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Influence of pH on the performance of anaerobic piggery wastewater treatment coupled with membrane-based NH_3 extraction

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ABSTRACT

The influence of the pH of piggery wastewater (PWW) on both ammonia recovery and anaerobic digestion performance during PWW treatment was evaluated in a continuous stirred tank reactor coupled with a membrane-based extraction module. The anaerobic digester was operated at a hydraulic retention time of 20 days at 37 °C, while the flat sheet PTFE membrane module operated continuously at liquid recirculation rates of 250 mL min^{-1}. The membrane module was able to gradually decrease the total ammoniacal concentration from 1.27 ± 0.01 to 0.62 ± 0.01 g L^{-1} after 360 days of operation. Membrane-based NH_3 extraction induced a CH_4 yield increase of 1.3-fold. Moreover, COD and VS removal efficiencies increased up to 1.2-fold and 1.5-fold, respectively, along with the increase in PWW pH from 7.5 to 12. Total VFAs removal efficiencies were higher at a PWW pH of 9.

1. Introduction

Piggery wastewater (PWW) is composed of liquid and solid swine excrements combined with water from rain, barn cleaning and water troughs, and fodder leftovers. Spain is the first European country in terms of pig farming, with more than 34 million heads per year. PWW production in Spain is estimated to be over 86 million m^3/year and represents nowadays a serious environmental problem [1,2]. While PWW has been traditionally considered a sustainable fertilizer, areas with intensive pig farming typically experience a limitation in cultivation lands to apply this PWW [3]. In this context, the high cost of PWW transportation restricts the use of PWW as a biofertilizer to the crop lands nearby the pig farms [4]. In addition, the direct spreading of PWW as a biofertilizer entails the loss of the energy contained in the residual organic matter and pernicious NH_3 emissions to the atmosphere.

Anaerobic digestion (AD) is a cost-effective technology to manage swine manure [5]. AD supports the biological conversion of residual organic matter into a methane-rich biogas and a nutrient-rich digestate in the absence of oxygen and nitrate/nitrite [6]. Biogas production from PWW entails a reduction in fossil fuel consumption in farms, which is crucial to climate change mitigation and to the economic sustainability of pig farming [7]. However, AD is not capable of removing significant concentrations of nitrogen and phosphorus from swine manure and gets partially inhibited at high NH_3 concentrations and pH values [8,9]. Indeed, total ammoniacal nitrogen concentrations ranging between 1700 and 14,000 mg TAN L^{-1} can cause a 50 % reduction in methane production during AD. Similarly, over 400 mg NH_3-N L^{-1} have been reported to cause inhibition issues during AD [8–11].

Nowadays, there are several technologies available, either at commercial scale or under investigation at laboratory/pilot scale, to reduce nitrogen concentrations from livestock wastewaters such as electrochemical cells, stripping, denitrification-nitrification, ion exchange, zeolite adsorption, partial nitritation-anammox, gas-permeable membranes, etc. [12,13]. Gas-permeable membranes can mediate the extraction of ammonia from PWW through a hydrophobic membrane in contact with an acidic solution that retains NH_3. The most common acids used in this process are H_2SO_4, H_3PO_4 and HNO_3, which can generate chemical fertilizers such as ammonium sulphate, ammonium phosphate and ammonium nitrate [14–16]. This process does not require high operational pressures, a pretreatment of the swine manure or a high consumption of energy. Therefore, gas-permeable membranes are a promising alternative for the cost-efficient and sustainable recovery of

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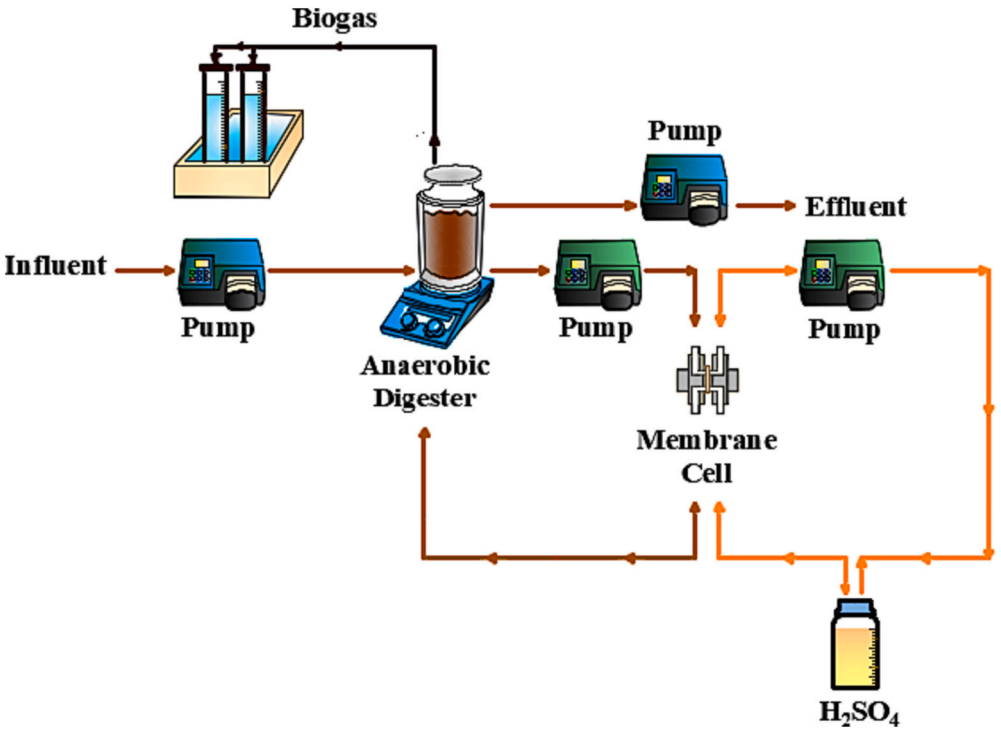

Fig. 1. Schematic diagram of the experimental anaerobic CSTR coupled to a membrane-based ammonia extraction process.

NH_3 from PWW, which is aligned with the new paradigm of circular bioeconomy. In addition, in-situ membrane-based NH_3 extraction can improve the performance of AD by in-situ extracting the NH_3 from the anaerobic broth [17].

One of the main operational parameters for the optimization of membrane-based NH_3 extraction processes is pH, which determines the share of NH_3 and NH_4^+ concentrations in PWW and therefore its mass transfer across the membrane. NH_4^+ ions are dissociated to form free ammonia and hydrogen under basic conditions. Thus, increasing the pH values in the PWW fed to the anaerobic digester can boost membrane-based NH_3 removal by shifting the NH_4^+/NH_3 equilibrium towards NH_3 [18]. Unfortunately, the influence of pH in PWW on the performance of membrane-based NH_3 extraction and organic matter conversion to methane has never been systematically assessed.

In this work, the performance of a continuous anaerobic reactor coupled with an external flat sheet PTFE hydrophobic membrane module was investigated during the anaerobic treatment of PWW with increasing pH values using NaOH. The influence of pH (7.7, 9, 10, 11 and 12) on TAN concentration, methane productivity yields and organic matter removal was systematically assessed for 360 days.

2. Materials and methods

2.1. Piggery wastewater and inoculum

Fresh PWW was collected from a nearby swine farm (Segovia, Spain) and stored at 4 °C prior to use (for no longer than 30 days). The average composition of the PWW was pH 7.7 ± 0.1, 65.2 ± 6.5 g COD/L (Chemical Oxygen Demand), 1.4 ± 0.1 g NH_4^+/L, 5.4 ± 0.1 g TKN L^{-1}, 35.1 ± 2.8 g TS/L and 25.1 ± 1.9 g VS/L. Digestate from an anaerobic continuously stirred tank reactor (CSTR) treating PWW was used as inoculum [19]. The composition of the anaerobic inoculum was pH 8.21 ± 0.01, 21.6 ± 0.6 g COD/L, 1.14 ± 0.02 g NH_3/L, 3.8 ± 0.1 g TKN L^{-1}, 23.8 ± 0.3 g TS/L and 13.2 ± 0.6 g VS/L.

2.2. Experimental set-up

The experimental set-up consisted of a 3 L CSTR magnetically stirred at 180 rpm and located in a room with controlled temperature of 37 °C (Fig. 1). A peristaltic pump (Watson Marlow 520, Spirax-Sarco Engineering plc, UK) tangentially recirculated at 250 mL min^{-1} the anaerobic culture broth from the CSTR over the active layer of a 44 cm^2 rectangular membrane cell with a hydrophobic PTFE flat sheet membrane, with pore size 0.22 μm, a nominal thickness of 175 μm, 70 % porosity, and a contact angle of 150 θ according to Rivera and coworkers [20]. The receiving solution of the extracted ammonia was sulfuric acid at 1 M, which was tangentially recirculated in the support layer of the membrane at 250 mL min^{-1} using a peristaltic pump (Watson Marlow 520, Spirax-Sarco Engineering plc, UK). A Watson–Marlow Sci-Q 323 peristaltic pump (Spirax–Sarco Engineering plc, UK) was also used to daily fed the 150 mL of fresh PWW and withdraw the same volume of anaerobic cultivation broth, which entailed a hydraulic retention time (HRT) of 20 days and a solid retention time (SRT) of 26 days.

2.3. Influence of the pH of the PWW on the NH_3 extraction process and AD performance

The experimental set-up was operated for 360 days under seven operational stages at increasing pH values in the PWW fed to the CSTR. The bioreactor was inoculated with 3 L of digestate from an anaerobic CSTR coupled with a NH_3-extraction module treating PWW [19]. Stage 1 was operated for 63 days at a HRT of 20 days without membrane-based NH_3 extraction. Stage 2 involved the continuous operation for 123 days of a PTFE flat sheet membrane module (44 cm^2 rectangular cell) coupled to the CSTR via anaerobic broth recirculation at 0.25 L min^{-1} using a 1 M H_2SO_4 solution to capture the dissolved NH_3. Process operation in stage 3, 4, 5 and 6 was similar to that in stage 2, but the pH of the PWW fed to the CSTR was stepwise increased to 9, 10, 11 and 12, respectively, via NaOH addition. The duration of these operational stages was 20, 40, 43 and 45 days, respectively. At the beginning of stage 7, NH_3 concentration in the anaerobic broth was increased using 7.5 g L^{-1} of NH_4Cl in order to achieve a total ammoniacal concentration of 1.2 g L^{-1} (values

observed in stage 2) and the operation of the membrane module was shut down in order to assess any potential NH_3-mediated inhibitory effect. The PTFE membrane was replaced every ≈ 25 days to guarantee an effective NH_3 extraction process due its gradual fouling. Liquid samples of 150 mL from the influent PWW and effluent of the CSTR were drawn twice a week to monitor the pH and temperature, and the concentrations of TAN, total Kjeldahl nitrogen (TKN), total nitrogen (TN), total chemical oxygen demand (COD), total and volatile solids (TS, VS), total organic and inorganic carbon (TOC, IC), volatile fatty acids (VFAs), NO_2^-, NO_3^-, PO_4^{-3} and SO_4^{-2}. The composition and production of biogas were also daily recorded. Samples of anaerobic broth of 15 mL were taken at the end of each operational stage and preserved at −20 °C in order to determine the structure of the bacterial and archaeal community.

2.4. Analytical methods

A 100 μL gas-tight syringe (Hamilton, 1710 SL SYR, USA) was used to determine biogas composition (CO_2, H_2S, O_2, N_2 and CH_4) using a gas chromatograph with a thermal conductivity detector (Varian CP-3800, USA). The GC-TCD was equipped with CP-Molsieve 5 A (15 m × 0.53 mm × 15 μm) and CP-PoraBOND Q capillary columns (25 m × 0.53 mm × 10 μm). The carrier gas was ultra-pure helium at 0.013 L min^{-1}. Dissolved total ammoniacal nitrogen was measured using the Nessler analytical method at 425 nm wavelength in a SPECTROstar Nano Absorbance Reader spectrophotometer (BMG LABTECH, Germany). Temperature and pH were monitored using a Basic 20 pH meter with a 50 14 T electrode (Crison Instruments, S.A., Spain). The concentrations of TOC, IC and TN were measured in a Shimadzu TOC-VCSH analyzer (Shimadzu, Japan) equipped with a TNM-1 chemiluminescence module. Concentrations of COD, TKN, TS and VS were analyzed according to Standard Methods for examination of water and wastewater (APHA, 2005). VFAs concentrations were determined in an Agilent 7820A GC-FID (Agilent Technologies, USA) equipped with a G4513A autosampler and a TEKNOKROMA NF29370-F packed column (2 m × 1/8″ × 2.1 mm) (Teknokroma, Spain) [21]. Cl^-, NO_2^-, NO_3^-, PO_4^{-3} and SO_4^{-2} concentrations were analyzed by high-performance liquid chromatography-ion conductivity (HPLC-IC) with a Waters 515 HPLC pump coupled to a Waters 432 conductivity detector and equipped with a Waters IC-Pak Anion HC column (150 mm × 4.6 mm) [22].

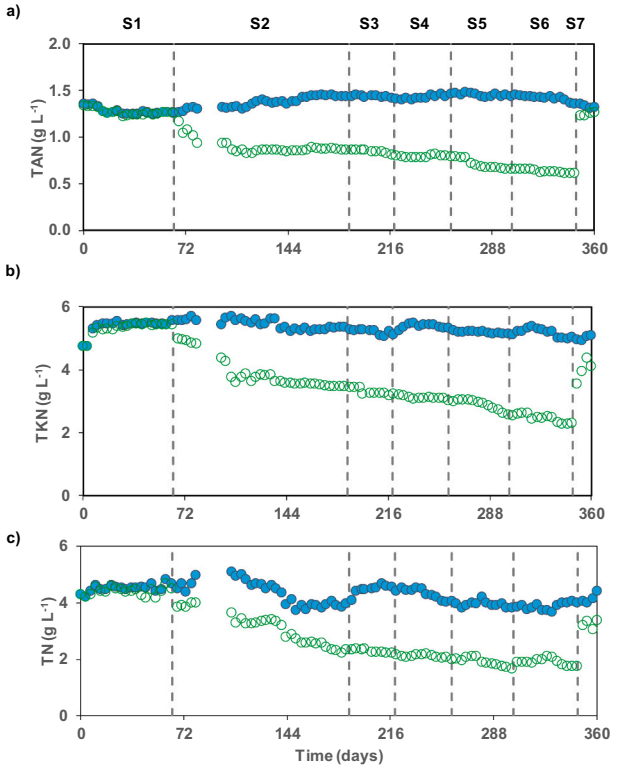

Fig. 2. Time course of the TAN (a), TKN (b) and TN (c) concentrations in the influent PWW (●) and anaerobic effluent (○) during the seven operational stages.

Sequencing and bioinformatic analyses of the bacterial and archaeal communities' structure was carried out by BIOPOLIS Science (ADM, Spain). The amplification of 16S ribosomal RNA gene (16S rRNA) was conducted by amplification of hypervariable region V3-V4 using the oligonucleotides 341F-805R for *Bacteria* and oligonucleotides combination 344F-1041R and 519F-806R for *Archaea* [23]. The libraries of 16S rRNA were sequenced using a MiSeq sequencer (Illumina, USA) according to the manufacturer's protocol 15,044,223 B [24]. Chimeric and denoising depletion were performed using the software DADA2 pipeline [25]. Clean amplicon sequencing variants were annotated using NCBI 16S rRNA database, while SILVA database (version 138) was used for amplicon sequencing variants assigned with less than 97 % identity.

3. Results and discussion

3.1. Influence of the influent PWW pH on nitrogen removal

The pH in the influent PWW and anaerobic broth is a key parameter influencing the process of NH_3 extraction with membranes and the microbiology of PWW degradation. During the stabilization of the bioreactor in the absence of membrane operation and adjustment of the PWW pH, the pH of the anaerobic broth remained constant at 8.30 ± 0.04. However, when the membrane module was interconnected, the pH of the anaerobic broth tended to decrease to 8.05 ± 0.05. PWW is a slightly basic solution supporting the occurrence of free NH_3 and H^+ when NH_4^+ dissociates, where this equilibrium depends on the temperature and the pH of the aqueous matrix. This initial acidification of the anaerobic broth, which was also recorded immediately after the periodic membrane replacement, can be mainly attributed to the active diffusion of H^+ across the PTFE membrane [26]. This H^+ diffusion was gradually hindered by membrane fouling and occurred regardless of the influent PWW pH. The pH in the anaerobic broth gradually increased from steady state values of 8.3 ± 0.1 in stage 2 to 8.38 ± 0.1 in stage 5. Interestingly, when the pH of the influent PWW increased from 11 to 12 in stages 5 and 6, the pH in the CSTR remained at 8.49 ± 0.08. and 8.49 ± 0.02, respectively. This highlights the high buffer capacity of the anaerobic digestion process. In this context, Zhang and co-workers analyzed the correlation between NaOH addition, salinity and pH in PWW due to the key influence of pH on the performance of biological treatment and ammonia dissociation [27]. The authors concluded that PWW typically exhibits a high buffer capacity as a result of its high alkalinity concentration (7000 mg$CaCO_3$/L) compared to other types of wastewaters [28].

Inhibition of the AD treatment of livestock wastewaters is typically caused by their high total ammoniacal concentrations. However, it also depends on the pH, temperature, organic substrates and type of microorganisms [11]. Reducing ammonia levels in the anaerobic broth below inhibitory concentrations enhances AD performance, which ultimately entails higher COD and VS removals, and therefore productivities of biogas [19,29]. In this context, Hejnfelt and Angelidaki (2009) reported that TAN concentrations of 1500–7000 mg N L^{-1} can inhibit the AD process [30]. pH and temperature are also crucial environmental factors during ammonia extraction in membrane-based processes since both parameters govern the mass transfer of NH_3 throughout the membrane. The increase in pH in the PWW feed caused a positive effect on ammonia removal during the continuous mesophilic treatment of PWW carried out in this work. Hence, steady state TAN concentrations decreased from 1.3 ± 0.1 mg N L^{-1} in the absence of membrane extraction (stage 1) to 0.9 ± 0.1 mg N L^{-1} in stage 2. The increase in the pH of the influent

PWW to 9, 10, 11 and 12 resulted in steady state total ammoniacal concentrations of 0.83 ± 0.02, 0.81 ± 0.01, 0.67 ± 0.01 and 0.62 ± 0.01 mg N L^{-1}, respectively. Therefore, TAN removal efficiencies at an inlet pH of 12 was ~1.4 – fold higher than at pH of 7.7 ± 0.1 (39.7 % ± 0.2 % versus 55.5 % ± 0.5 %, respectively). These findings confirmed that hydrophobic membranes effectively removed ammoniacal nitrogen from anaerobic cultivation broths. Similarly, concentrations of TKN and TN of 5.42 ± 0.01 and 4.5 ± 0.1 gN L^{-1} were recorded under steady state in the absence of NH_3 extraction. Process operation with the membrane module and no pH control induced a decrease in TKN and TN concentrations to 3.46 ± 0.01 and 2.3 ± 0.1 gN L^{-1}. Steady state concentrations during process operation with a PWW pH of 9, 10, 11 and 12 induced a decrease in TKN and TN concentrations to 3.22 ± 0.05 and 2.24 ± 0.01 gN L^{-1}, 3.09 ± 0.01 and 2.08 ± 0.01 gN L^{-1}, 2.63 ± 0.09 and 1.71 ± 0.05 gN L^{-1}, and 2.28 ± 0.01 and 1.77 ± 0.01 gN L^{-1}, respectively. These concentrations corresponded to TKN removals of 31.1 % ± 0.2 %, 36.9 % ± 1.9 %, 42.4 % ± 0.6 %, 48.9 % ± 1.5 % and 54.4 % ± 0.2 %, and TN removals of 41.34 % ± 0.8 %, 51.3 % ± 0.6 %, 49.2 % ± 1.1 %, 55.8 % ± 0.6 % and 56.1 % ± 0.4 % in stages 2, 3, 4, 5, and 6, respectively. The significantly higher TKN and TN removals compared to TAN eliminations under steady state suggests that the implementation of an in-situ NH_3 extraction unit in the anaerobic CSTR promoted the ammonification of organic nitrogen in the anaerobic broth. By the end of stage 7, where the membrane module was shut down and the TAN concentration in the anaerobic broth was artificially increased via direct NH_4CL addition in the CSTR up to 1.26 ± 0.01 g N L^{-1}, TKN and TN concentrations of 4.1 ± 0.2 and 3.27 ± 0.17 gN L^{-1} were achieved (Fig. 2). This ammonia concentration increase hindered biogas production and induced VFAs accumulation, which resulted in the deterioration of the anaerobic digestion performance [31]. Previous studies with a similar experimental set-up reported a decrease of 1200 mg L^{-1} of TAN concentration [19]. Other studies with poultry manure as substrate in a leach-bed membrane integrated anaerobic system with ammonia extraction reported a capacity of TAN extraction of up to 2000 mg L^{-1} [32]. Recent studies by García-González and co-workers (2015) reported a 26 % TAN recovery in batch experiments using tubular gas permeable membranes for TAN capture in the absence of pH adjustments [26].

In this context, the molar fluxes of ammonia throughout the membrane module in stage 2 averaged 0.01 mol TAN m^{-2} h^{-1} and in stages 3 to 6 it accounted for 0.03 mol TAN m^{-2} h^{-1}. The main factors that affect the ammonia flux through the membrane are pH, membrane type and temperature, the latter determining ammonia's partial pressure. A previous study with a similar experimental set-up reported a comparable molar flux of 0.05 mol TAN m^{-2} h^{-1} [19]. Similarly, a recent study with membrane-based NH_3 extraction from poultry manure reported a molar flux of 0.07 mol TAN m^{-2} h^{-1} [31]. The fact that TAN fluxes did not increase linearly with the inlet pH of the PWW was mainly attributed to the membrane fouling under long-term operation, which hindered NH_3 permeation through the membrane, and to the high buffer capacity of the AD process, which resulted in moderate increases in the pH of the anaerobic broth at increasing PWW pH values. The rapid membrane fouling was evidenced by the short-term acidification of the anaerobic broth following membrane replacement. Membrane fouling is caused by deposition of microorganisms, or organic and inorganic materials on the membrane surface, and represents one of the major problems of membrane technology in biotechnological applications. This deposition ultimately entails pore blocking and a partial loss of membrane hydrophobicity, which decreases the efficiency of NH_3 extraction [33,34]. However, it is possible to restore membrane functionality to its optimal performance by applying both physical and chemical cleaning procedures [35,36].

3.2. Influence of the influent PWW pH on organic matter removal

The removal efficiencies of COD and VS under steady state in the absence of membrane-based TAN extraction accounted for 58.7 % ± 0.5 % and 36.1 % ± 2.6 %, respectively (Fig. 3). The implementation of the membrane module and the gradual increase in the pH of the inlet PWW via NaOH addition enhanced the removal efficiencies for both COD and VS. Thus, COD and VS removals of 64.1 % ± 0.6 % and 41.9 % ± 0.4 % were recorded during stage 2, 65.8 % ± 1.9 % and 42.4 % ± 0.5 % during stage 3 at pH 9, and 66.3 % ± 0.1 % and 59.2 % ± 1.7 % during stage 4 at pH 10. Those results agreed with those reported by Rivera an co-workers (2022) in a similar experimental set-up in the absence of pH control [19], where COD and SV removals of 61.8 % ± 1.3 % and 37.9 % ± 1.8 %, respectively, were recorded. Similar COD removals of 62 % were achieved by [37] in the absence of pH control with a gas permeable tubing located inside the anaerobic digestor under batch operation. Likewise, Molinuevo-Salces and co-workers (2018) achieved COD removal efficiencies of 68.8 % with pretreated PWW using tubular PTFE gas permeable membranes [38]. Outstanding results were achieved in this particular study at an inlet pH of 11, where COD was removed by 78.6 % ± 0.8 % and VS by 64.3 % ± 1.4 %. Interestingly, no significant improvements in COD and VS removal were achieved at pH 12 (76.2 % ± 0.3 % and 64.1 % ± 2.9 %, respectively). The improvement in COD and VS removal with increasing pH was likely mediated by the decrease in the ammonia concentration in the anaerobic broth, which reduces the inhibition and improves the microbial biodegradation performance. Moreover, the addition of NaOH and the inherent exposure of the PWW at pH 9, 11 and 12 likely caused a hydrolysis of organic matter. In this regard, a previous study of PWW pretreatments reported that the anaerobic biodegradability of PWW was enhanced by 78 % when using alkali as a pretreatment [39]. Finally, the removal efficiencies of COD and VS in the presence of TAN concentrations of 1.26 g N L^{-1} in the anaerobic broth under process operation with an influent PWW pH of 12 in the absence of membrane-based NH_3 extraction accounted for 49.4 % ± 0.3 % and 39.8 % ± 8.0 %, respectively. This finding confirmed the pernicious effects of ammonia during PWW AD and highlighted the need to implement in-situ NH_3 extraction strategies to enhance PWW treatment. Hence, Resch and coworkers (2011) reported an increase of 55 % in COD removal and an improvement in VFAs digestion when a reduction in TKN concentration by 47 % was achieved by NH_3 stripping in an AD plant treating animal-by products. However, in-situ NH_3 extraction should be carefully controlled since methanogenesis does not occur when ammonia concentrations remain in the range of 10–100 mg NH_4^+-N L^{-1}) [40,41].

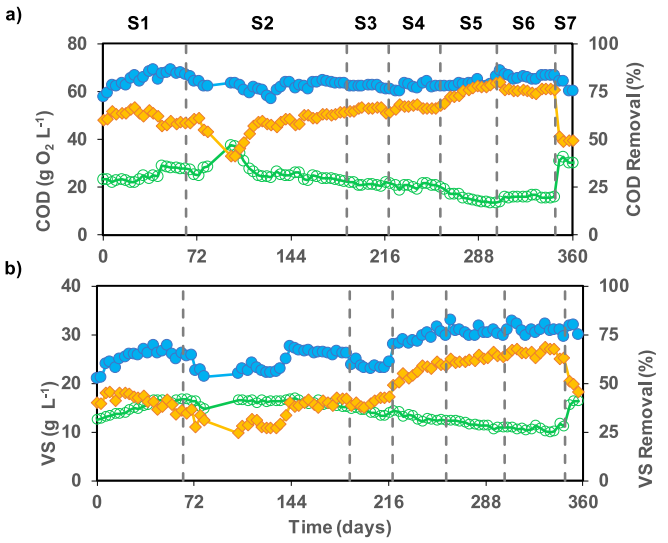

Fig. 3. Time course of the concentrations of COD (a) and VS (b) in the influent PWW (●) and anaerobic effluent (○), and their corresponding removals (◆) during the seven operational stages.

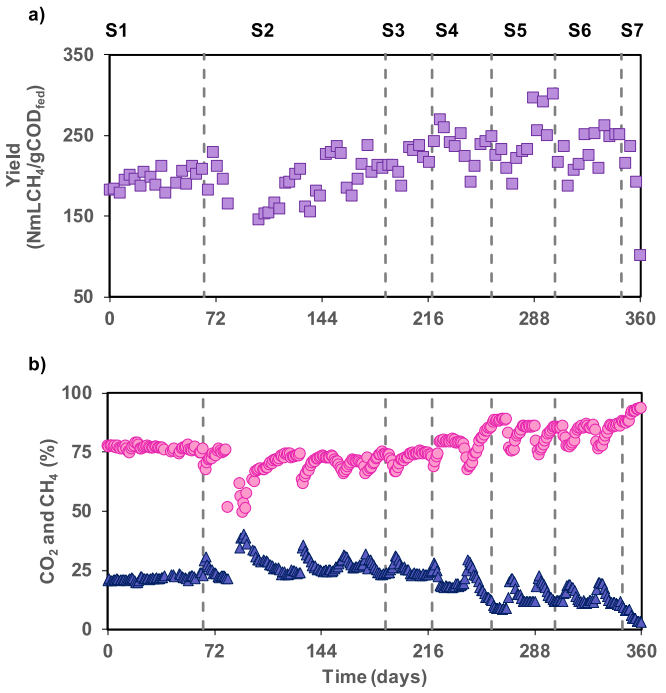

Fig. 4. Time course of the biogas yield (a) and concentrations of CO_2 (▲) and CH_4 (●) (b) in the biogas generated during the seven operational stages.

3.3. Influence of the influent PWW pH on biogas production and VFAs rate

A methane yield of 207.1 ± 4.5 $NmLCH_4$ g COD_{fed}^{-1} was recorded under steady state in the absence of membrane-based NH_3 extraction (Fig. 4a). The implementation of the membrane module did not significantly enhance methane yields, which remained at 209.1 ± 4.1 $NmLCH_4$ g COD_{fed}^{-1}. Methane yields at an influent PWW pH of 9, 10, 11 and 12, which corresponded to stages 3 to 6, averaged 225.5 ± 10.8, 231.0 ± 17.2, 274.6 ± 25.6 and 253.7 ± 6.1 $NmLCH_4$ g COD_{fed}^{-1}, respectively. The methane yield in stage 7, where the extraction unit was stopped and the ammonia concentration was artificially raised, averaged 146.4 ± 63.8 $NmLCH_4$ g COD_{fed}^{-1}. Thus, the highest methane yield in this study was reached in stage 5 when the pH was 11, where the pH in the cultivation broth averaged 8.5, which entailed an increase in the methane yield by 33 % compared to stage I. A recent study by Gonzalez-Garcia and co-workers (2021) compared the performance of two bioreactors, with and without membrane ammonia extraction unit using PWW as a feedstock, and reported an increase in the methane yield by 9 % when operating with the ammonia extraction unit [42]. Similarly, an increase in methane productivity by 13 % was recorded when using NaOH as a PWW pretreatment during batch biochemical methane production assays [39].

Biogas composition under steady state in the absence of membrane-based NH_3 extraction in terms of CO_2 and CH_4 content averaged 22.5 % ± 1.0 % and 76.1 % ± 1.1 % respectively (Fig. 4b). However, the implementation of the membrane module in stage I resulted in CO_2 and CH_4 concentrations of 26.5 % ± 2.8 % and 70.9 % ± 2.9 %, respectively. CO_2 concentrations at inlet pHs of 9, 10, 11 and 12 averaged 23.5 % ± 0.5, 18.6 % ± 5.0 %, 16.6 % ± 3.7 % and 13.2 % ± 3.2 %, respectively,

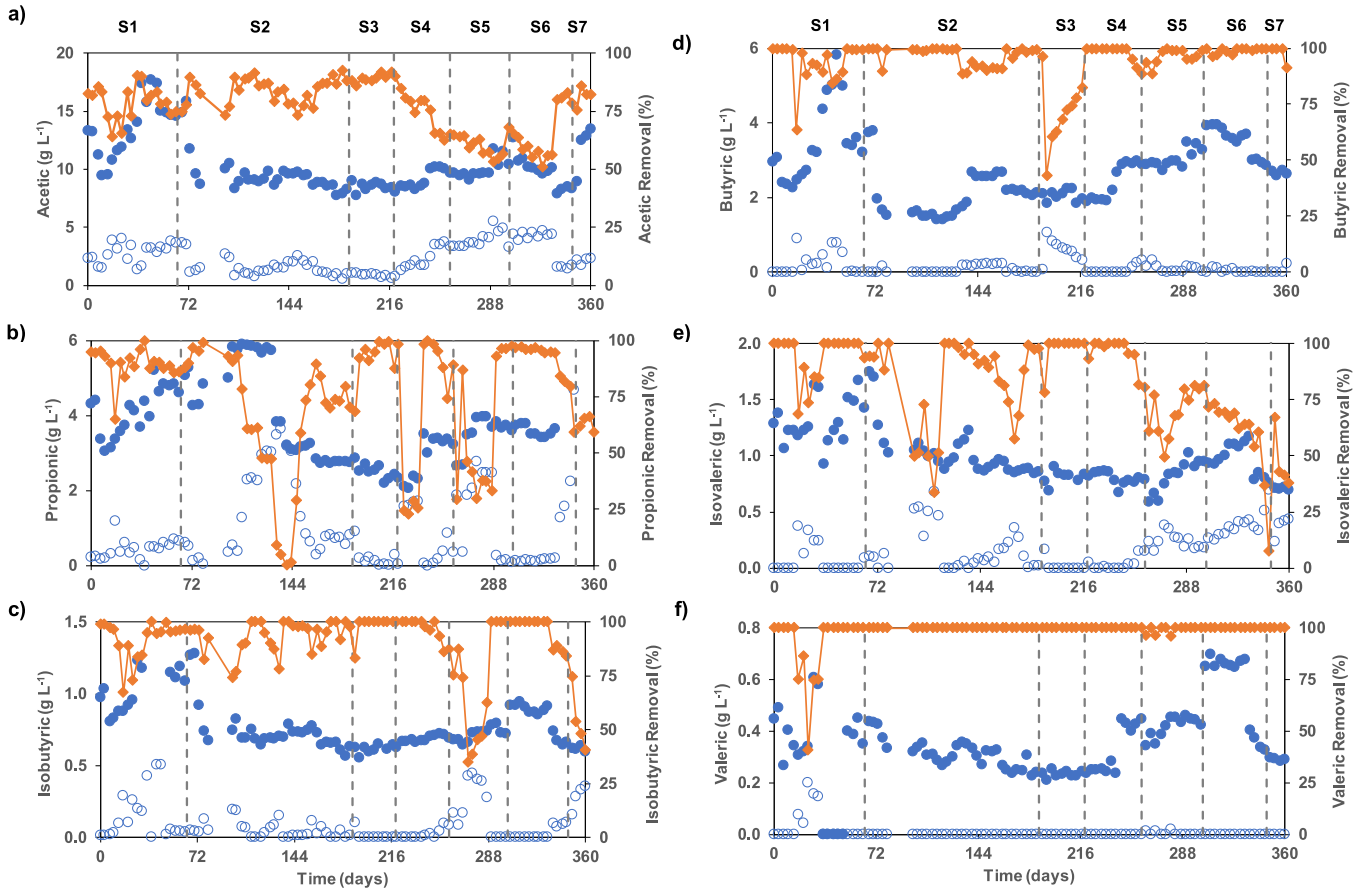

Fig. 5. Time course of the concentrations of acetic (a), propionic (b), isobutyric (c), butyric (d), isovaleric (e) and valeric (f) acids in the influent PWW (●) and anaerobic effluent (○), and their corresponding removal efficiency (♦) during the seven operational stages.

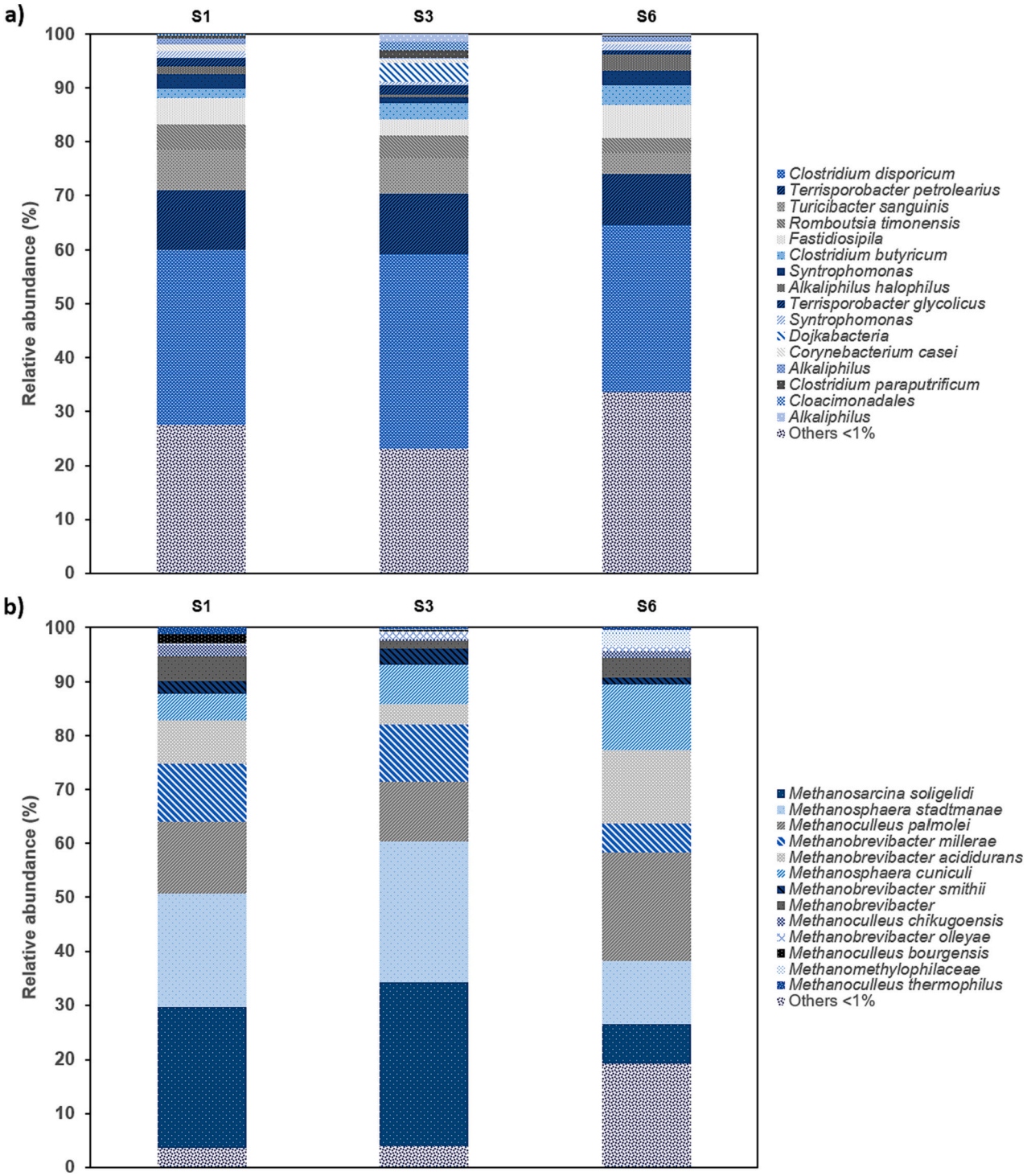

Fig. 6. Relative abundance (%) in the culture broth of the CSTR of Bacteria (a) and Archaea (b) during anaerobic digestion of PWW in stages 1, 3 and 6.

with associated CH_4 concentrations of 74.5 % ± 0.5 %, 78.7 % ± 5.4, 80.5 % ± 4.0 % and 84 % ± 3.3 %, respectively. Biogas compositions in stage 7, where the NH_3 extraction unit was stopped and ammonia concentration was artificially raised, was characterized by CO_2 and CH_4 contents of 3.8 % ± 0.7 % and 93.0 % ± 0.5 %, respectively. The increase in pH of the influent PWW induced by the alkali pretreatment with NaOH in the absence of membrane-based NH_3 extraction resulted in the gradual increase in the pH of the anaerobic broth and an active CO_2 absorption, with the corresponding high CH_4 concentrations [39]. In this context, periodic increases in the concentration of CO_2 in the biogas, concomitant with a decrease in CH_4 concentrations, were observed as a result of membrane replacement, which supported a slight acidification of the anaerobic broth mediated by a rapid H^+ permeation. A periodic membrane replacement was implemented to prevent membrane fouling, which enhances TAN recovery and proton transfer from the sulfuric acid container to the anaerobic cultivation broth. Previous studies have also reported pH variations in the anaerobic cultivation broth following membrane replacement [19,32].

VFAs removal efficiencies under steady state in the absence of membrane-based NH_3 extraction accounted for 75.9 % ± 2.7 % for

acetic acid, 86.7 % ± 1.6 for propionic acid, 96.2 % ± 0.3 % for isobutyric acid, and 99.5 % ± 0.2 % for butyric acid (Fig. 5). Furthermore, the implementation of membrane-based NH_3 extraction during stage 2 resulted in removals of acetic acid, propionic acid, isobutyric acid and butyric acid of 89.2 % ± 3.1 %, 89.4 % ± 4.7 %, 96.5 % ± 4.2 % and 98.9 % ± 0.6 %, respectively. The implementation of the membrane extraction unit reduced the concentrations of VFAs in the anaerobic broth, especially for acetic acid. This was mainly attributed to the decrease in the TAN concentration, which is associated with the microbial assimilation of VFAs. At influent PWW pH of 9, 10, 11 and 12, the acetic acid removals averaged 91.1 % ± 1.3, 64.6 % ± 1.6 %, 59.95 % ± 7.3 % and 80.9 % ± 2.3 %, respectively, the propionic acid removals 95.1 % ± 6.4, 85.8 % ± 10.6 %, 96.6 % ± 0.7 % and 73.6 % ± 12.6 %, respectively, the isobutyric acid removals 100.0 % ± 0.1, 93.2 % ± 6.9 %, 100.0 % ± 0.1 % and 86.40 % ± 2.4 %, respectively, and the butyric acid removals 82.7 % ± 4.1, 95.5 % ± 4.3 %, 96.9 % ± 1.8 % and 100.0 % ± 0.1 %, respectively. Therefore, process operation at an inlet pH of 9 supported the highest removal efficiencies of VFAs. Finally, when the extraction unit was stopped and the ammonia concentration was artificially raised in stage 7, the removals of acetic acid, propionic acid, isobutyric acid and butyric acid accounted for 83.6 % ± 2.3 %, 63.5 % ± 13.8 %, 47.5 % ± 6.6 % and 97.1 % ± 5.0 %, respectively. Propionic acid is the most toxic VFAs affecting AD performance [43], with ratios of propionate/acetate greater than 1.4 deteriorating AD performance [44]. In this study, propionate/acetate ratios in stages 1 to 7 amounted 0.2, 0.15, 0.16, 0.13, 0.03, 1.8 and 3.9, respectively. The increase in VFAs concentration recorded during stage 7 was likely due to NH_3 inhibition, which inhibited methanogenesis and entailed an accumulation of VFAs in the anaerobic broth.

3.4. Influence of the influent PWW on microbial population structure

The analysis of sequencing gene 16S rRNA revealed that the cultivation broth of the CSTR was dominated by *Bacillota* at the bacteria phylum level (Fig. 6). The dominant bacterial specie was *Clostridium disporicum,* with relative abundance of 32.5 %, 36.2 % and 30.9 % during stages 1, 3 and 6, respectively. *C. disporicum* has been associated with PWW treatment and to the degradation of fermentative organic compounds in literature [45]. In addition, *Terrisporobacter petrolearius* was also detected with abundances of 10.9 %, 11.1 % and 9.4 % in stages 1, 3 and 6, respectively. *T. petrolearius* is known for its remarkable capacity to assimilate different carbon sources and transform them mainly into acetate or carbon dioxide [46]. On the other hand, the analysis of sequencing of the gene 16S rRNA specific to A*rchaea* revealed that the anaerobic broth was dominated by *Euryarchaeota* in stages 1, 3 and 6, which is related mainly to methanogenic *Archaea.* The most abundant *Archaea* species in stage 1 during the operation without membrane were *Methanosarcina soligelidi*, *Methanosphaera stadtmanae*, *Methanoculleus palmolei* and *Methanobrevibacter millerae,* with a relative abundance of 26.0 %, 21.1 %, 13.2 % and 10.8 %, respectively. These methanogenic *Archaea* are commonly found in AD processes [47]. For instance, *M. soligelidi* is an *Archaea* able to produce CH_4 using different pathways, both from CO_2 reduction, acetoclastic and methylotrophic pathways [48]. In addition, the presence of *Methanosarcina* sp. has been reported in continuously stirred digesters treating feedstocks with high TAN and VFAs concentrations [31]. This selective pressure promotes the growth of *Methanosarcina* sp., thus favouring an efficient methane production [49]. Similarly, the most abundant archaea during stage 3 were *M. soligelidi* (30.2 %), *M. stadtmanae* (26,1 %), *M. palmolei* (11.2 %) and *M. millerae* (10.6 %). Thus, the continuous stirring and the high TAN and VFAs concentrations present in PWW favoured the dominance of *M. soligelidi* in both stage 1 and 3. However, the increase in the pH of PWW up to 12 in stage 6 caused variations in the archaeal diversity during stage 6, with *M. palmolei* as dominant specie with a relative abundance of 20.1 %, followed by *Methanobrevibacter acididurans* (13.5 %), *Methanosphaera cuniculi* (12.3 %) and *M. stadtmanae* (11.8 %). *M. palmolei* synthesizes CH_4 mainly via CO_2 reduction growing in syntrophic associations with fermentative anaerobes during the AD of PWW. Thus, the increase in pH in the influent PWW caused a change in the archaeal communities present in the CSTR. Interestingly, this pH increase enhanced ammonia removal from the cultivation broth via membrane extraction and prevented methanogenic *Archaea* inhibition during PWW anaerobic digestion [47].

4. Conclusions

The in-situ extraction of ammonia via membrane permeation from the anaerobic broth mediated a significant improvement in the performance of PWW AD at increasing pH values. Organic matter removal and biogas production were enhanced at increasing influent PWW pHs, with an optimum PWW pH of 11. The removal of ammonia in the broth from 1.3 to 0.67 mg N L^{-1} boosted VS, COD and VFAs removals up to 64 %, 80 % and 92 %, respectively. Moreover, the CH_4 content and yield increased from 76 to 84 % CH_4 and from 207 to 275 $NmLCH_4$ g COD_{fed}^{-1}, respectively.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jwpe.2023.104226.

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