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Up-flow anaerobic sediment trapped (UAST) reactor as a new configuration for the enrichment of anammox bacteria from marine sediments

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Abstract

A novel reactor configuration for the enrichment of anammox bacteria from marine

sediments was developed. Marine sediments were successfully kept inside the bioreactors

during the enrichment process by strategically installing traps at different depths to prevent

the wash-out of sediments. Three up-flow anaerobic sediment trapped (UAST) reactors

were set up (α, β and ω supplied with 50, 150 and 300 mg Ca^{2+}/L, respectively). Nitrogen

removal rates (NRR) of up to 3.5 g N/L-d and removal efficiencies of >95% were reached.

Calcium enhanced biomass production as evidenced by increased volatile suspended solids

and extracellular polymeric substances. After the long-term operation, dominant families

detected were *Rhodobacteracea*, *Flavobacteracea*, and *Alteromonadacea*, while the main

anammox genera detected in the three reactors were *Candidatus Kuenenia* and *Candidatus*

Anammoximicrobium. The UAST reactor is proposed as suitable technology for the

enrichment of anammox bacteria applicable for the treatment of saline industrial wastewaters with high nitrogen content.
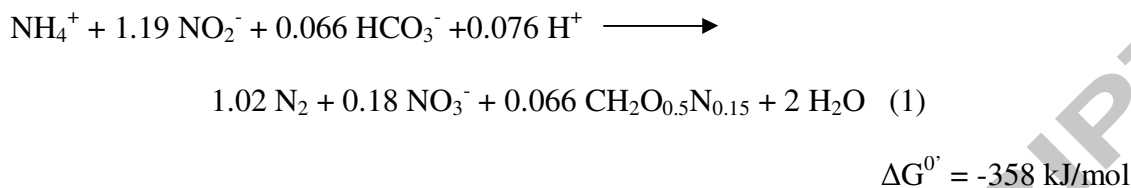
1. Introduction

Intensive discharge of nitrogen present in wastewaters to water bodies may cause serious environmental and public health problems. Specifically, wastewaters derived from textile dyeing, aquaculture, chemical and pharmaceutical sectors, as well as landfill leachates and seafood processing factories, contain large amounts of ammonium and salts (Dapena-Mora et al., 2006). Conventionally, sequential nitrification and heterotrophic denitrification are the typical biological treatment processes used for nitrogen removal from wastewaters, which require external organic matter, chemical reagents and aeration (Cervantes, 2009). For these reasons, anaerobic ammonium oxidation (anammox) based technologies have been considered as promising alternatives for the traditional technologies because they are cost saving and energy-efficient (Ali and Okabe, 2015). The anammox process uses nitrite as an electron acceptor to achieve ammonium oxidation to nitrogen gas (van de Graaf et al., 1995). In addition, anammox is an autotrophic process without requiring external organic matter as a carbon source. Energy consumption can be decreased by 60% and sludge generation minimized in industrial applications, when anammox processes are implemented

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(Jetten et al., 1997). The stoichiometry of the anammox process was calculated according to Kleerebezem and Van Loosdrecht (2010) as follows:

$$NH_4^+ + 1.19\ NO_2^- + 0.066\ HCO_3^- + 0.076\ H^+ \longrightarrow$$

$$1.02\ N_2 + 0.18\ NO_3^- + 0.066\ CH_2O_{0.5}N_{0.15} + 2\ H_2O \quad (1)$$

$$\Delta G^{0'} = -358\ kJ/mol$$

Some of the main challenges in the development of the anammox process are the complex composition of wastewater, including inhibitory components (Dapena-Mora et al., 2007; Jin et al., 2012; Leal et al., 2016), and the long time required for starting-up an anammox bioreactor due to the very slow growth rate of anammox bacteria with typical doubling time between 10 and 12 days (Strous et al., 1998). Although the anammox process has been investigated for two decades, the long start-up period of anammox reactors remains as one of the main obstacles to the widespread implementation of the process. Different bioreactors configurations have been tested for the enrichment and retention of anammox biomass including lift gas reactor (Sliekers et al., 2003), sequential batch reactor (SBR) (Dapena-Mora et al., 2004; Strous et al., 1998), rotating biological contactors (van de Graaf et al., 1996), membrane bioreactors (MBR) (van der Star et al., 2008; Wang et al., 2016) and UASB (up-flow anaerobic sludge blanket) reactors (Cervantes et al., 1999; Ni et al., 2010; Tang et al., 2011). The proper selection of reactor configuration is essential to decrease the start-up period of an anammox process. Biomass retention is also important to develop anammox processes intended for high nitrogen loading rates (NLR). Most studies have evaluated the feasibility of freshwater anammox consortia when adapting to high salinity wastewater (Dapena-Mora et al., 2010; Kartal et al., 2006) but a few studies have been focused on the enrichment of anammox bacteria from marine sediments for

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biotechnological applications (Kindaichi et al., 2011; Nakajima et al., 2008). It is interesting from the engineering and from the ecological point of view to investigate nitrogen removal from saline wastewater and to elucidate the role of anammox in marine environments. Hence the main purpose of this study was the enrichment of anammox bacteria from marine sediments in a novel reactor configuration, referred to as up-flow anaerobic sediment trapped (UAST) reactor. In addition, the effect of calcium on the performance of the anammox process was also studied as well as the taxonomic characterization of the anammox enrichments.

2. Materials and Methods

2.1 Inocula and basal medium

Marine sediments were collected from bays of the states of Baja California (BC) and Sinaloa (SIN) located in the Mexican littoral at the Pacific Ocean. Marine sediments were selected based on a previous study, showing anammox activity in batch experiments, in which specific characteristics and location are reported (Rios-Del Toro and Cervantes, 2016). In the present study, a mixture of fresh sediments from BC and SIN were used, in a proportion of 80%/20% v/v, respectively, with an initial concentration of 3.2 % of volatile suspended solids (VSS). Basal medium used for the operation of the UAST reactors was prepared with the following composition (g/L): $KHCO_3$, 1.25; NaH_2PO_4, 0.029; $CaCl_2 \cdot 2H_2O$, 0.3; $MgSO_4 \cdot 7H_2O$, 0.2; EDTA, 0.00625; $FeSO_4$, 0.00625; organic salt, 35; and 1.25 mL/L of trace elements solution. It should be noted that 35 g/L of organic salt contained the following composition (mmol/kg): Na, 462; K, 9.4; Mg, 523; Ca, 9.4; Cl,

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521; and Sr, 0.19. The trace element solution is reported in Rios-Del Toro and Cervantes (2016). Ammonium and nitrite were added to the basal medium as needed in the form of NH_4Cl and $NaNO_2$, respectively.

2.2 Bioreactors set-up

Three UAST reactors were referred to as alpha (α), beta (β) and omega (ω). Bioreactor configuration is schematically represented in Figure 1. Characteristics of each UAST reactor are as follows: They are glass columns with a height of 60 cm, volume of 1 L and an internal diameter of 5.5 cm. The hydraulic retention time (HRT) was set at 12 h throughout all experiments. The reactors were designed with two traps strategically located at different heights (at 30 cm and at 45 cm). Traps prevented the wash out of marine sediments throughout the enrichment process to contribute to the well establishment of biomass in the bioreactors. The up-flow velocity was established at 1 m/h by a recirculation line. Each trap contained 7 holes with an internal diameter of 8 mm each, which promoted disruption of sediment blocks carried by biogas accumulated. By disrupting sediment blocks transported by biogas produced inside the bioreactors, allowed sediments to settle back to the bottom. The reactors were placed in the darkness in a controlled temperature room at 28 °C and the pH of the influent was maintained in the range of 7.5–7.7. The different concentrations of ammonium and nitrite used throughout the different periods of operation of bioreactors are presented in Table1. Calcium was also added at concentrations of 50, 150 and 300 mg Ca^{2+}/L for α, β and ω reactors, respectively. Calcium was added to study the effect of this element both on the performance of the anammox enrichments and on the physical-chemical properties of the sediments. In order to compare the performance of all reactors, Kruskal-Wallis test was carried out for nitrogen removal rate (NRR), as well

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as for NH_4^+-N/NO_2^--N (Rs) and NO_3^--N/ NH_4^+-N (Rp) ratios, using software R version 3.3.1.

2.3 Biomass characterization

Biomass from all reactors was characterized based on VSS, extracellular polymeric substances (EPS), calcium content, X-ray diffraction (XRD) analysis, scanning electron microscopy (SEM) and Illumina's 16S Metagenomic Sequencing. All samples were collected and analyzed immediately by triplicate.

2.3.1 Extracellular polymeric substances extraction

EPS extraction was carried out according to Liu and Fang (2002). To each sediment sample (6 g), 10 mL of deionized water and 0.06 mL of formaldehyde were added. Samples were left for 1 h at 4 $^{\circ}$C and then 4 mL of 1 N NaOH were added and let stand for 5 h at 4 $^{\circ}$C. Samples were centrifuged at 13,000 rpm for 20 min. Supernatants were filtered through a membrane of 0.22 µm and further purified with a dialysis membrane (3500 Da) for 24 h or until pH was 7.5 by constant washings with deionized water. Finally, samples were frozen and lyophilized at -50 $^{\circ}$C. To the EPS purified fraction, carbohydrates and proteins were measured. Carbohydrate concentration was measured according to the proposed method by Dubois et al. (1956). Protein concentration was measured by Bradford's method based on the formation of a complex between the dye, brilliant blue G, and proteins in solution (Bradford, 1976).

2.3.2 Calcium Content

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Calcium content was obtained by inductively coupled plasma-optic emission spectroscopy (ICP-OES, Varian 730-ES). Samples of 2 g of biomass were in contact with a mixture of HNO_3 and HCl at a ratio of 1:3 v/v until boiling. The mixture was washed several times with distilled water.

2.3.3 X-ray diffraction (XRD) analysis

X-ray diffraction analysis of sediments was carried out in an X-Ray diffractometer Bruker D8 Advance. XRD patterns were recorded from 20° to 90° 2θ with a step time of 2 s and step size of 0.01° 2θ.

2.3.4 Scanning electron microscopy

SEM/EDS (energy dispersive spectroscopy) spectra were measured in an environmental scanning electron microscope ESEM FEI-QUANTA 200. Samples (2 g) were dried at room temperature for 12 h. Afterwards, a portion of each sample was placed on the top of a pin covered with carbon tape. Finally, samples were covered with a thin layer of gold to make their surface conductive.

2.3.5 DNA extraction and Illumina's 16S Metagenomic Sequencing

Composed sediment samples were obtained by mixing equal portions (10 g each) from the bottom, the medium and the top part of sediment blanket in each UAST reactor. Total DNA was extracted from homogenized samples using the Power Soil DNA extraction kit (MO BIO Laboratories, Carlsbad, CA, USA) following the protocol described by the manufacturer. DNA isolated from each sample was amplified using primers 341F and 785R, targeting the V3 and V4 regions of the 16S rRNA gene fused with Illumina adapter

overhang nucleotide sequences32. Polymerase chain reactions (PCRs) were performed in 50 µl reactions using Phusion Taq polymerase (ThermoScientific, USA) under the following conditions: denaturation at 98 ℃ for 60 s, followed by 5 cycles of amplification at 98 ℃ for 60 s, 50 ℃ for 30 s and 72 ℃ for 30 s, followed by 25 cycles of amplification at 98 ℃ for 60 s, 55 ℃ for 30 s and 72 ℃ for 30 s, followed by a final extension of 72 ℃ for 5 min. Two independent PCR reactions were performed for each sample. The products were indexed using Illumina's 16S Metagenomic Sequencing Library Preparation protocol and Nextera XT Index Kit v2 (Illumina, San Diego CA). Libraries were deep sequenced with the Illumina MiSeq sequencer. Analysis of 16S rRNA gene libraries was carried out using Mothur open source software package (v 1.34.4)33. Sequences containing homopolymer runs of 9 or more bases, those with more than one mismatch to the sequencing primer and Q-value average below 25 were dismissed. Illumina single end reads were analyzed for potential chimeric reads using the UCHIME algorithm. Group membership was determined prior to trimming of the barcode and primer sequence. High quality screened sequences were aligned against the SILVA 123 16S/18S rRNA gene template using the nearest alignment space termination (NAST) algorithm, and trimmed for the optimal alignment region. A pairwise distance matrix was calculated across the non-redundant sequence set, and reads were clustered into operational taxonomic units (OTUs) at 3% distance using the furthest neighbour method. Sequences and OTUs were categorized taxonomically using Mothur's Bayesian classifier and the SILVA 123 reference set. Obtained sequences have been submitted to NCBI GeneBank database.

2.4 Analytical Methods

Measurements of NO_3^--N, NO_2^--N, NH_4^+-N, pH, and VSS were performed according to the standard methods (APHA, 1998). Details can be found in Rios-Del Toro and Cervantes (2016). Measurement of pH was done using a pH meter (Thermo Scientific, Orion 4-star).

3. Results and discussion

3.1 Anammox activity in UAST reactors

Enrichment of anammox bacteria from marine sediments was carried out in three UAST reactors (α, β and ω). All UAST reactors were operated under the same experimental conditions (HRT, up-flow velocity, pH, medium composition and temperature), except for the content of calcium supplied. Reactors α, β and ω were supplemented with 50, 150 and 300 mg Ca^{2+}/L, respectively, in order to assess the effects of this element to promote granulation of flocculent sediment during the enrichment of anammox bacteria. The UAST reactors were operated for nearly one year and their performance is shown in Fig. 2. Different stages can be distinguished depending on nitrite and ammonium concentrations in the influent (Table 1). Stage 1 was characterized mainly by the absence of ammonium consumption in the three reactors. At this stage only nitrite consumption was observed with endogenous substrates promoting denitrification. When nitrite concentration was doubled in stage 2, endogenous substrates were finally depleted and anammox activity started. Once ammonium consumption started in parallel with nitrite reduction, nitrate production also occurred in all UAST reactors. After about three months of operation, stoichiometric anammox activity could be established (Supplementary Data (SD), Table S1) in all UAST reactors and a very good performance was observed in all of them achieving a total NRR of

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0.56 g N/L-d when NLR was 0.70 g N/L-d in stage 2 (Table 1). In the following operational stages, ammonium and nitrite concentrations were gradually increased until reaching 1 g/L of nitrite at stage 6. Robust anammox activities could be established in all UAST reactors achieving nitrogen removal efficiency >95% throughout the remaining operational periods. Both Rs and Rp ratios appeared very close to the stoichiometric values throughout the experiments for all UAST reactors (SD, Table S1).

Different reactor configurations have been explored for the enrichment of anammox bacteria, and the vast majority have been operated with fresh water anammox biomass, but anammox consortia are also demanded for the treatment of wastewaters containing high concentrations of ammonium and salinity, such as those generated from aquaculture activities. Several efforts to adapt fresh water anammox consortia to high salinity concentrations have been made (Jin et al., 2011; Kartal et al., 2006), but this adaptation is time consuming and costly. Thus, anammox consortia derived from marine environments represent a suitable option to develop anammox biomass adapted to high salinity conditions. The present study introduces the novel bioreactor configuration called UAST as an emerging system for the enrichment of anammox bacteria. The use of UAST configuration offers several advantages. First, excellent biomass retention can be achieved during the start-up period. The sandy and flocculent composition of marine sediments represents a challenge to maintain sediment particles inside bioreactors since accumulation of biogas can wash out fine particles and flocculent flocs. The strategic position of traps integrated inside the UAST reactors allowed sediment expansion and well mass transfer. The immobilizing mechanism (traps) of biomass inside the UAST reactor demands simple and cheap materials. No clogging occurred due to a proper combination of high up-flow

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velocity and relatively large open holes through the traps that allow disruption of flocs carried by biogas. The operation of the UAST reactor is easy and there is no need to change the operational conditions frequently as with others configurations. The efficient system designed to keep particles inside the UAST reactor could be a suitable technology to start up bioreactors inoculated with sediments or flocculent sludge for the treatment of different industrial wastewaters. The UAST reactor could be considered as a modified UASB reactor; however, the main novelty of the proposed design is that no matter the nature of the inoculum (granular or flocculent sludge, as well as marine sediments), the traps installed inside the UAST reactor will allow to keep biomass inside the reactor.

3.2 Biomass characterization

Biomass from the three UAST reactors was characterized as a function of VSS, EPS (SD, Figure S1), calcium content, X-ray diffraction, SEM/EDS (SD, Figure S2) and massive sequencing analysis (Figures 3 and SD Figure S3). The highest biomass growth, based on VSS concentration (9.7%) was measured in reactor ω, which received the highest calcium supplied (SD, Figure S1). Reactor ω also showed the highest EPS content (9.1 mg/g VSS) at the end of the experiments. In fact, there was a direct correlation between calcium supplied and production of EPS in the UAST reactors with reactors α (with 50 mg Ca^{+2}/L) and β (with 150 mg Ca^{+2}/L) containing 3.9 and 5.8 mg/g VSS, respectively, at the end of the operation. Further analysis of extracted EPS revealed a drastic change on the protein/carbohydrate ratio, with values of 6.55, 5.11 and 8.43 for reactors α, β and ω, respectively, after nearly one year of operation, which is very different from the value

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found in the original inoculum of 0.48. Total calcium concentrations in sediments collected from the three UAST reactors was also measured at the end of the experimental period. Results (SD, Figure S2) show an increase on calcium concentration in the sediments of bioreactors (1.41, 1.89 and 2.27 mg Ca/g for of α, β and ω reactors, respectively) compared with the original inoculum (0.53 mg Ca/g). Deep examination of sediments by XRD and EDS analysis revealed that calcium carbonate and calcium aluminum silicate were the main minerals sequestering calcium inside the UAST reactors (SD, Figure S2). On the other hand, calcium did not have any effect on reactors performance according to Kruskal-Wallis test (P=0.62, 0.19 and 0.35 for NRR, Rs and Rp values, respectively).

Taxonomic characterization of microbial communities developed in the UAST reactors was performed based on 16S rRNA gene sequencing of sediment samples. A total of 675,419 high quality reads of 16S rRNA gene were obtained for all libraries. For a better assessment, libraries were normalized to equal abundance of the sample with the least sequencing efficiency (131,430 reads per sample). The most abundant phyla (Figure 3) were *Proteobacteria*, *Bacteroidetes*, *Actinobacteria* and *Gemmatimonadetes* in the three reactors, as well as in the original inoculum. The abundance of *Proteobacteria* was 62, 64 and 67 %, *Bacteroidetes* contributed with 23, 17 and 12 %, *Actinobacteria* with 9, 11 and 9 % and *Gemmatimonadetes* with 1, 1.3, and 1.9 % in reactors α, β and ω, respectively. *Bacteroidetes* is a phylum frequently reported in anammox reactors and our results agree with abundance levels reported by Egli et al. (2003) and by Li et al. (2009).

Relative abundance of all genera belonging to *Planctomycetes*, which have been related to the anammox process, are presented in Figure 3. Due to the selection imposed by operational conditions, the abundance of *Planctomycetes* decreased in the three reactors,

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compared to the inoculum. However, collected data suggest a correlation between the relative abundance of specific groups of *Planctomycetes* with respect to the amount of calcium added (0.51, 0.76 and 0.95 % for reactors α, β and ω, respectively). Some genera of *Planctomycetes*, such as *Rhodopirellula*, *Pir1 lineage*, *Planctopirus*, and an *unclassified* genus, were more abundant in the three reactors after the enrichment as compared to the original inoculum. Results also showed a decrease on the relative abundance of *Candidatus Anammoximicrobium* when calcium supplied increased (Figure 3). The opposite occurred with *Candidatus Kuenenia*, which increased 18 times its relative abundance in reactor α and 85 times in reactor β as compared to its prevalence in the original inoculum.

Changes in the microbial community composition at family level with respect to the inoculum are showed in SD, Figure S3. Among the principal families decreasing their abundance with respect to the inoculum were *Piscirickettsiaceae*, *Helicobacteraceae*, *Campylobacteraceae*, *Rhodospirillaceae* and *Anaerolineaceae*. Clear enrichment of *Rhodobacteraceae* family was observed. Reactor ω obtained 46 % abundance of this family, followed by reactor β with 32 % and reactor α with 27 %. These results suggest that increased supply of calcium promoted a selective increase of *Rhodobacteraceae* family. *Rhodobacteraceae*, a big family which members had been reported as aquatic bacteria frequently thriving in marine environments (Pujalte et al., 2014). Enrichment of this family in anammox reactors has been previously reported (Shu et al., 2016). Within the main genera found belonging to *Rhodobacteraceae*, we obtained *Sediminomonas* (around 40%) and *Roseibacterium* (around 25%). *Sediminomonas* are strictly halophilic, require NaCl for growth, and are able to use a variety of carbon sources. Moreover, they are able to reduce nitrate to nitrite, thus they might have contributed to recycle the electron acceptor require

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for anammox (e.g. nitrite) in the UAST reactors, using endogenous substrates present in the sediments. *Roseibacterium* members have the genetic potential to utilize methylated amines as alternative nitrogen sources and requires Na^+ ion (or sometimes combined marine salts) for growth (Pujalte et al., 2014). Conversely, families *Flavobacteriaceae*, *Alteromonadaceae*, *Incertae Sedis*, and *OM1 clade*, negatively responded to the addition of calcium. Certainly, the relative abundance of these families was higher in reactor α supplemented with the lowest calcium content (50 mg Ca^{2+}/L) as compared to reactor ω, which received the highest calcium supply (300 mg Ca^{2+}/L). Members of *Flavobacteriaceae* are chemoorganotrophic and widespread in nature (McBride, 2014). *Alteromonadaceae* are mostly from marine origin and require sodium to grow. Members of this family do not form endospores or microcysts, are chemoorganotrophs, and can use oxygen or nitrate as electron acceptors (López-Pérez and Rodriguez-Valera, 2014). These results suggest that anammox and other heterotrophic bacteria coexisted in the UAST reactors, supporting the idea that a diverse microbial community is useful and probably necessary for the enrichment of anammox bacteria and for the stability of the anammox process (Gonzalez-Gil et al., 2015).

4. Conclusions

Enrichment of slowly growing anammox bacteria from marine sediments was possible in the novel reactor configuration, UAST. The treatment concept was shown to be suitable for biomass retention and to establish anammox biomass to achieve high nitrogen removal rates. Addition of Ca^{+2} promoted greater production of biomass and enrichment of *Planctomycetes* in UAST reactors. The new reactor configuration could be a suitable option

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for the treatment of nitrogen-rich saline wastewaters, such as those generated from aquaculture.

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Figure Captions

Figure 1. Schematic diagram of the up-flow sediment trapped (UAST) reactor used for the enrichment of anammox bacteria from marine sediments.
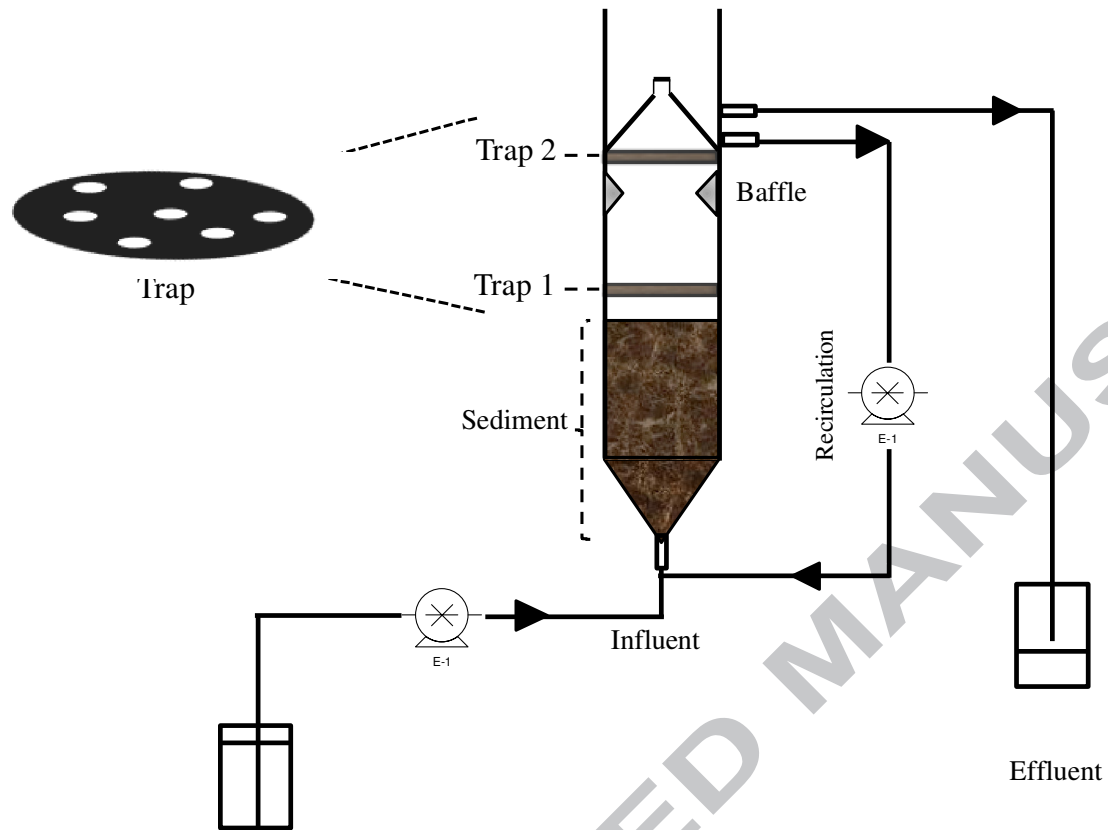
Figure 2. UAST reactors performance. A) reactor α with 50 mg Ca^{+2}/L, B) reactor β with 150 mg Ca^{+2}/L and C) reactor ω with 300 mg Ca^{+2}/L. Legends, (△), Nitrite influent; (▲), nitrite effluent; (○), ammonium influent; (●), ammonium effluent and (♦), nitrate effluent. Dotted lines indicate the different operational stages of the reactors.
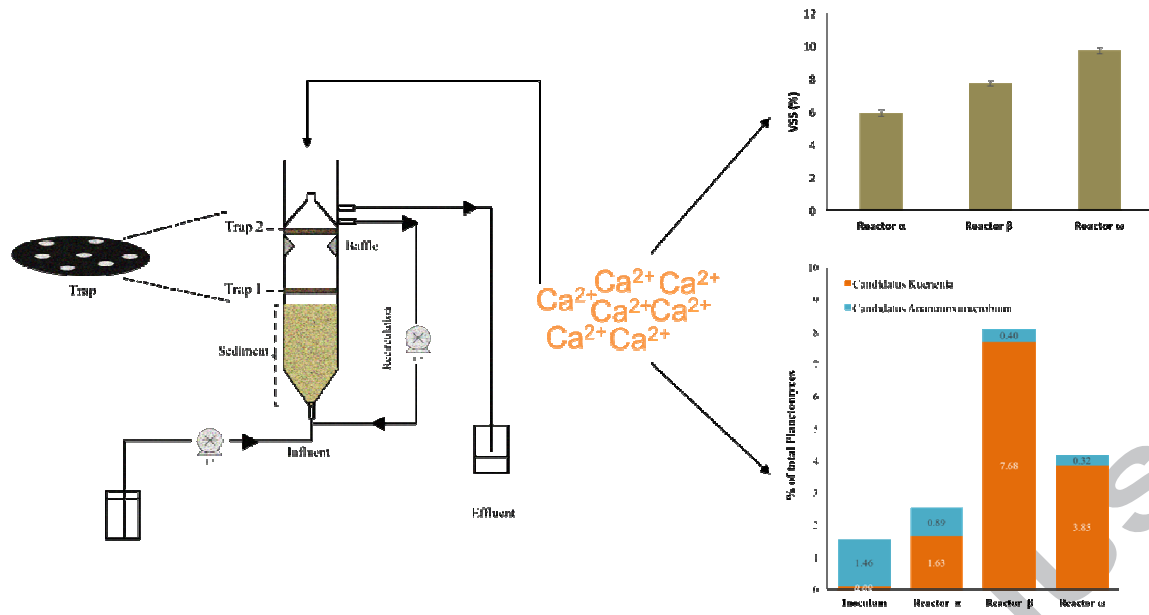
Figure 3. Microbial community composition of inoculum and from the UAST reactors at the end of operational period based on Illumina's 16S Metagenomic Sequencing. A) Relative abundance of all genera belonging to *Planctomycetes*, B) Relative abundance of phyla and C) Enriched anammox genera.

Table 1. Performance of UAST reactors at different influent substrate concentrations and at a constant HRT (12 h)

Stage	Period (days)	Influent Concentration (mg-N/L)		NLR and NRR (g N/L-d)		NRE (%)	NLR and NRR (g N/L-d)		NRE (%)	NLR and NRR (g N/L-d)		NRE (%)
				Reactor α			Reactor β			Reactor ω		
		NO_2^--N	NH_4^+-N	NLR	NRR	NRE	NLR	NRR	NRE	NLR	NRR	NRE
1	0 - 65	98.4 ± 3.4	97.9 ± 3.7	0.39 ± 0.01	0.18 ± 0.07	46.1	0.39 ± 0.01	0.18 ± 0.07	46.7	0.39 ± 0.01	0.20 ± 0.01	50.9
2	66 – 194	197.1 ± 8.7	150.7 ± 6.9	0.70 ± 0.03	0.58 ± 0.06	91.5	0.69 ± 0.02	0.56 ± 0.07	91.4	0.70 ± 0.02	0.54 ± 0.10	81.9
3	195 – 227	304.6 ± 3.7	230.4 ± 6.5	1.07 ± 0.01	0.92 ± 0.06	99.4	1.07 ± 0.02	0.91 ± 0.06	99.4	0.95 ± 0.36	0.78 ± 0.37	95.3
4	228 – 248	510.8 ± 6.2	380.1 ± 9.7	1.77 ± 0.04	1.48 ± 0.10	99.5	1.79 ± 0.01	1.51 ± 0.08	99.8	1.78 ± 0.02	1.50 ± 0.08	96.2
5	249 - 259	762.78± 7.3	567.4 ± 10.5	2.68 ± 0.05	2.28 ± 0.10	99.5	2.66 ± 0.03	2.34 ± 0.10	99.8	2.64 ± 0.02	2.16 ± 0.01	99.1
6	260 - 346	1006± 9.0	766.3 ± 10.4	3.55 ± 0.02	3.08 ± 0.09	99.7	3.54 ± 0.02	3.10 ± 0.05	99.7	3.41 ± 0.70	2.89 ± 0.71	99.4

Nitrogen Loading rate (NLR) was calculated taking into account the ammonium and nitrite supplied. Nitrogen removal rate (NRR) was calculated as the total nitrogen removed. NRE = nitrogen removal efficiency. Reactor α with 50 mg Ca^{+2}/L, Reactor β with 150 mg Ca^{+2}/L, and Reactor ω with 300 mg Ca^{+2}/L.

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Trap 2

Baffle

Trap

Trap 1

Recirculation

Sediment

E-1

Influent

E-1

Effluent

ACCEPTED MANUSCRIPT

Figure 2

Highlights

- Novel UAST reactor proposed for the enrichment of anammox bacteria.
- UAST design allowed biomass retention for treating N rich saline wastewaters.
- Supplemented Ca^{2+} increased biomass production.
- High nitrogen removal efficiencies accomplished in the UAST reactors.
- *Kuenenia* and *Anammoximicrobium* main anammox bacteria enriched.

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