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Evaluation of corncob as a bio-carrier for the enrichment of anammox bacteria using activated sludge as seed

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ABSTRACT

Anammox is the most sustainable way to remove inorganic nitrogen from wastewater. The feasibility of corncob as bio-carrier for the enrichment of anammox bacteria using activated sludge as inoculum in lab-scale sequencing batch reactors (SBRs) was studied. Control experiments using Levapor® carrier and without carrier were also conducted. Nitrite was completely removed without utilizing NH_4^+-N when corncob used as carrier in SBR. These results revealed that corncob is not a suitable carrier for anammox enrichment as it supports the growth of denitrifying bacteria. NH_4^+-N removals were observed after 80 d in control SBRs (with and without Levapor® carrier). The average NH_4^+-N removals in control and Levapor® carrier set-ups were almost similar (11.92% and 9.17%, respectively) between 80 and 119 d. However, the nitrite removal was significantly higher using Levapor® carriers (36.47%) than control experiment (3.33%). These preliminary results indicate the feasibility of activated sludge as seed for the enrichment of anammox bacteria.

1. Introduction

In order to meet the stringent effluent limits and protect the aquatic fauna and flora from the deleterious effects of excess nitrogen in the receiving water bodies, nitrogen removal has become an important priority of the wastewater treatment plants. Conventionally, nitrogen removal in wastewater treatment plants is achieved via nitrification-denitrification, a two-step process. This traditional approach is quite costly and energy intensive as it requires extensive aeration for nitrification and addition of external carbon source for denitrification to occur (Gut, 2006). Additionally, in such systems large amount of sludge and reject water from sludge dewatering process is produced. In the recent past, anaerobic ammonium oxidation (anammox) process has emerged as a sustainable and eco-friendly technique for nitrogen removal from wastewaters and has gained much attention in this field due to its cost-effectiveness and high nitrogen removal efficiency. Anammox, a short-cut in the conventional nitrogen cycle is carried out by a specialized group of microorganisms (anammox bacteria), which belongs to the phylum *Plantomycetes* (Fernandes et al., 2018). The anammox bacteria have potential to transform ammonium and nitrite to nitrogen (N_2) gas under anoxic conditions. The stoichiometry of the anammox process is given in Eq. (1) (Kuenen, 2008). The process has various advantages such as: (1) high nitrogen removal efficiency is achieved; (2) low energy consumption due to anaerobic process; (3) no need of external organic carbon as anammox bacteria are autotrophic in nature; (4) less sludge production (Xiong et al., 2013).

$$NH_4^+ + 1.32\ NO_2^- + 0.066\ HCO_3^- + 0.13\ H^+ \rightarrow 0.066\ CH_2O_{0.5}N_{0.15} + 1.02\ N_2 + 0.26\ NO_3^- + 2.03\ H_2O \quad (1)$$

Besides, it's several advantages the process has certain limitations as well. Anammox bacteria has a very slow growth rate varying between 7 and 11 d (Oshiki et al., 2011) and requires suitable environmental conditions of dissolved oxygen less than 0.1 mg/L (Third et al., 2005), temperature between 30 and 40 °C and pH of ~8 (Oshiki et al., 2011) for its proper growth. Besides, anammox bacteria are quite sensitive to certain other factors as well. The substrate nitrite at levels above 50–150 mg-N/L, high concentrations of free ammonia (FA) and free nitrous acid (FNA), and presence of organic matter inhibit the anammox bacteria (van der Star et al., 2007; Daverey et al., 2013; Chamchoi et al., 2008).

All these limitations summed up with the unavailability of large volumes of enriched anammox sludge gives rise to long start-up problem and thus critically limits the practical application of anammox process for nitrogen removal in most of the countries worldwide. However, recent studies have shown that active anammox cultures could be enriched successfully from local mixed activated sludge seeds and that the use of local sludge has significant effect on the start-up of anammox reactors in comparison to enriched anammox seeds

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transported from abroad (Kocamemi et al., 2018). It is well established that biofilm systems are highly effective in retaining biomass within the reactor and protect microbes from toxic chemicals. Therefore, the aim of this study is to evaluate the feasibility of corncob as low-cost biocarrier to enrich anammox bacteria using activated sludge collected from local sewage treatment plant (STP).

2. Materials and methods

2.1. Inoculum or sludge source and media composition

Activated sludge samples from the aeration tanks of two different STPs namely, Kedarpur STP, Mothrowala Road, Dehradun and Kargi STP, Dehradun were used as inoculum for anammox enrichment experiment. The sludge samples were washed thoroughly with phosphate buffer (pH 7.0) for removing any dead cell microorganisms and organic matter present in it. Synthetic wastewater used as medium for anammox enrichment was prepared as per the method described in Third et al. (2005) with slight modification. The synthetic wastewater contained (in mg/L, except trace element solution): $KHCO_3$, 1250; KH_2PO_4, 25; $CaCl_2.2H_2O$, 300; $MgSO_4.7H_2O$, 200; $FeSO_4$, 6.25; EDTA 6.25 and 1.25 mL/L of trace elements solution. The trace element solution was added after autoclaving and contained (g/L): EDTA, 15; $ZnSO_4.7H_2O$, 0.43; $MnSO_4.H_2O$, 0.99; $CuSO_4.5H_2O$, 0.25; H_3BO_4, 0.014. Ammonium sulphate and sodium nitrite were used as source of NH_4^+-N and NO_2^--N in the study.

2.2. Carriers used

Two different carriers, namely, corncob and Levapor® were used to evaluate their impact on the anammox enrichment. The corncob carriers were prepared from the corncob waste collected from a local market in Dehradun as described by Le et al. (2016). In brief, the corncob was washed several times with distilled water, cut into 2 × 2 × 2 cm sizes and then dried at 105 °C for two days in an oven to remove moisture. The Levapor® carriers were kindly provided by the LEVAPOR GmbH, Biofilm Technologies. These Levapor® carriers are basically polyurethane foam impregnated with activated carbon and have high adsorbing surface and high porosity.

2.3. Reactor set-up and operation

The study was conducted in conical flasks (five sets) with 500 ml working volume, which were operated as sequencing batch reactors (SBRs). In each flask, 400 ml medium (synthetic wastewater) and 100 ml of activated sludge (inoculum) was added to start the enrichment study. The detailed description of the SBR set-ups are presented in Table 1. The hydraulic retention time (HRT) of the SBRs was 20 d for the initial 28 d and later it was shortened to 10 d for the remaining days of reactor operation. The initial pH of the synthetic wastewater was kept at 8.0 in all the reactors and it was not maintained during the reactor operation. Every fifth day, the wastewater sample (125 ml or 250 ml depending on HRT) was withdrawn from the reactor and replaced with fresh synthetic wastewater with equal volume. The flasks were purged with argon gas for 5–7 min to maintain anoxic conditions in the reactor after the wastewater replacement. The flasks were incubated at 37 °C and manually shaken 4–5 times in a day.

2.4. Analytical methods

Nitrogen analysis (NH_4^+-N, NO_2^--N and NO_3^--N) of the samples withdrawn was done according to the Standard Methods for the Examination of Water and Wastewater (APHA, 2005). Ammonia was measured by using Phenate method [4500-NH_3 (F)], nitrite was analysed by applying the Colorimetric method [4500-NO_2^- (B)] and nitrate was measured using the UV Spectrophotometric Screening method [4500-NO_3^- (B)]. Effluent pH was measured by using pH electrode. The concentrations of FA (free ammonia) and FNA (free nitrous acid) were calculated using the Equations (2) and (3), respectively) described by Anthonisen et al. (1976).

$$FA(NH_3\ mg\ L^{-1}) = \frac{17}{14} \times \frac{[NH_3 - N + NH_4^+ - N] \times 10^{pH}}{e^{\left[\frac{6344}{273+T}\right]} + 10^{pH}} \quad (2)$$

$$FNA(HNO_2\ mg\ L^{-1}) = \frac{46}{14} \times \frac{[HNO_2 - N + NO_2 - N]}{e^{\left[\frac{2300}{273+T}\right]} + 10^{pH}} \quad (3)$$

3. Results and discussion

3.1. Nitrogen (NH_4^+-N, NO_2^--N and NO_3^--N) removal performance of the reactors

All the SBRs were operated with an influent NH_4^+-N concentration of 70 mg/L. The influent concentrations of NO_2^--N were between 35 and 70 mg/L. Fig. 1 shows the temporal variations of inorganic nitrogen in influent and effluent of different SBRs. It is clearly evident from Fig. 1d that NH_4^+-N was not at all utilized in SBR using corncob as carrier (Kargi AT, Corncob). In fact, the effluent NH_4^+-N concentrations were always remained higher than influent, probably due to the lysis of cell which released ammonia in the reactor. Also, the nitrite was completely removed without converting to nitrate in the reactor (Fig. 1d). The nitrite and nitrate concentrations in effluent remained negligible despite increasing their influent concentrations to 70 mg-N/L and 50 mg-N/L, respectively (Fig. 1d). This clearly indicates the dominance of heterotrophic denitrifying microorganisms in the reactor. The organic carbon released due to cell lysis of aerobic bacteria and from the corncob was utilized by the heterotrophic microorganisms, which reduced nitrite/nitrate to nitrogen gas. The release of organic carbon was not studied in this study. However, released soluble carbon concentrations from the corncob carrier in a nitrification-denitrification lab-scale reactor were around 1.2–1.5 g/L, as reported in literature (Le et al., 2016). Other studies also suggested the release of soluble organic carbon from corncob and its utilization by heterotrophic denitrifying microorganisms (Li et al., 2012; Feng et al., 2017).

The colonization of heterotrophic microorganisms on the corncob cubes was also observed in both experimental set-ups (Kargi AT, Corncob and Kedarpur AT, Corncob, data not shown). These results indicate that corncob cubes are not suitable for the enrichment of anammox bacteria. However, corncob can be used as low-cost carbon source for heterotrophic denitrifying bacteria for nitrate removal.

In control experiment (SBR without carrier, Fig. 1 a), effluent concentrations of NH_4^+-N slowly decreased from 120 mg/L to 48 mg/L.

Table 1
Description of experimental set-ups used for anammox enrichment.

Sl. No	Experimental Set	Inoculum	Inoculum source	Carrier
1	Kargi AT (Control)	Activated sludge	Kargi STP, Dehradun	No
2	Kargi AT (Corncob)	Activated sludge	Kargi STP, Dehradun	Corncob cubes
3	Kargi AT (Levapor®)	Activated sludge	Kargi STP, Dehradun	Levapor®
4	Kedarpur AT (Corncob)	Activated sludge	Kedarpur STP, Dehradun	Corncob cubes
5	Kedarpur AT (Levapor®)	Activated sludge	Kedarpur STP, Dehradun	Levapor®

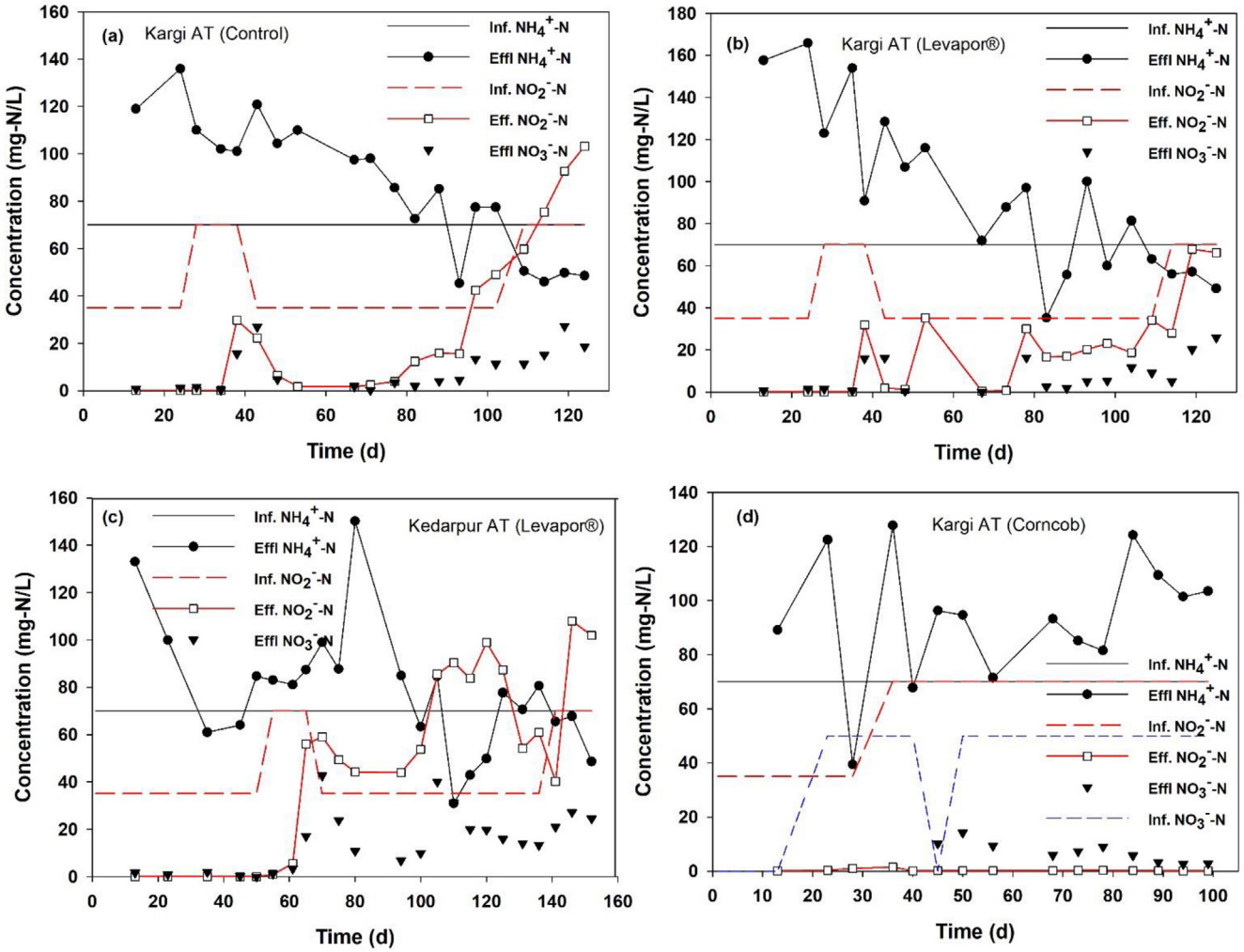

Fig. 1. Temporal variation in influent and effluent inorganic nitrogen in SBRs (a) Control (no carrier, sludge from Kargi STP); (b) Levapor® (sludge from Kargi STP); (c) Levapor® (sludge from Kedarpur STP); (d) Corncob carrier (sludge from Kargi STP).

Nitrite was almost utilized completely till 80 d in the reactor and it started accumulating after that. The small amount of nitrate (averaged 11.9 mg-NO_3^--N between 80 and 124 d) was also observed in the effluent. Slightly better results were obtained when Levapor® carriers used in SBR (Fig. 1b). The average NH_4^+-N removals in SBR without carrier (11.92%) and SBR with Levapor® carrier (9.17%) using activated sludge as seed from aeration tank of Kargi STP were almost similar between 80 and 120 d. However, the nitrite removal was significantly higher when Levapor® carriers (36.47%) were used in SBR than control (no carrier) SBR (3.33%). Similar results of NH_4^+-N removals were observed in SBR inoculated with activated sludge from Kedarpur STP (Fig. 1 c). However, nitrite accumulation was observed when influent concentration increased to 70 mg- NO_2^--N/L.

A three phase (cell lysis, propagation and cultivation) enrichment of anammox bacteria from activated sludge has been explained in literature (Chamchoi and Nitisoravut, 2007). In cell lysis phase heterotrophic bacteria remain dominant, which feed on the organic carbon released due to the cell lysis. In this phase ammonia removal remains negligible. In propagation phase, activity of heterotrophic bacteria diminishes due to the exhaustion of organic carbon. The population of anammox bacteria increases in propagation phase, which usually extends 10–12 weeks. The optimal removal of nitrogen as per the anammox stoichiometry is usually observed in cultivation phase (Chamchoi and Nitisoravut, 2007). However, in our study SBRs were operated for only 4–5 weeks of propagation phase.

The influent ratio of substrates NH_4^+-N to NO_2^--N is important and it should be 1:1.32 according to Eq. (1). In literature, the substrate ratio was usually maintained in the range of 1:1 to 1:1.5 for anammox enrichment (Chamchoi and Nitisoravut, 2007; Lu et al., 2018; Chi et al., 2018). However, the ratio of NH_4^+-N: NO_2^--N was 1:0.5 in present study, that might also be the reason for delayed response in nitrogen removal in our study.

3.2. *Effluent pH, and concentrations of free ammonia (FA) and free nitrous acid (FNA)*

The influent pH was adjusted to 8.0 in all the reactors in order to provide suitable conditions for the enrichment of anammox bacteria. However, effluent pH in all the reactors were fluctuated between 8.0 and 8.6 during 30–120 d (Fig. 2 a). This increased pH further revealed the dominance of denitrifying microorganisms, which produce alkalinity. It has been reported that presence of FA (> 20 mg/L) and FNA (> 1 μg/L) inhibits anammox activity (Daverey et al., 2013). Therefore, the concentrations of FA and FNA in all the SBRs were calculated. The results indicated that concentrations of FNA remained well below the inhibitory levels (< 1 μg/L) in all reactors except in experimental set-up Kedarpur AT (Levapor®) (average 1.13 μg -FNA/L during 95–155 d). In case of FA concentrations, sometimes it crossed the inhibitory levels (> 20 mg/L) in all reactors (Fig. 2b). The average FA concentrations were 25 mg/L during 78–99 d when corncob was used as carrier

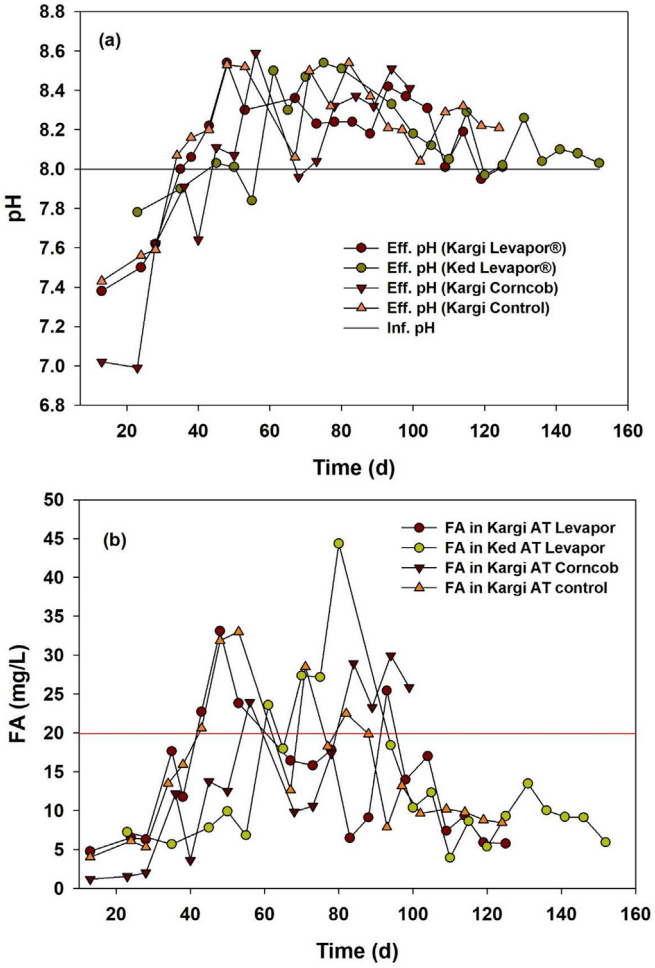

Fig. 2. Temporal variations in pH (a) and free ammonia (FA) concentrations (b) in SBRs.

probably due to high pH (average 8.38 during 78–99 d) and ammonia concentrations in this phase. The highest FA concentration (44.3 mg-FA/L on 80 d) was observed in experimental set-up Kedarpur AT (Levapor®). The inhibition due to FA (average 26.46 mg-FA/L during 61–94 d) and FNA (average 1.13 µg-FNA/L during 95–155 d) was clearly evidenced as nitrite kept accumulating in the reactor from 61 d onwards (Fig. 1c). Therefore, it is essential to keep the concentrations of FA and FNA below the inhibitory levels for fast start-up of anammox bacteria by maintaining the suitable pH in the reactor.

4. Conclusions

This preliminary study revealed that corncob is not a suitable biomass carrier for the enrichment of anammox bacteria. However, it can be a good source of carbon for denitrification studies. Better nitrogen removal was observed when Levapor® carrier used as supporting media than control set-up (without carrier). The activated sludge can be used as potential seed biomass for anammox enrichment. Maintaining the concentrations of FA and FNA well below the inhibitory levels is the key for fast and successful anammox start-up.

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