Chemical Engineering Journal 313 (2017) 355–363

Contents lists available at ScienceDirect

Chemical Engineering Journal

journal homepage: www.elsevier.com/locate/cej

Symbiosis of denitrification, anammox and anaerobic pathways – An innovative approach for confiscating the major bottlenecks of anammox process

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HIGHLIGHTS

- Proficient symbiosis was established amongst anammox, anaerobes & denitrifiers.
- Optimal seed ratio to facilitate simultaneous removal of OM and nitrogen was 0.6.
- Synergistic association of communities resulted 97.4% N_2 & 95.4% OM removal.
- MLR model predicted NRE with least error of precision (0.89 ± 4.45%).
- A new mathematical model predicted N_2 and biogas with least error of precision.

ARTICLE INFO

Article history:
Received 20 October 2016
Received in revised form 5 December 2016
Accepted 13 December 2016
Available online 18 December 2016

Keywords:
Anammox
Symbiosis
Pathways
Modelling
Kinetics

ABSTRACT

Co-occurrence of organic matter (OM) and nitrogen in industrial and domestic wastewaters is a major obstacle in field scale application of anammox process- a novel technology for biological nitrogen removal. This study explored a new symbiosis amongst different consortium i.e., denitrifiers, anammox and anaerobes, which together minimized the inhibition caused due to OM through its concurrent utilization via other metabolic pathways. This symbiosis was achieved by seeding anaerobic granular sludge in existing anammox hybrid reactors at different seed ratios, i.e., 0.2, 0.4, 0.6 and 0.8. COD/TN and seed ratio played a vital role in controlling the overall dynamics of the three different pathways, i.e., denitrification, anammox and anaerobic degradation. The seed ratio of 0.6 was found ideal for synergistic removal of both OM (95.4%) and nitrogen (97.4%). At higher COD/TN ratio, anaerobes were more prevalent and outcompeted anammox bacteria which showed predominance at lower COD/TN ratio. The process performance data were analysed using multi-linear regression (MLR) and non-linear regression models. Biasness of the models were analysed using *t*-test which dictated MLR model was unbiased and statistically more precise than non-linear regression model. A new mathematical model coceptualised on the mass balance of substrate predicted N_2 and biogas with least error of precision.

1. Introduction

Anammox is a novel microbiological approach that has changed the traditional concept of biological nitrogen removal. The process facilitates direct oxidation of ammonium nitrogen into nitrogen gas under anoxic conditions with nitrite as an electron acceptor [1]. Significant reduction in aeration costs, exogenous electron donor saving and low sludge production makes the process techno-economically feasible over existing conventional treatment technologies [2]. While, the newly discovered anammox process opens up the new possibilities for nitrogen removal from wastewater, the industrial applications are constrained due to co-existence of organic matter (OM) and nitrogen in the actual wastewater and slow growth rate of anammox bacteria [3,4]. Industrial effluents, such as coke ovens, fertiliser, antibiotics, and digested black water contain high concentration of both OM (COD-46.6–2200 mg/l) and nitrogen (123–800 mg/l [5–11]. Several researchers [12–15] have investigated the effects of OM on anammox process and revealed

Abbreviations: AHR, anammox hybrid reactor; OM, organic matter; NRE, nitrogen removal efficiency; CRE, COD removal efficiency; MLR, multi-linear regression.

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http://dx.doi.org/10.1016/j.cej.2016.12.053
1385-8947/

that it poses severe threat to anammox bacteria. Molinuevo et al. [14] observed complete inhibition of anammox process at COD concentration of 292 mg/l. Guven et al. [16] revealed that even 0.5 mM of methanol resulted in immediate and complete inactivation of anammox activity. The study revealed that anammox bacteria are no longer able to compete with heterotrophic denitrifiers at C:N ratios above 1:1. This may be due to higher affinity of denitrifiers to nitrite than OM leading to depletion of nitrite which is essentially required as a co-substrate for smooth functioning of anammox process.

The effect of inhibition can significantly be minimised by extensive acclimation and use of granular anammox sludge in bioreactor [17–20]. Ni et al. [13] revealed that granular anammox sludge demonstrated higher tolerance to OM and was capable of delivering 80% ammonium removal even at a higher COD/N ratio of 3:1. Seeding of granular sludge significantly contributed towards enhanced COD removal efficiency (CRE) in addition to its capability to sustain high organic loads [14,21–23]. Chen et al. [24] investigated the performance of single stage UASB reactor for the treatment of modified greenhouse turtle breeding wastewater and revealed simultaneous removal of both nitrogen and organic carbon with nitrogen removal efficiency (NRE) > 85% and CRE 56.5 ± 7.9%. The study concluded that anammox bacteria, denitrifiers and ammonia-oxidizing bacteria coexist for synergetic removal of nitrogen. However, the anammox contribution to nitrogen removal was always dominant leading to relatively lower CRE. There are numerous studies reported in literature which described the biological removal of both OM and nitrogen [25–27]. But, the requirement of a pre-treatment process and relatively low CRE are the major bottlenecks of the process and restrict its field scale applicability. Hence, there is a need to evolve a single stage biological system which can efficiently remove both OM and nitrogen from wastewater. This may be possible by establishing symbiosis amongst different bacterial communities, viz., anammox, denitrifiers and anaerobes. The strategy of symbiosis has ubiquitously been applied for the treatment of various kinds of pollutants. Borde et al. [28] and Muñoz et al. [29] used algal-bacterial symbiosis for the treatment of agricultural and industrial wastewater wherein, O_2 secreted by algae was used by heterotrophic bacteria to degrade organic compounds, and the CO_2 required for algal photosynthesis was produced by bacterial decomposition of organic pollutants. This symbiosis simultaneously helped in reducing aeration cost and mineralization of produced CO_2. Blender et al. [30] investigated symbiotic relationship between soil fungi and plants and showed significant reduction in N_2O emissions from the soil. Eio et al. [31] also found that symbiotic association between algae and bacteria successfully helped in biodegradation of bisphenol-A (BPA) and its intermediates. In view of the above facts, the prime objective of this study was to test a new symbiosis amongst three different pathways i.e. denitrification, anammox, and anaerobic degradation for synergistic removal of both OM and nitrogen in anammox hybrid reactor (AHR).

2. Material and methods

2.1. Experimental set-up of AHR

The experimental setup of AHR is shown in Fig. 1. The reactor was fabricated of transparent acrylic plastic with an internal diameter of 10 cm and height 65 cm. The total working volume was 5 L. Corrugated polyvinyl chloride (PVC) pipes of length 2.25 cm and diameter 2.25 cm were used as filter media. Total 55 Nos. of PVC carriers were added to the reactor to constitute an attached growth system. The sludge blanket in the lower half of the reactor constitutes suspended growth system while filter media in the upper part provides attached growth for the microorganisms. The reactor was also completely covered with black cloth to avoid the growth of phototrophic organisms and oxygen production [32]. The reactor was fed with the synthetic wastewater [33] using a peristaltic pump to maintain a constant flow rate.

AHR was inoculated with mixed seed culture of anoxic and activated sludge, 1:1 (v/v) to maintain an optimum sludge loading rate of 0.1 mg N/mg VSS d. The mixed inoculum sludge was brownish-black in colour with a VSS/TSS ratio and SVI of 0.22 and 60–75 ml/g, respectively. Gradual establishment of anammox activity resulted in transformation of flocculant sludge to granular anam-

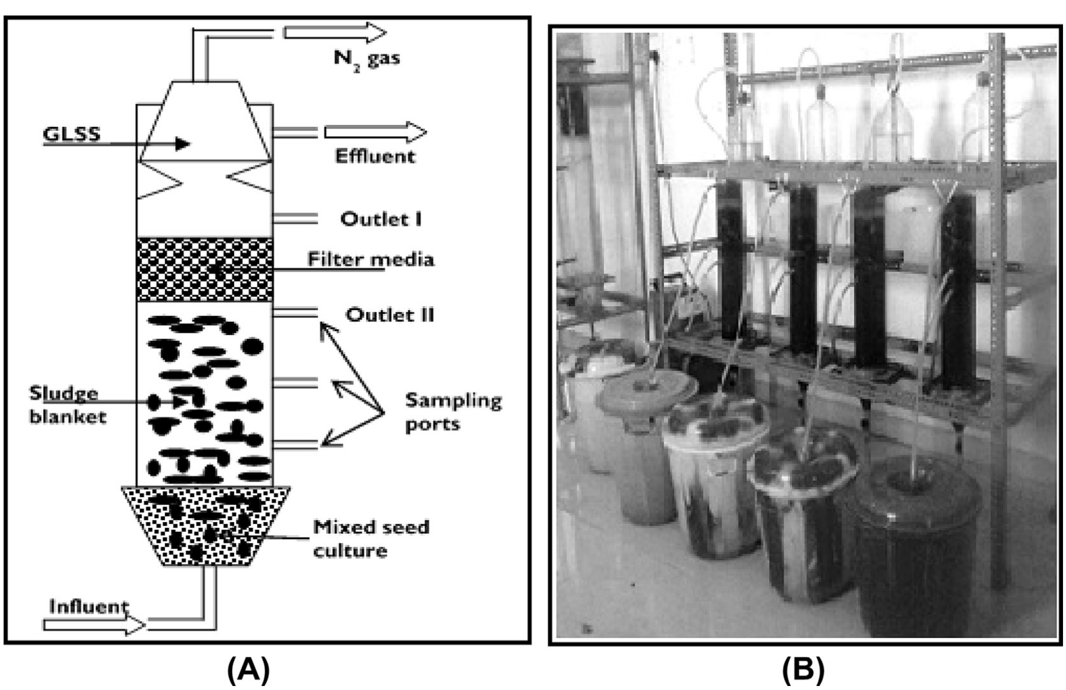

Fig. 1. (A) Schematic diagram of AHR (B) Experimental set-up of AHR.