydroxide (NaOH), calcium hydroxide ($Ca(OH)_2$), and magnesium hydroxide ($Mg(OH)_2$) were tested. The operational parameters are detailed in the top table of Fig. 3. To monitor the performance of the bioreactor, samples were taken periodically from the influent and effluent.

2.4 Physicochemical water, biomass, and microbiome analyses

The NH_4^+-N, NO_2^--N, NO_3^--N, and phosphate (PO_4^{3-}) were measured with the San++ Automated Wet Chemistry Analyzer. The TN, total phosphorus (TP), total chemical oxygen demand (TCOD), soluble chemical oxygen demand (SCOD) were photometrically determined using NANOCOLOR test tubes (Macherey-Nagel, Germany). The VSS was measured using standard methods (Clesceri et al., 1998). Elements of potassium (K), calcium (Ca), magnesium (Mg), and sodium (Na) were measured by inductively coupled plasma mass spectrometry (Thermo Scientific™ iCAP

Q, Thermo Fisher Scientific).

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

2.5 Nutrient balancing strategy and economic assessment

The effluent of the bioreactor was filtered through the 0.2 μm filters to simulate the function of a membrane module. The filtrate was regarded as the BNS. Hoagland nutrient solution (HNS) is considered one universal nutrient solution for plant growth in hydroponics. The concentration of major elements in HNS is 210 mg NO_3^--N L^{-1}, 31 mg P L^{-1}, 235 mg K L^{-1}, 200mg Ca L^{-1}, and 48 mg Mg L^{-1} (Jones Jr, 2014). To model the nutrient balancing according to the HNS recipe, a nutrient balancing strategy was illustrated in Fig. 5a. Water of low salinity (< 0.75 mS cm^{-1}) is recommended in the dilution step (van Os et al., 2016). Commonly used chemical compounds for making nutrient solutions such as calcium nitrate ($Ca(NO_3)_2$), magnesium nitrate ($Mg(NO_3)_2$), magnesium sulfate ($MgSO_4$), calcium sulfate ($CaSO_4$), potassium sulfate (K_2SO_4) and dipotassium phosphate (K_2HPO_4) were selected to supplement relevant nutrients to the

BNS (in Supplementary Material (SM), Table S1). The market reference prices of chemicals were obtained from two large global suppliers (i.e., https://www.alibaba.com/ and https://www.ec21.com/). The selection of compounds followed two major principles: firstly, the dosage of each compound should not exceed water solubility after the final dilution to HNS; secondly, the cheaper compounds according to the indicative market price should always be selected in priority, only using the relative expensive chemicals once other nutrient elements in the cheaper chemicals reached the required level. Based on the three types of pH control reagents used in phase VII, VIII, and IX, the nutrient balancing strategy was applied to three organic scenarios (i.e., NaOH, $Ca(OH)_2$, and $Mg(OH)_2$). Subsequently, the concentration of nonessential element sodium (Na) and the electric conductivity (EC) in balanced organic scenarios were checked.

Following the strategy to prepare HNS from the BNS organic scenarios, two types of commercially available inorganic fertilizers, YaraTera Kristalon Scarlet and Floraflex B1™/B2™ (composition and price in SM Table S2), were used to design the HNS as commercial scenarios for the convenience of further cost comparison with organic scenarios. Besides, HNS composed of individual chemical compounds was set as a control scenario.

The OPEX was calculated based on the cost of CAB, commercial hydroponic fertilizers, supplemented chemical compounds, the aeration for COD removal and nitrification, and the saved cost of CO_2 production for CO_2 fertilization. The price for CAB was 1 € g^{-1} dry matter, communicated by the company Avecom. The O_2 consumption for complete nitrification was assumed to be 4.57 g g^{-1} NO_3^--N (Metcalf & Eddy, 1991). The average O_2 transfer efficiency and electricity cost were taken as 2kg kWh^{-1} and 0.117€ kWh^{-1} (Eurostat, 2018; Roman & Mureşan, 2014).

3. Results and discussion

3.1 Batch test screen for optimal mineralization conditions

In the batch tests, the performance of inorganic nitrogen (i.e., NH_4^+-N, NO_2^--N, and NO_3^--N) release was employed to indicate the mineralization and nitrification of BAPN and CAB (Fig. 2). The first-order kinetics fitted the total inorganic nitrogen (TIN) releasing performance in all the batch tests ($R^2 \geq 0.92$), and k is the kinetic rate constant. In the anaerobic tests, only NH_4^+-N was detected as TIN. The mineralization performance of both BAPN and CAB at 35°C was better than at 20°C with higher rate constants and efficiencies (Fig. 2a and 2b). In the anaerobic tests at 35°C, the TIN releasing efficiency of CAB reached 43% in two days, which was much higher than BAPN with only 9% released at day 2. Based on the better mineralization performance at 35°C, aerobic tests were implemented at 35°C for BAPN and CAB. There was negligibly further improvement of mineralization in the BAPN batch (Fig. 2c). By contrast, the aerobic incubation further improved the mineralization efficiency of CAB to 91% and stabilized in 9 days. Therefore, CAB was more promising than BAPN as organic fertilizer in the bioconversion process. Fig. 2d showed the dynamic changes of NH_4^+-N, NO_2^--N, and NO_3^--N during the aerobic batch test of CAB. There was no NO_2^--N detected during the whole batch period. NH_4^+-N was seen in the first 5 days. Since day 6, only NO_3^--N could be detected in the reactor, indicating the nitrification process could be completed under aerobic mineralization. Moreover, the maximum and average nitrification rates of the CAB aerobic test were calculated based on the increase in TIN concentrations over the corresponding periods (the first 4 and 9 days), which were 59 and 38 mg N L^{-1} d^{-1}, respectively.

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

et al., 2014; Kraus et al., 2003) or by cellobiose from cellulose degradation (Duff & Murray, 1996). Besides the improvement via increasing temperature to 35°C, sufficient aeration could break the bottleneck of CAB hydrolysis. In our batch study, aerobic mineralization at 3±0.5 g O_2 L^{-1} substantially improved the NH_4^+-N released and completely converted the NH_4^+-N to NO_3^--N. Spanoghe et al. (2020) showed more than 70% of the organic nitrogen mineralized from CAB into ammonium and nitrate in 77 days. This prolonged mineralization process should be attributed to the poor incubation conditions via blending CAB with a commercial organic growing medium and storing it at a relative humidity of 75% and temperature of 16 °C.

3.2 Nitrate production in the mineralization and nitrification bioreactor

In the influent tank, TIN only accounted for 3±1% of TN (SM Fig. S1). The performance of NO_3^--N production in the bioreactor was divided into nine phases (I to IX, Fig. 3a). A new phase started when a parameter was changed to improve the performance of NO_3^--N production or balance the nutrient quality in BNS. In the bioreactor, the accumulation of NH_4^+-N and NO_2^--N was detected only for short periods at the beginning of phases I, III, and IV (Fig. 3a).

3.2.1 Performance of nitrate production under various controlling parameters

The bioreactor was started with an influent loading rate of 100 mg TN L^{-1} d^{-1} in phase I. The concentration of NO_3^--N in the bioreactor gradually increased and stabilized at 275±8 mg N L^{-1} with a conversion efficiency of 55±2% after three rounds of HRT (i.e., 15 days) (Fig. 3a). Under the same operational condition, doubling the loading rate to 200 mg TN L^{-1} d^{-1} in phase II (via increasing the influent concentration) further increased the NO_3^--N concentration. In this phase, the bioreactor obtained a stable NO_3^--N conversion efficiency of 54±2% after three rounds of HRT again, with 540±16 mg NO_3^--N L^{-1}. Due to the poor settleability of sludge with 6.5g 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 NO_3^--N ratio reached
307 around 3.2. The influent K content of 1581±49 mg L^{-1} could obtain a K to NO_3^--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 NO_3^--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 PO_4^{3-} concentration dropped

to 19 mg P L^{-1} (Fig. 3b). The P recovery efficiency in the effluent was less than 10%, much lower than previous phases with around 50% recovery. Presumably, the loss of PO_4^{3-} could be attributed to the chemical precipitation of calcium phosphates such as dicalcium phosphate ($CaHPO_4$) and tricalcium phosphate ($Ca_3(PO_4)_2$) at high calcium concentration (Carlsson, 1997). After replacing $Ca(OH)_2$ with $Mg(OH)_2$ as a pH control reagent, the PO_4^{3-} concentration in effluent gradually increased to 106±5 mg P L^{-1}, and its recovery efficiency reached around 50% again (Fig. 3b).

From phase I to III, stepwise increasing the TN loading rate could always obtain over 50% TIN production. The decreased NO_3^--N concentration (phase V) and the relatively low TIN releasing efficiency (phase VI-IX) compared to phase III were probably related to the influence of cations (e.g., K^+ and Na^+ from pH regents) on microbial activity and community. The nitrogen mineralization was suggested to be less tolerant to Na^+ than K^+ (Sindhu & Cornfield, 1967). Hence, the decreasing trend of NO_3^--N concentration in phase V was probably due to the switching of pH regent from KOH to NaOH.

Nevertheless, under the influent loading rate of 400 mg TN L^{-1} d^{-1} at 5-day HRT and SRT, NO_3^--N production efficiency and rate could stabilize in a range of 44-53% and 175-212 mg N L^{-1} d^{-1}, respectively. The NO_3^--N production rate in the bioreactor was significantly improved over the long-term operation, compared to the CAB aerobic batch test of 59 mg N L^{-1} d^{-1} (section 3.1). By contrast, mineralization rates of organic fertilizers in soils or growing media were considerably slower (SM, Table S4). Laboratory incubation of common organic fertilizers (e.g., thermally-dried biosolids, manures, and composts) in soils only obtained the nitrogen mineralization rates in a range of 0.13-1.88 mg N L^{-1} d^{-1}) (Lazicki et al., 2020; Masunga et al., 2016; Smith & Durham, 2002). In most cases, even if under these low rates, complete nitrification could not be guaranteed.

Previous mineralization tests for CAB realized complete nitrification only at a rate of 5.12 mg N L^{-1} d^{-1} in organic growing media (Spanoghe et al., 2020). These slow nitrogen transformation rates should mainly be attributed to the relatively low incubation temperature (16-25°C) as well as water and oxygen availability (Geisseler et al., 2019; Rigby et al., 2016). Similarly, the carbon mineralization rates in soil incubations were only 36-158 mg CO_2-C L^{-1} d^{-1} (Guo et al., 2019; Ribeiro et al., 2010). Assuming the microbial cell formula is $C_5H_7O_2N$, the corresponding carbon mineralization rates of CAB were 750-909 mg CO_2-C L^{-1} d^{-1} during the NO_3^--N production (DİNÇER & ARAL, 2020). Therefore, the proposed well-controlled bioreactor approach produced liquid nitrate-rich fertilizer more rapidly than was realized in soils. Additionally, the performance of SCOD removal was excellent over the whole operation period, especially the phase V-IX with above 90% removal (SM Fig. S2), which could benefit the hydroponic cultivation by decreasing the risk of the proliferation of heterotrophic pathogens and biofilms (Lee et al., 2015; Seo et al., 2017).

3.2.2 The microbial community in the bioreactor

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

NaOH, $Ca(OH)_2$, and $Mg(OH)_2$ scenario, respectively, and the salinity of HNS was 4.4, 3.0, and 2.8 mS cm^{-1}. Since the allowable nonessential element sodium and the salinity should not exceed 180 mg Na L^{-1} and 4 mS cm^{-1} (Jones Jr, 2014; Sonneveld & Voogt, 2009), the NaOH scenario was evidently out of the ranges.

The Sankey diagrams based on the nutrient mass flow in each section of the $Ca(OH)_2$ and $Mg(OH)_2$ organic scenarios are shown in Fig. 5b and 5c. In the bioreactor section, the observed accumulative dosing of $Ca(OH)_2$ and $Mg(OH)_2$ until the sampling time (around 14 days) introduced 1656 mg Ca and 552 mg Mg into 1 L bioreactor content, respectively. As a result of the membrane filtration, nutrients are separated into liquid and solid fractions. A share of 57.5% or 1150 mg TN was retained in the solid fraction (i.e., retentate), which can be applied to land as a fertilizer. According to the typical microfiltration process, the volume ratio of permeate and primary flow was assumed to be 0.98 (Metcalf & Eddy, 1991). After the membrane section, soluble nutrients could be recovered in 0.98 L permeate as the BNS. In the nutrient balancing section, element K was the limiting factor of $Ca(OH)_2$ scenario, and extra NO_3^--N (67 mg), P (117 mg), Ca (136 mg), and Mg (164 mg) should be supplemented into the BNS tank (Fig. 5b). Eventually, after 4.5 times of dilution by H_2O, 4.38 L HNS could be obtained in the $Ca(OH)_2$ scenario, and the NO_3^--N sourced from CAB mineralization accounted for 92.7% of NO_3^--N in HNS. While in the $Mg(OH)_2$ scenario, element Mg was the limiting factor, and the additional NO_3^--N (1427 mg), P (231 mg), K (1524 mg), and Ca (2129 mg) were supplemented (Fig. 5c). After 11.1 times dilution, 10.88L HNS could be obtained for the $Mg(OH)_2$ scenario. Compared to the $Ca(OH)_2$ scenario, the proportion of organic-sourced NO_3^--N (i.e., NO_3^--N_{OG}) in HNS of $Mg(OH)_2$ scenario was much lower (only 37.4%).

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

to obtain 1 L BNS. In the $Ca(OH)_2$ organic scenario, there was roughly 0.19 g NO_3^--N_{OG} in HNS. Calculated from the stoichiometry of nitrification (i.e., 4.57g O_2 g^{-1} N), 0.9 g O_2 L^{-1} HNS was needed. Besides, the O_2 consumption for TCOD removal was around 14.9 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 efficiency and electricity cost, the total aeration cost of 2.5×10^{-4} € L^{-1} HNS could be calculated. According to the microbial cell formula of $C_5H_7O_2N$, the aerobic mineralization of CAB was assumed to release CO_2 of 15.71 g g^{-1} NO_3^--N_{OG} (DİNÇER & ARAL, 2020). Therefore, around 3.1g CO_2 L^{-1} HNS could be produced in the $Ca(OH)_2$ scenario. Since the market price of liquid CO_2 is in a range of 0.08-0.15 € kg^{-1} (Mikunda et al., 2015), the HNS produced in the $Ca(OH)_2$ scenario could save a maximum of 4.6 $\times10^{-4}$ € L^{-1} for the CO_2 fertilization. As a result of the lower NO_3^--N_{OG} concentration in the $Mg(OH)_2$ organic scenario (around 0.08 g L^{-1} HNS), the total aeration cost and CO_2 fertilization saving were only 1.0×10^{-4} and 1.8×10^{-4} € L^{-1} HNS, respectively.

Among the three cost categories, the cost of CAB dominated the OPEX of the organic scenarios, while chemical costs were four to seven orders of magnitude lower than that of fertilizers for either of the suppliers investigated (SM Table S3). The OPEX of commercial scenarios only included the costs of fertilizer and supplemented compounds. As a result, the OPEX of YaraTrea and FloraFlex was 1×10^{-3} and 34×10^{-3} € L^{-1} HNS, respectively. The cost of the control scenario was the lowest ($1.7\text{-}1.8\times10^{-8}$ € L^{-1} HNS), but individually purchasing every chemical compound is not practically feasible.

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

4. Conclusion

This study delivered a proof of concept for an approach based mainly on a bioreactor and a nutrient balancing step to produce a nitrate-rich liquid fertilizer from a solid organic fertilizer. Batch tests showed that the best operating condition was aerobic conversion at 35°C. The nitrification performance of the microbial fertilizer could reach an efficiency above 90% and a maximum rate of 59 mg N L^{-1} d^{-1}, while completely oxidizing the released NH_4^+-N to NO_3^--N. Subsequently, 35°C was chosen to operate a bioreactor, reaching a nitrate production rate of 212 mg N L^{-1} d^{-1}, yet at a lower efficiency of 51%. A nutrient balance estimation to simulate the Hoagland nutrient solution showed a higher proportion of NO_3^--N (92.7%) in the $Ca(OH)_2$ scenario was organically sourced. The CO_2 generated in the bioreactor can be used for greenhouse CO_2 fertilization to cut the OPEX. The Hoagland solution produced from the microbial fertilizer is potentially cost-competitive compared to the commercially available inorganic fertilizer nutrient solution (2.2×10^{-3} versus 34×10^{-3} € L^{-1} in total OPEX).

Supplementary Material

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

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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.

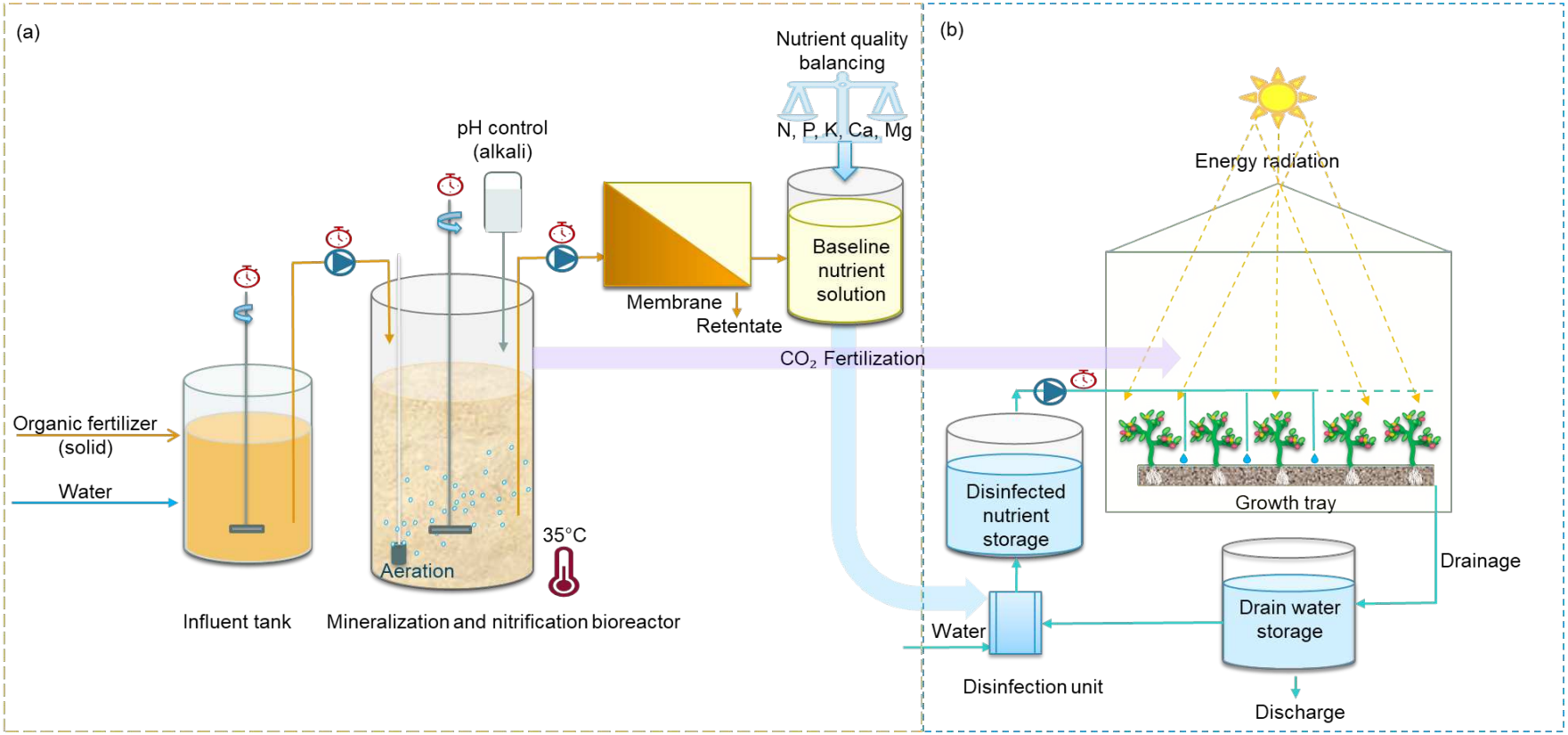


Figure 2. Performance evaluation of batch tests; anaerobic mineralization with ADS at 20°C and 35°C (a) BAPN and (b) CAB; (c) aerobic mineralization with AS at 35°C on BAPN and CAB; (d) dynamic changes of specific inorganic nitrogen during "AS-35°C-CAB" test. "k" is the kinetic rate constant. "EFF" means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

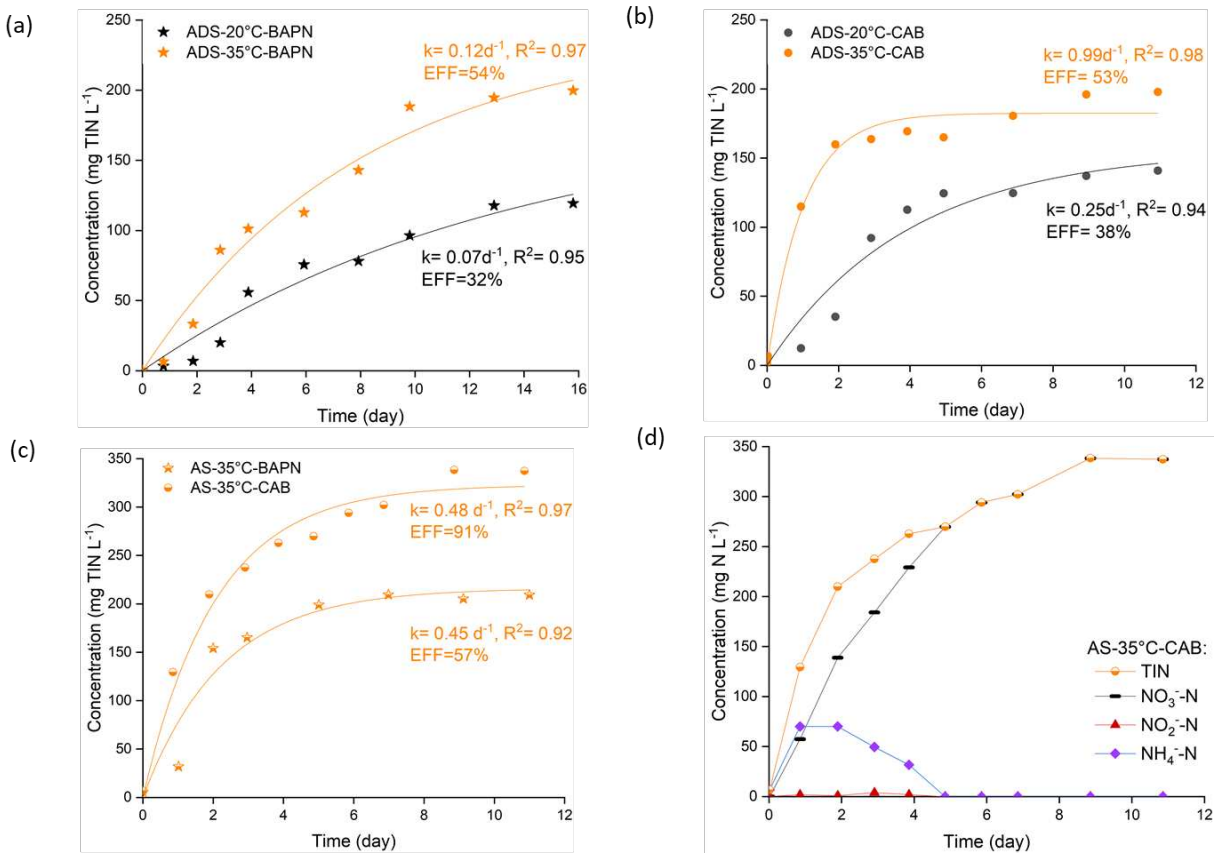


Figure 3. The profile of the inorganic nitrogen conversion (a) and phosphate recovery (b) in the effluent of mineralization and nitrification bioreactor from day 0 to day 170. The major variables are shown on top. TIN conversion efficiency and P recovery efficiency represent the concentration ratios of TIN and PO_4^{3-}-P in the effluent to TN and TP in the influent, respectively.

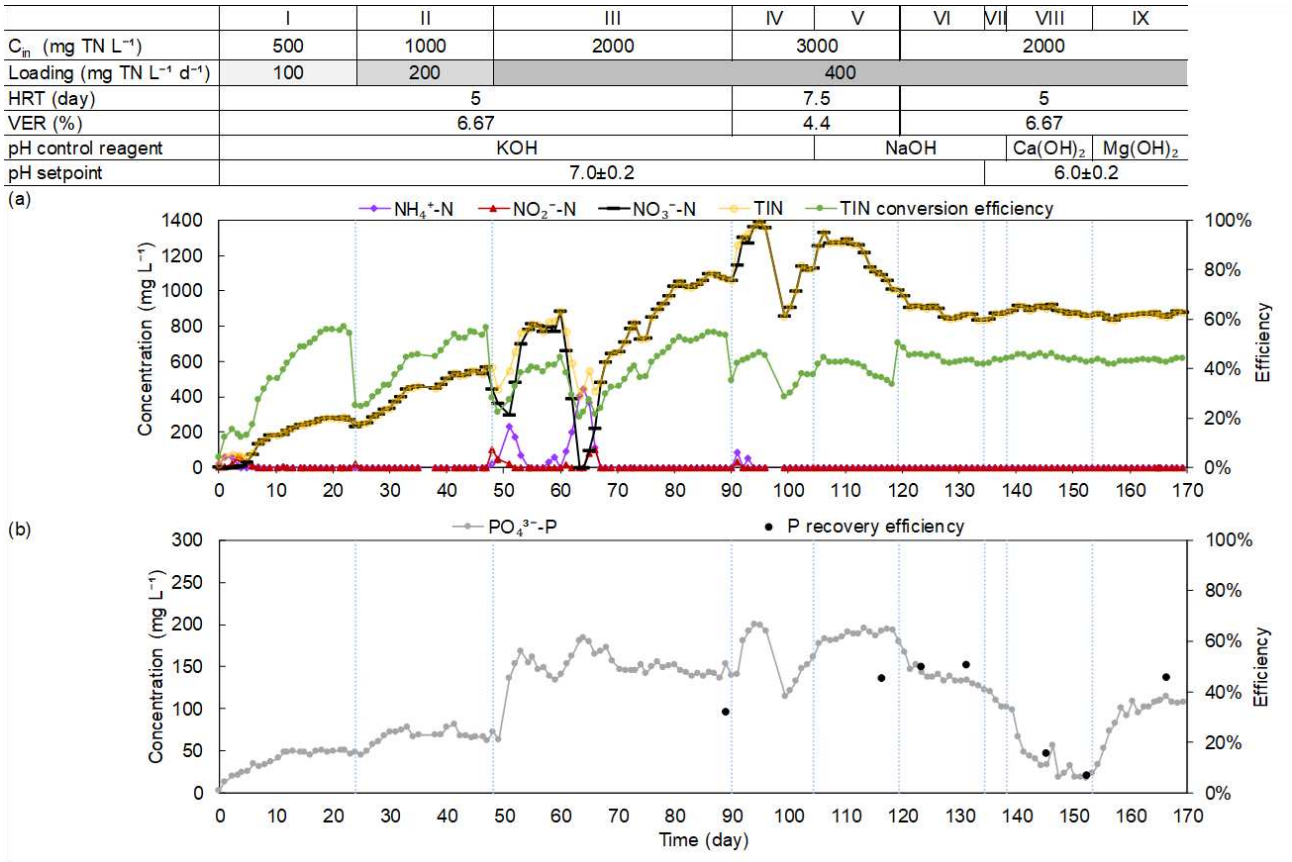
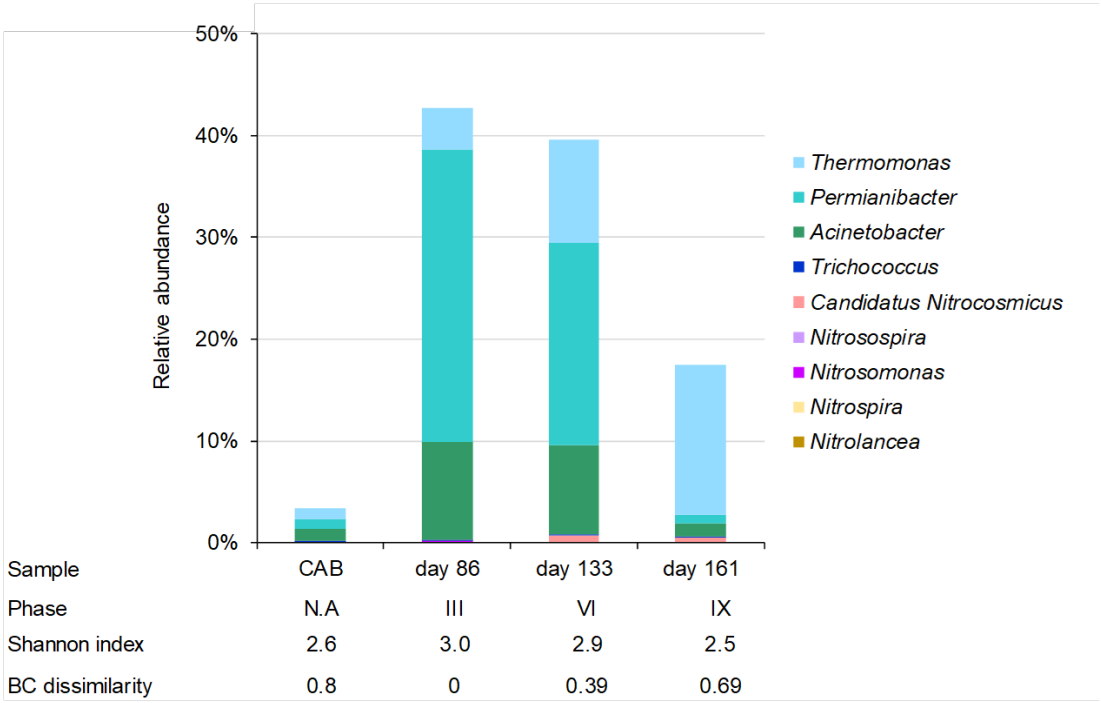
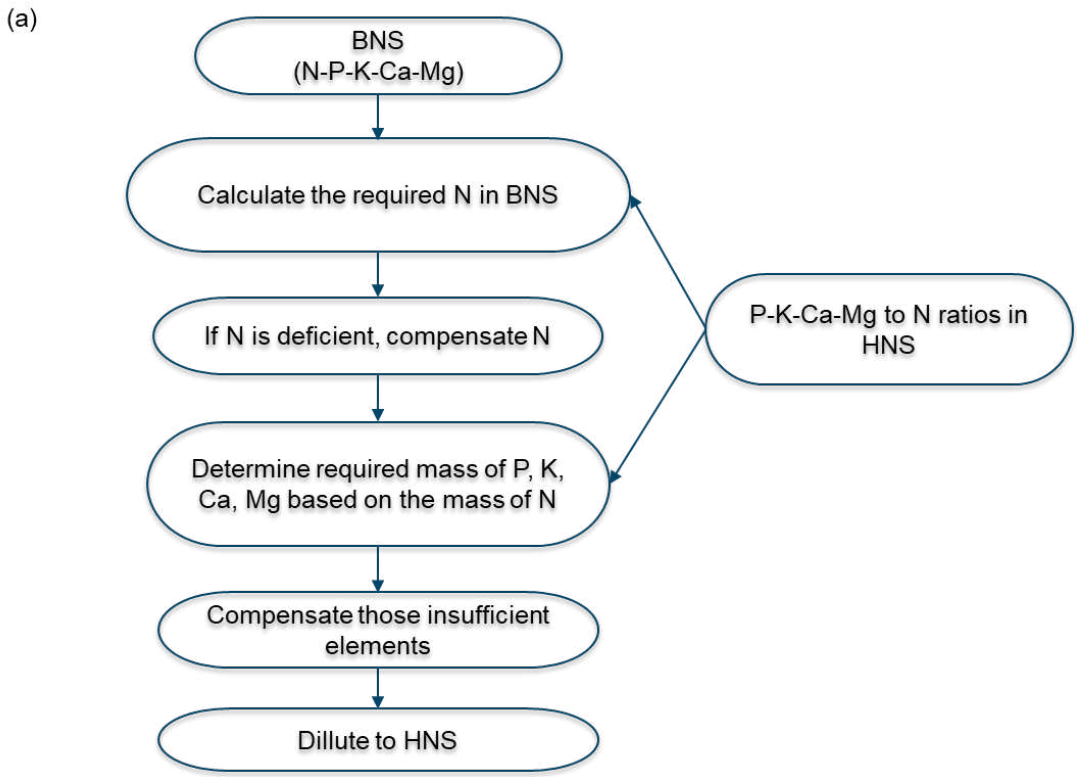


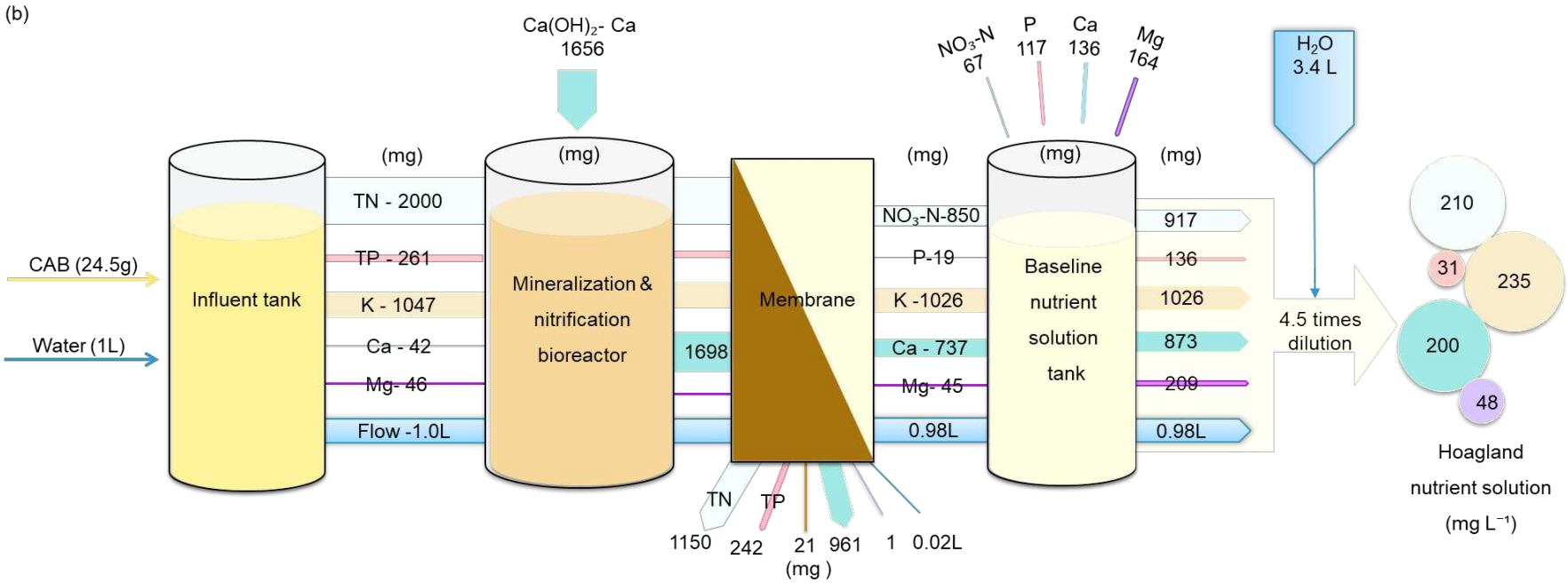
Figure 4. The relative abundance of microbes in the mineralization and nitrification bioreactor at genus levels. CAB means the influent composed of CAB. BC dissimilarity represents the Bray-Curtis dissimilarity between day 86 and each other sample.



Sample	CAB	day 86	day 133	day 161
Phase	N.A	III	VI	IX
Shannon index	2.6	3.0	2.9	2.5
BC dissimilarity	0.8	0	0.39	0.69

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 $Ca(OH)_2$ organic scenario, (c) the $Mg(OH)_2$ organic scenario. TN and TP mean the total nitrogen and total phosphorus, respectively.




(b)
Ca(OH)2- Ca
1656
NO3-N
67
P
117
Ca
136
Mg
164
H2O
3.4 L
(mg)
(mg)
(mg)
(mg)
(mg)
CAB (24.5g)
Water (1L)
Influent tank
TN - 2000
TP - 261
K - 1047
Ca - 42
Mg- 46
Flow -1.0L
Mineralization & nitrification bioreactor
1698
Membrane
NO3-N-850
P-19
K -1026
Ca - 737
Mg- 45
0.98L
Baseline nutrient solution tank
917
136
1026
873
209
0.98L
4.5 times dilution
210
31
235
200
48
Hoagland nutrient solution (mg L⁻¹)
TN
TP
1150
242
21
961
1
0.02L
(mg)

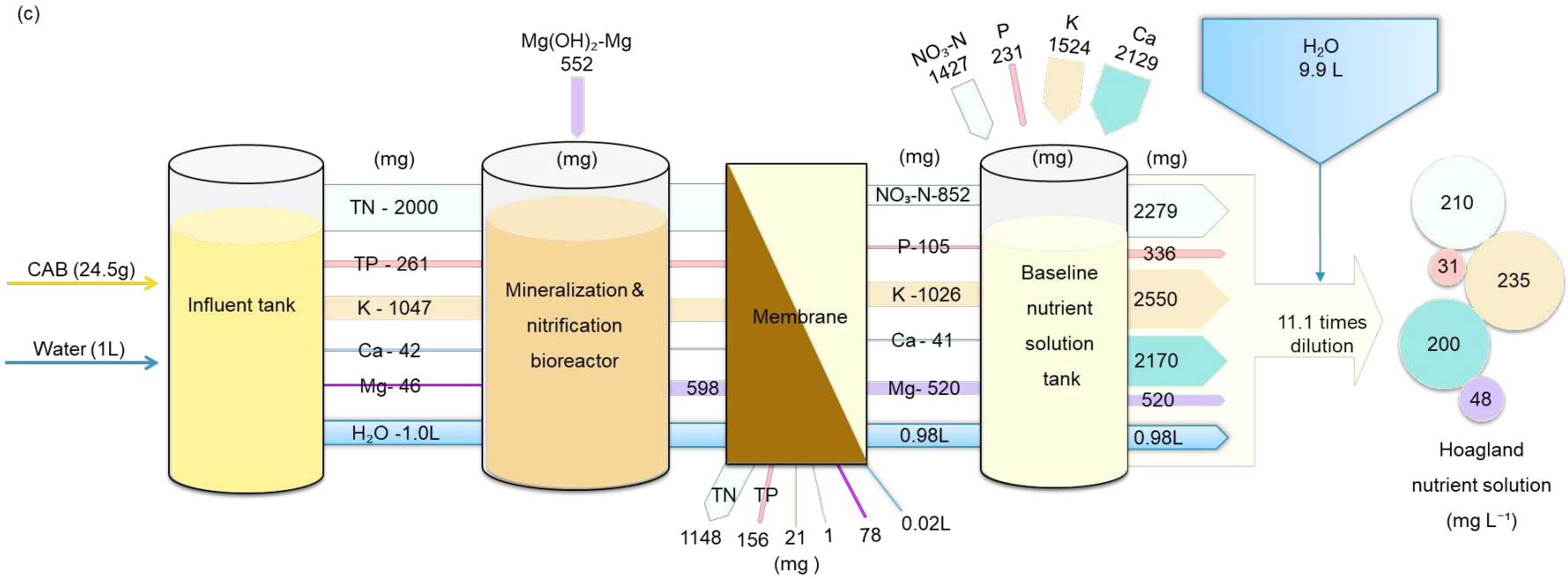

(c)
Mg(OH)2-Mg
552
NO3-N
1427
P
231
K
1524
Ca
2129
H2O
9.9 L
(mg)
(mg)
(mg)
(mg)
(mg)
CAB (24.5g)
Water (1L)
Influent tank
TN - 2000
TP - 261
K - 1047
Ca - 42
Mg- 46
H2O -1.0L
Mineralization &
nitrification
bioreactor
598
Membrane
NO3-N-852
P-105
K -1026
Ca - 41
Mg- 520
0.98L
Baseline
nutrient
solution
tank
2279
336
2550
2170
520
0.98L
11.1 times
dilution
210
31
235
200
48
Hoagland
nutrient solution
(mg L-1)
TN
TP
1148
156
21
1
78
0.02L
(mg)

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		N.A.	
	g O_2 L^{-1} HNS (nitrification)	0.9	0.4			
	g O_2 L^{-1} HNS (COD removal)	3.3	1.3			
	€ 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	€ L^{-}HNS	5.4×10^{-3}	2.2×10^{-3}	1.0×10^{-3}	3.4×10^{-3}	1.8×10^{-8}/ 1.7×10^{-8}