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Development of a high rate biological system (Anammox + phosporous) for the treatment of low strength wastewaters

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ABSTRACT

Since their detection and abundance are disrupted by a number of unidentified conditions, anaerobic ammonium oxidising bacteria (anammox) play a vital role in wastewater. Low Strength Wastewaters (LSW) are those that have a chemical oxygen demand (COD) < 1000 ppm. Nitrogen and phosphorus are the major nutrients present in LSW in addition to the organic carbon. The biological nitrogen removal was studied through using a recent process of anaerobic ammonium oxidation (Anammox) process. A thorough explanation of the anammox substrate removal mechanism and denitrification phosphorus removal is provided. Denitrification was studied in a high rate anoxic hybrid reactor (HR) which yielded NO_3^--N removal in the range of 92–100% till a Hydraulic Retention Time (HRT) of 0.3 d and 79% at 0.24 d respectively for an inlet NO_3^--N concentration of 500 ppm. Total kjedahl nitrogen (TKN) removal of 91%, 77%, 69% and 61% were obtained in the HR containing anammox and denitrifying granules at a HRT of 2.14, 0.94, 0.56 and 0.4 per day respectively for an inlet TKN concentration of 500 ppm. Phosphorus removal of 100, 83 and 72% was obtained for an inlet PO_4^{3-}- P concentration of 50, 75 and 100 ppm, respectively at an optimum anaerobic HRT of 0.06 d (1.5 h) and aerobic HRT of 0.94 d. It is evident that the phosphorus removal efficiency is better in continuous mode than in the batch mode.

1. Introduction

Wastewater is classified as the low strength wastewater when the COD is <1000 ppm. Several important industries such as leather industry, dye industry, sugar industry, even septic tank in residential areas are having large amount of contaminated low strength wastewater [1]. Recently, the Anammox process was identified in denitrifying pilot plant reactor. Anammox is an innovative, promising, and low-cost alternative to traditional denitrification techniques [2,3]. Ammonium is transformed to nitrogen gas using the Anammox process, which uses nitrite as an electron acceptor. [4]. Because the Anammox process is autotrophic, no COD is required to sustain denitrification. Furthermore, when the Anammox process is combined with a prior nitrification phase, only a portion of the ammonium must be nitrified to nitrite, and the Anammox process then combines the remaining ammonium with the nitrite to produce nitrogen gas. This will lower the oxygen demand in the nitrification reactor and cost effective. The Anammox process produces very little sludge since the biomass output is so low [5]. The third feature that contributes to the significantly lower operation costs compared to conventional denitrification systems is the low sludge generation. The limited biomass production, on the other hand, necessitates efficient sludge retention. The bacteria that perform the Anammox process are members of the phylum planctomycetes, which includes the genera planctimycees and pirellula. Brocadia, anammoxoglobus [6], kuenenia (all fresh water species), and scalindua are the four genera of Anammox bacteria that have been (provisionally) defined (marine species) (kuypers) [7].

Recently coupling mechanism are used for treating water with the combination of more than two phenomenon such as. (1) Anammox has access to carbon sources and substrates through denitrification. When NO3 is used as an electron acceptor during the denitrification process, it inhibits NO2 reductase to diminish the generation of N2O, which is used to consume

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organic materials and release CO2 [8,9] In order to provide a low-oxygen environment for anammox, partial denitrification consumes dissolved oxygen (DO) while consuming organic matter [2] CO2 from denitrification is used as a carbon source for anammox. (3). Anammox eats the NO2 substrate, lessening the impact of NO2 buildup on denitrification and preventing further NO2 reduction to N2O. Anammox bacteria employ NO2 and + as substrates that are reduced and oxidised to N2, and the energy that is generated is used for the organic matter's CO2 production and other life-sustaining processes [10,11].

Currently four genera of Anammox bacteria have been (provisionally) defined: brocadia, anammoxoglobus [12], kuenenia (all fresh water species), and scalindua (marine species) (kuypers). Biological phosphorus removal is carried out by bacteria, which are able to take up more phosphorus than what is required for their growth [13]. An anaerobic–aerobic sequence and the presence of short chain fatty acids in the anaerobic phase are essential to encourage the growth of phosphate-accumulating organisms in an activated sludge system [14]. Under anaerobic conditions, the phosphate accumulating organisms use the energy released from the hydrolysis of intracellular polyphosphate to transport Volatile Fatty Acids (VFAs), mainly acetic acid, across their cell membranes and hence produce Poly HydroxyAlkonates (PHAs) and Poly Hydroxy Butyrate (PHB). The phosphate is released during the storage of organic matter in anaerobic environments. PHB acts as an energy source for cell development and polyphosphate storage in aerobic environments. Enhanced biological phosphorus removal, or Bio-P removal, is the process of accumulating phosphorus beyond that required for normal cell growth and disposing of it with surplus biomass [15]. Organisms of acinetobacter-moraxella group are mainly responsible for phosphate removal by activated sludge [16]. Other bacteria like p*seudomonas, aeromonas, lampropedia* species and *microlunatusphosphovorus* are also important phosphate removers. Temperature, pH, dissolved oxygen (DO) concentration and sludge retention time (SRT) are the important parameters in biological phosphorus removal. For biological phosphorus removal to occur, an anaerobic stage is required for the production of volatile fatty acids (VFAs). As a result, if nitrification is taking place, denitrification must first take place before improved biological phosphorus removal can take place. The system is anoxic rather than anaerobic if this does not happen when nitrite or nitrate is present. As a result, when biological phosphorus removal is required, a low DO concentration must be maintained for a longer period than when denitrification is required [17].

The current research study investigates the performance of anammox and nitrification for the removal of nitrogen and phosphorus separately from LSW at high hydraulic loading rates. An attempt is made to develop the optimal combined treatment system for robustness of the developed system with respect to variations in the feed characteristics can be studies.

2. Materials and methods

Effluents: The basic composition of the synthetic effluents used in this study is adapted from an earlier study [18].

Synthetic wastewater containing organic nitrogen in the form of urea, sodium nitrite as the nitrite source and other nutrients is used as the effluent to experimentally study the Anammox process. This effluent has an inlet concentration of 500 ppm TKN and 700 ppm NO_2^-- N. In the continuous mode, when denitrifying granules are also added to the Anammox reactor, a COD of 700 ppm was used in the effluent by using methanol as the carbon source.

Synthetic wastewater containing phosphate in the form of di-potassium hydrogen orthophosphate, potassium di-hydrogen orthophosphate (50 to 150 ppm) as the phosphorus source, sodium acetate (COD- 500 ppm) as the carbon source and other nutrients is used as the influent to study the biological phosphorus removal process. The composition of the synthetic effluent used is given in the Table 1.

Inoculums: The Anammox culture was enriched using the leachate from a landfill. The inoculum used for biological phosphorus removal was a mixture of 50% acinetobacter calcoaceticus and 50% of activated sludge from STP.

Table 1
Composition of effluents for denitrification, Anammox and biological phosphorus removal.

Anammox		Biological phosphorus removal	
component	g/L	component	g/L
NH_2CONH_2	1.0	CH3COONa	0.65
$NaNO_2$	3.45	NH_4Cl	0.84
Yeast Extract	0.340	Yeast Extract	0.34
$KH_2\ PO_4$	0.134	$KH_2\ PO_4$	Desired Amounts
K_2HPO_4	0.234	K_2HPO_4	Desired Amounts
$MgCl_2.6H_2O$	0.084	$MgCl_2.6H_2O$	0.084

Anammox: A Hybrid Reactor (HR) made of glass was used to study the nitrogen removal by the Anammox microbial process. The HR is inoculated using enriched Anammox culture and operated in batch mode at room temperature for the formation of Anammox granules; subsequently, the anoxic denitrifying granules are also added to the Anammox HR. The HR containing Anammox and denitrifying granules is operated in continuous mode and at a HRT of 2.14, 0.94, 0.56 and 0.4 per day. Two peristaltic pumps are used, one to circulate the wastewater through the reactor with an upflow velocity of 4 m/h and the other with a variable flow-rate to feed the synthetic wastewater. All the connections are made with leak proof silicon tubes.

Biological Phosphorus Removal: Batch experiments are conducted in shake flask reactors to study the phoshphate removal kinetics and optimize the anerobic and aerobic exposure/reaction cycle. An anaerobic Continuous Stirred Tank Reactor (CSTR) followed by an Activated Sludge Process (ASP) are used at room temperature to study the phosphorous removal in continuous mode. Diffused aeration is used in the aeration tank of ASP. All the connections are made with leak proof silicon tubes.

The stainless steel reactor had a taper at the top to slow the up-flow velocity and keep the smaller grains inside the reactor. The reactor had a working volume of 15 L and a height of 100 cm. To maintain an upflow liquid velocity of 4 m/h, the wastewater was recycled using a centrifugal pump. In order to keep the DO concentration in the reactor above 2 ppm, the recycled wastewater was aerated in a separate container. The synthetic wastewater was fed using a peristaltic pump with a variable flow rate (Fig. 1).

Total Kjeldahl Nitrogen (TKN) Estimation

TKN was estimated as per the Standard Methods [24].

Ammonical Nitrogen (NH_4^+-N) Estimation

Ammonical Nitrogen concentration was estimated by the colorimetric method- Berthelot reaction [25,26]. 2 g/L ammonium chloride solution was taken as stock solution. Dilutions were made to obtain ammonium chloride concentration in the range of 0 to 2 g/L. 5 ml of reagent A (10 g/L phenol, 50 mg/L sodium nitroprusside) was taken in a test tube, 10 μL of standard solution was added and mixed properly. 5 ml of reagent B (5 g/L sodium hydroxide, 8.4 g/L sodium hypochlorite {100 ml/l of 4% w/v chlorine}) was then added to the test tube and mixed again. Reagent blank of 5 ml solution A and 5 ml solution B was taken. The samples were incubated at 37^0C for 30 min. The absorbance at 630 nm was measured. The absorbance at 630 nm was plotted versus ammonium chloride concentration to obtain the standard curve.

Nitrate Nitrogen (NO_3^-N) Estimation

Nitrate Nitrogen concentration was estimated by the colorimetric method.

Nitrite Nitrogen (NO_2^-N) Estimation

Nitrite Nitrogen concentration was estimated by the colorimetric method (APHA, 1992).

Chemical Oxygen Demand (COD) Estimation

COD was estimated as per the Standard Methods (APHA, 1992).

Phosphate Estimation

Phosphate concentration in the supernatant was estimated using a colorimetric assay based on the formation of a blue colour complex with molybdate ions (Rout et al. 2021). Molybdate mixed reagent was prepared

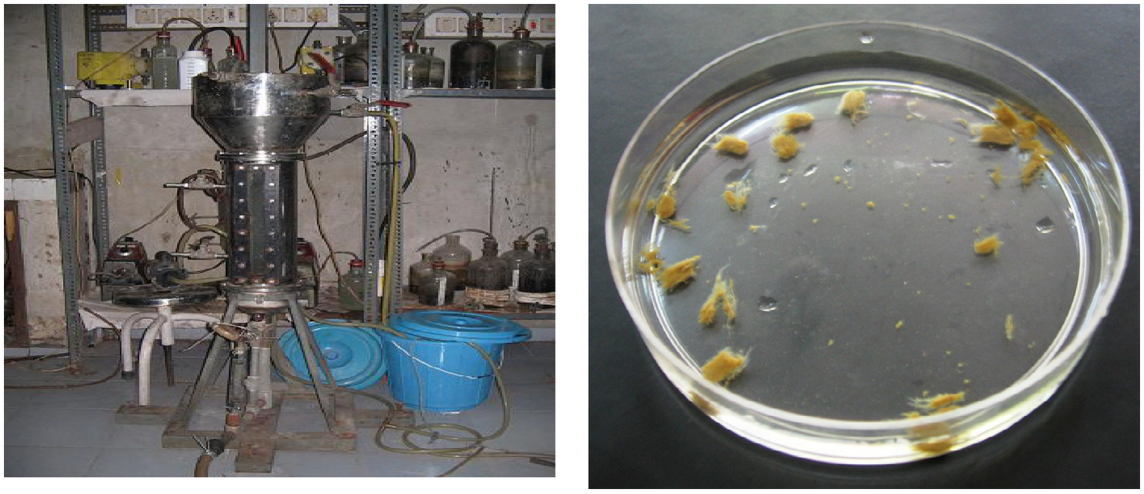

Fig. 1. (a) Experimental Set-up of anoxic HR, (b) Nitrifying granules in aerobic HR.

by mixing 20 mL of 3.0% (w/v) Ammonium Paramolybdate, 50 mL of 5 N Sulfuric Acid, 5.4% (w/v), 20 mL of Ascorbic Acid and 10 mL of 0.136% (w/v) Potassium Antimony Tartrate (freshly prepared). Standard NaH_2PO_4 solution of concentration 1 g/L is made 1 mL sample was taken and diluted with 5 mL of distilled water. NaH_2PO_4 solution of known concentration (0.02 g/L, 0.04 g/L, 0.06 g/L, 0.08 g/L, 0.1 g/L) was prepared. Mixed reagent (0.75 mL) was added to the diluted sample and was incubated for 30 min at 25 °C before measuring the absorbance at 880 nm using UV–Visible spectrophotometer (PerkinElmer, USA). The absorbance plotted versus NaH_2PO_4 concentration to obtain the standard curve.

pH

pH was measured using a pH meter (Eutech Cyberscan 500).

3. Results and discussion

3.1. Anammox

The Anammox culture was enriched using the leachate from a landfill in a 2 l glass bottle using synthetic wastewater. The enriched Anammox culture is transferred into a HR and operated in batch mode for the formation of Anammox granules. The results obtained in batch mode are shown in Fig. 2.

It takes nearly 60 days for the Anammox process to yield TKN and Nitrite removal of 90%, which implies that the growth rate of Anammox bacteria is very less (Fig. 2). It can also be observed that the NO_3^-- N concentration increases with time indicating the conversion of TKN or NO_2^- into NO_3^-. The pH decreases from 8 to 7 also confirming the formation of NO_3^-. The ratio of TKN removal, NO_2^-N removal and NO_3^--N formation in the batch mode is 1:1.4:0.22 which correlates with the stoichiometric ratios of the anammox process i.e. 1:1.32:0.26 [19].

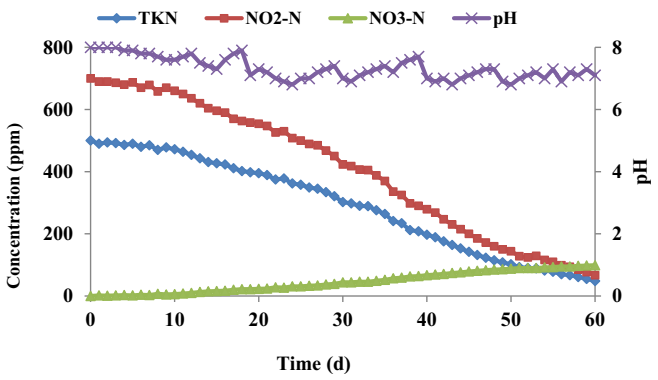

Fig. 2. Anammox in a HR (Batch Mode).

3.2. Anammox & denitrification (Continuous mode)

Since some nitrate is formed in the Anammox process, the anoxic denitrifying granules are also added to the Anammox HR and the HR containing Anammox and denitrifying granules is shifted into continuous mode. The continuous mode is started with a feed rate of 0.28 l/d, i.e. a HRT of 2.14 d. The feed rate used is based on the variation that can be made using the peristaltic pump. The results obtained at this operating condition are given in Fig. 3(a).

From the Fig. 3(a), it can be observed that the TKN removal is 91% at steady state under these operating conditions. The nitrite removal was proportional to the TKN removal, i.e. 1:1.46 and also corresponds to the stoichiometric equation of anammox, i.e. 1:1.32. The negligible concentration of nitrate in the effluent shows that the denitrifiers are active by utilizing the COD and can coexist with the anammox in the HR. The pH is noted to decrease from 8 to 7.2. Since the performance of the HR with respect to nitrogen removal is >90% at 2.14 d HRT, the feed rate is changed to a higher value (lower HRT) while keeping the same inlet TKN, NO_2^--N concentration and COD.

It can be observed from Fig. 3(b), that the TKN removal is 77% at steady state. The nitrite removal is proportional to the TKN removal and concentration of nitrate in the effluent is negligible. The COD removal is proportional to the amount of nitrate formed, which in turn is proportional to the TKN removal. The pH decreases from 8 to 7.2. In order to study the performance of the HR at different HRTs, the feed rate is changed to a higher value (lower HRT) while keeping the same inlet TKN, NO_2^--N concentration and COD.

From the Fig. 3(c), it can be observed that the TKN removal is 69% at steady state. As observed earlier, the nitrite removal is proportional to the TKN removal, i.e. 1:1.46 and also corresponds to the stoichiometric equation of anammox, i.e. 1:1.32. The negligible concentration of nitrate in the effluent shows that the denitrifiers are active by utilizing the COD and can coexist with the anammox in the HR. The pH decreases from 8 to 7. Since the performance of the HR with respect to nitrogen removal was nearly 70% at 0.56 d HRT, the feed rate is changed to a higher value (lower HRT) while keeping the same inlet TKN, NO_2^--N concentration and COD with same operating condition: HRT = 0.4 d, Inlet TKN = 500 ppm, Inlet NO_2^-- N = 700 ppm, Inlet COD = 700 ppm.

From Fig. 3(d), it can be observed that the TKN removal is 61% at steady state. The nitrite removal is proportional to the TKN removal and concentration of nitrate in the effluent is negligible. The pH decreases from 8 to 7.1. Since the anammox are autotrophs, they do not utilize the COD and

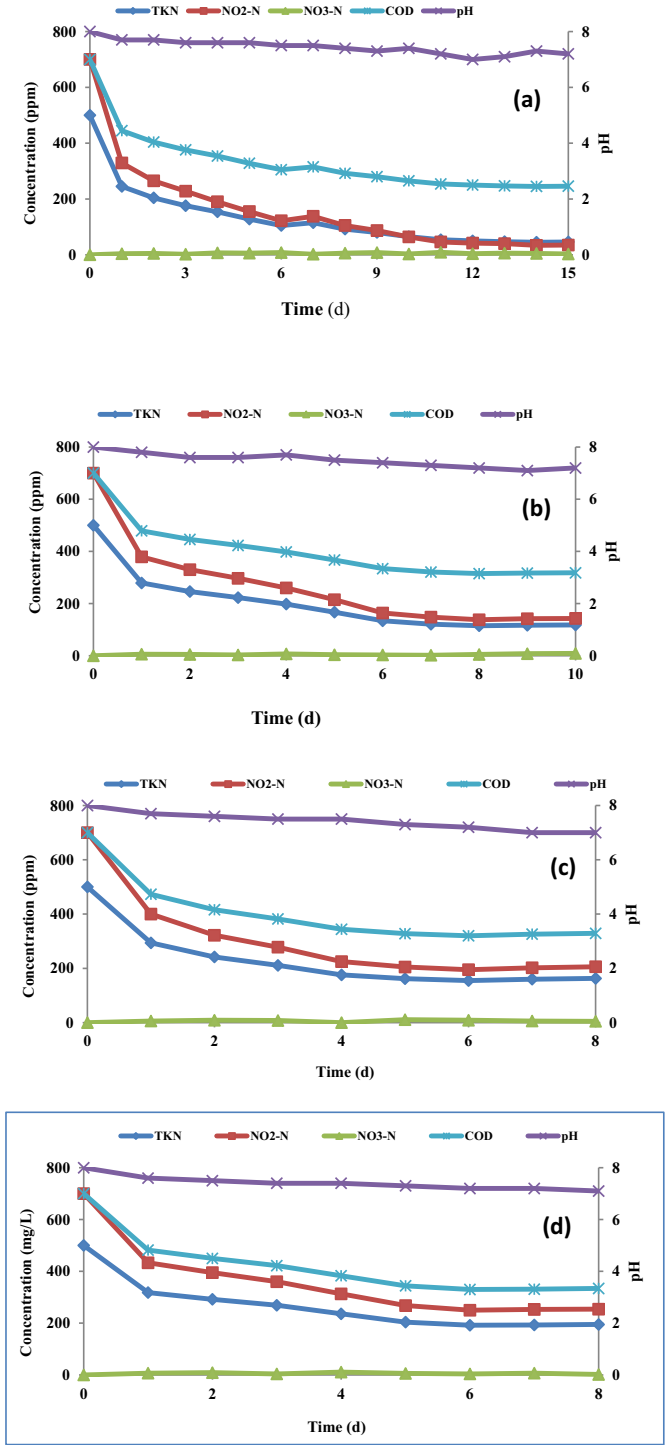

Fig. 3. Anammox and Dentrification in HR at (a) 2.14, (b) 0.94, (c) 0.56 and (d) 0.4 day of HRT.

the denitrifiers being heterotrophs use the COD for removal of NO_3^--N. The COD removal is proportional to the amount of nitrate formed, which in turn is proportional to the TKN removal. The relatively lower TKN removal at 0.4 d HRT shows the effect of HRT on the nitrogen removal using anammox.

3.3. Biological phosphorus removal

A two step process, i.e. anaerobic phase followed by aerobic phase is used for removal of phosphorus from LSW. Various batch experiments are

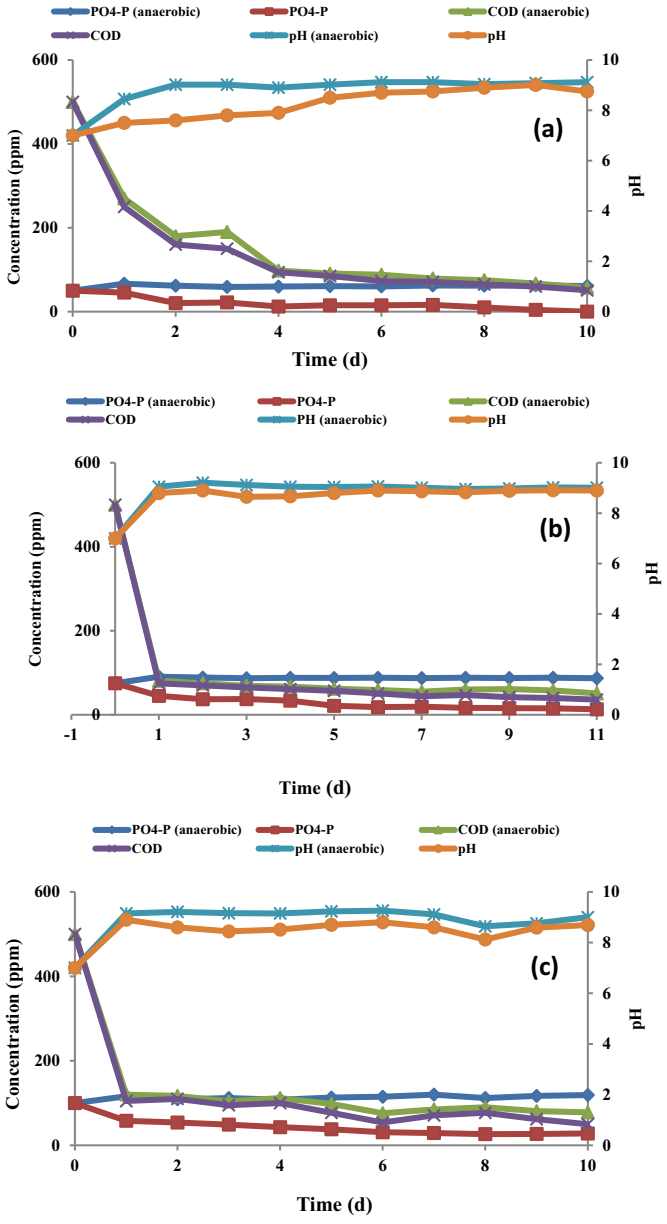

Fig. 4. Biological phosphorus removal for (a) 50 ppm, (b) 75 ppm and (c) 100 ppm PO_4^{3-}- P.

carried out in shake flasks to optimize the anaerobic and aerobic exposure/reaction time of the phosphorus removing microorganisms. It is found that the optimum anaerobic reaction time is 0.06 d (1.5 h) and the aerobic reaction time is 0.94 d. Phosphorus removal of 90, 60 and 30% is obtained for an initial PO_4^{3-}- P concentration of 50, 100 and 150 ppm, respectively, in the batch mode.

The biological phosphorus removal process is studied in continuous mode in an anaerobic Continuous Stirred Tank Reactor (CSTR) followed by an ASP. A HRT of 0.06 d (1.5 h) was maintained in the anaerobic CSTR and a HRT of 0.94 d is maintained in the aeration tank of the ASP. Since the phosphorus removal obtained in the batch mode at 50 & 100 ppm was 90 & 60% respectively, the phosphorus concentrations of 50, 75 and 100 ppm were used in the synthetic wastewater in the continuous mode. The optimal pH for biological phosphorus removal is 7 and hence the pH is adjusted to 7 in the beginning of each operating condition.

From Fig. 4 (a), it can be observed that the PO_4^{3-}- P removal is around 100% at steady state. It can be observed that the phosphorus is

released in the anaerobic CSTR resulting in an increased concentration than the inlet concentration. The COD profile indicates that the COD is mainly consumed/removed in the anaerobic CSTR for the formation of poly hydroxy alkanoates which also facilitate the release of phosphorus in the anaerobic CSTR. Since the phosphorus removal is 97% for wastewater containing 50 ppm phosphorus, the phosphorus concentration is increased to 75 ppm, keeping the same HRTs in the anaerobic and aerobic reactors. Fig. 4(b) shows that the PO_4^{3-}- P removal is around 83% at steady state. The COD profile indicates that the COD is being utilized primarily in the anaerobic CSTR. The pH profile shows a gradual increase in pH due to the consumption of acetate resulting in the production of alkalinity. The pH values are higher in the anaerobic phase than the aerobic phase. Since the phosphorus removal is >80% for wastewater containing 75 ppm phosphorus, the phosphorus concentration is increased to 100 ppm, keeping the same HRTs in the anaerobic and aerobic reactors [20,21].

Fig. 3(c) shows that the PO_4^{3-}- P removal is around 72% at steady state. As observed earlier, the COD profile indicates that the COD is being utilized primarily in the anaerobic CSTR and the increase in pH is due to the consumption of acetate resulting in the production of alkalinity. The relatively lower phosphorus removal at 100 ppm PO_4^{3-}- P can be attributed to the substrate inhibition. The phosphorus removal efficiency is found to be better in continuous mode than in the batch mode [22,23].

4. Conclusions

Nitrogen and phosphorus are the major nutrients present in LSW in addition to the organic carbon. The present study was undertaken to develop an optimal combined treatment system for removing organic carbon, nitrogen and phosphorus from LSW at high hydraulic loading rates as there are hardly any reports on the same. The enrichment/start-up time of anammox is longer, the anammox granules remove nitrogen efficiently (60%–90%) at shorter HRTs (0.4 d – 2.14 d) and can coexist with denitrifiers which take care of the nitrate and COD removal. Phosphorus removal of 97, 83 and 72% can be achieved for an inlet PO_4^{3-}- P concentration of 50, 75 and 100 ppm, respectively, at an optimum anaerobic HRT of 0.06 d (1.5 h) and aerobic HRT of 0.94 d. The phosphorus removal efficiency is better in continuous mode than in the batch mode.

Author credit statement

Author 1 and Author 2 carried out the work.
Author 3 and author 4 consolidated/ interpreted and wrote the paper.

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