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Investigating the role of co-substrate–substrate ratio and filter media on the performance of anammox hybrid reactor treating nitrogen rich wastewater

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This study explored the feasibility of using the anammox hybrid reactor (AHR), which combines suspended and attached growth media, for the biodegradation of ammonical nitrogen in wastewater. The study was performed in four laboratory-scale AHRs, inoculated with mixed seed culture (1:1). The anammox process was established by feeding the AHR with synthetic wastewater, containing NH_4–N and NO_2–N (1:1), at hydraulic retention time (HRT) of 1 day. The reactors were gradually acclimated to a higher ammonium concentration (1200 mg/l) until the pseudo-steady state was attained. Subsequently, the reactors were operated at various HRTs (0.25–3.0 days) to optimize the HRT and nitrogen loading rate (NLR). The study demonstrated that HRT of 1 day, corresponding to 95.1% of nitrogen removal was optimal. Pearson correlation analysis indicated the strong and positive correlation of HRT and sludge retention time (SRT), whereas the NLR and biomass yield correlated negatively with the nitrogen removal efficiency (NRE). The mass balance of nitrogen showed that a major fraction (79.1%) of the input nitrogen was converted into N_2 gas, and 11.25% was utilized in synthesizing the biomass. The filter media in the AHR contributed to an additional 15.4% of ammonium removal and a reduction of 29% in the sludge washout rate. The nitrogen removal kinetics in the AHR followed the modified Stover–Kincannon model, whereas the Lawrence–McCarty model best described the bacterial growth kinetics. The study concludes that the hybrid configuration of the reactor demonstrated promising results and could be suitably applied for industrial applications.

[**Key words:** Anammox; Anammox hybrid reactor; Nitrogen removal; Kinetics; Modeling; Bacterial growth; Mass balance]

In view of the escalating world population, the contribution of human activities to nitrogen pollution is constantly increasing. Nitrogen pollution is acerbated by the discharge of large volumes of effluents from various industrial sources, including abattoirs, pharmaceuticals, coke ovens, and tanneries. Fertilizer effluent, landfill leachate, and the digestate of anaerobic digesters used for the processing of manure are also potential sources of nitrogen pollution. The concentrations of NH_4–N in these industrial effluents vary significantly, ranging from 220 to 2600 mg/l (1–6). Conventional methods to remove nitrogen from wastewater comprise a two-stage nitrification–denitrification process. However, this process is energy intensive, requires an external carbon source, and the operating costs are high. Moreover, the failure of any of the stages results in the complete failure of the process. Therefore, the nitrification–denitrification process is techno-economically infeasible. However, simultaneous carbon and nitrogen removal by biological denitrification has increasingly gained attention in recent years. Li et al. (7) investigated the high-rate denitrifying automation circulation reactor (DAC) and reported exceptionally high nitrogen removal efficiency (NRE) of >99% at a nitrogen loading rate (NLR) of 35 kg N/m^{3}d. The effect of granular sludge bulking on the performance of the high-rate denitrifying process was also investigated (8). The high-rate DAC reactor achieved appreciably high nitrogen and COD removal rates of 25 kg N/m^{3}d (9) and 67.5 kg COD/m^{3}d (10), respectively. Another alternative often used in the industry is the ammonia stripper, which, however, is less efficient and causes air emission problems. The ion exchange process and by-product recovery have also been reported. The selective ion exchange process has the inherent advantages of high efficiency, insensitivity to temperature fluctuations, and the removal of ammonia with a minimal addition of dissolved solids (11). However, the process is expensive in view of the chemical regeneration of the resins. The by-product recovery process involves crystallization of nitrogen and phosphorus to form magnesium ammonium phosphate (MAP), which is used as a fertilizer and serves as a source of revenue (12). However, the substantial investment for the expensive chemicals required, is the major constraint of the process. As other economically viable methods to produce fertilizers are available, this technology has not attracted much attention. Hence, these conventional processes are considered techno-economically infeasible because of various constraints and other associated problems.

Anammox is one such microbial process that has transformed the traditional concept of biological nitrogen removal. The process facilitates direct oxidation of ammonical nitrogen under anaerobic conditions, with nitrite as an electron acceptor without the addition of external carbon sources. A significant reduction in the aeration costs, savings on the exogenous electron donors, and low sludge production contribute to the process being regarded as techno-economically feasible compared with the existing

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conventional treatment technologies. While the newly discovered anammox process affords additional possibilities for removing nitrogen from wastewater, the industrial applications are usually hindered by the slow growth rate of the anammox bacteria, excess nitrate production, and the requirement of nitrite as a co-substrate (5,13).

The anammox process has been investigated by several researchers, using various reactor configurations. These configurations include the up-flow anaerobic sludge blanket (UASB), continuous stirred tank reactor (CSTR), anaerobic membrane bioreactor (AnMBR), sequential batch reactor (SBR), membrane sequential batch reactor (MSBR), fluidized bed reactor (FBR), and the rotating biological contactor (RBC) (14–16). However, the hybrid configuration of the bioreactor, which combines the dual advantages of attached and suspended growth, has not been investigated to date. The potential for high sludge retention and good granulation renders this configuration superior to the other reactor configurations (17–19). Hence, in the present study, the hybrid reactor configuration was investigated to assess its feasibility and efficacy for treating nitrogen rich wastewater.

A sound knowledge of process kinetics and mathematical models is essential to exercise better control on the process design and operation of the reactor (20). These models provide a rational basis for designing and predicting the reactor performance. A number of mathematical models, e.g., the first-order substrate removal model (21), Grau second-order model (22), Stover–Kincannon model (23), and the Monod model (24) have been employed to evaluate the substrate removal kinetics in the anammox reactors. Abbas et al. (25) investigated the internal-loop-airlift bio-particle (ILAB) reactor and reported that the Grau second-order model and the modified Stover–Kincannon model could adequately predict the substrate removal kinetics. Ni et al. (26) indicated that these two models could be applied for determining the substrate removal kinetics in the UASB reactor. On the other hand, Chen et al. (27) reported the applicability of the Monod and the Haldane models in expanded granular sludge blanket reactor (ESGB) for the treatment of nitrogen laden wastewater. An extensive review of these models suggests that their suitability depends on various factors, i.e., the substrate concentration, reactor configuration, and other operational parameters. The process kinetics and modeling of this reactor configuration has not been attempted to date. Hence, the study also emphasized on evaluating the kinetics and assessing the applicability of the different kinetic models in the anammox hybrid reactor (AHR). The effect and contribution of the filter media (FM) was also assessed. Pearson correlation analysis was carried out to elucidate the impact of various operational parameters on the performance of the AHR.

MATERIALS AND METHODS

Experimental set-up of AHR The experimental set-up of AHR is shown in Fig. 1B. The reactor was fabricated from transparent acrylic plastic, with an internal diameter of 10 cm, height of 65 cm, and a total working volume of 5 L. Corrugated polyvinyl chloride (PVC) pipes, 2.25 cm long and with a diameter of 2.25 cm, were used as the FM. Fifty-five such PVC carriers were added to the reactor, constituting an attached growth system. The sludge blanket in the lower half of the reactor constitutes a suspended growth system, while the FM in the upper part provides an attached growth system for the microorganisms. To assess the effect of the FM, two outlets (outlets I and II) were provided, one above and the other below the FM, to facilitate the sample collection (Fig. 1A). The reactor was completely covered with a black cloth to prevent the growth of phototrophic organisms and the production of oxygen (28).

Origin of inoculum sludge A mixed seed culture of anoxic and activated sludge at 1:1 (v/v) was used as an inoculum for the AHR. The anoxic sludge was collected from the bottom of a waste stabilization pond used for treating municipal sewage. The activated sludge was collected from the Durgapur Coke Oven Effluent Treatment Plant, West Bengal, India. The mixed inoculum sludge was greyish black in color and the volatile suspended solids (VSS) content was 1.68 g/l. The reactor was fed with the synthetic wastewater, using a peristaltic pump to maintain a constant flow rate. The composition of the synthetic wastewater used in this study was adopted from Van de Graaf et al. (14).

Strategy of operation The reactors were started at a hydraulic retention time (HRT) of 1 d, with initial influent ammonium and nitrite concentrations of 100 mg/l each to maintain the optimal NO_2–N/NH_4–N ratio of 1:1. The reactor was operated for a period of 30 days until the pseudo-steady state condition was attained. Later, the ammonium and nitrite concentrations were gradually increased to 600 mg/l each, after which they were maintained constant. The performance of the AHR was monitored at different HRTs, varying from 3.0 to 0.25 d, to study the effect of the HRT and to investigate the substrate removal and bacterial growth kinetics. For this study, the reactors were operated for a minimum period of 15 days at each HRT, and the average values of the pseudo-steady state removals were reported. The reactors were also operated at different NO_2–N/NH_4–N ratios (0.5–1.32) to arrive at the optimal ratio for their efficient functioning. The effect and contribution of the FM toward nitrogen removal and biomass retention were assessed by analyzing the effluent samples from outlets I and II. The study was performed for a minimum period of three weeks (161–182 days) and the average contribution of the FM towards nitrogen removal and reduction in the sludge washout rate was obtained. A mass balance study was

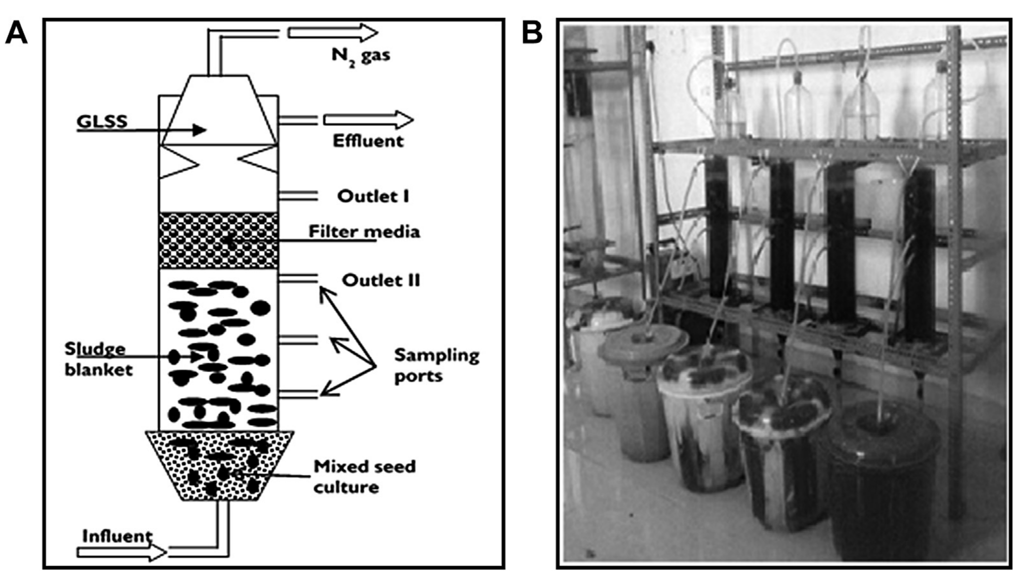

FIG. 1. AHR to treat nitrogen rich wastewater. (A) Schematic diagram, (B) experimental set-up.