



# Anoxic–aerobic SBR system for nitrate, phosphate and COD removal from high-strength wastewater and diversity study of microbial communities



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## ABSTRACT

Anoxic–aerobic sequencing batch reactor (SBR) system was operated for 180 days (under ambient temperature, 20 days SRT, 24 h HRT, influent COD/nitrate: 4 and COD/phosphate: 137) to treat a high strength wastewater (1000 mg/L nitrate and 4000 mg/L COD). The unique aspect was elimination of anaerobic cycle due to availability of nitrate (NO<sub>3</sub>) and oxygen as electron acceptors in anoxic and aerobic phases respectively. Simultaneous removal of nitrate (98%), phosphate (86%), and COD (72%) was achieved in anoxic phase. The subsequent aerobic phase experienced 26% of residual COD removal along with phosphate release (~3.4 mg/L), reducing the overall P-removal to 76%. A long anoxic phase (18 h) could sustain denitrifying dephosphatation with less MLSS generation. Pyrosequencing data were analyzed through Ribosomal database project (RDP) and DECIPHER while diversity of sampling was analyzed using Chao1 and Shannon index. Rarefaction curve reflected adequacy of sampling for total species diversity study. Overall analysis revealed *Proteobacteria*, *Alphaproteobacteria*, *Rhodobacterales*, *Rhodobacteraceae* and *Paracoccus* as the prominent phylum, class, order, family, and genus respectively.

Surplus electron donor and acceptor in anoxic phase (feasting) were advantageous for enrichment of DNPAOs over OHOs while nitrate exhaustion in the aerobic phase provided adequate fasting condition to maintain DNPAOs dominance. Low specific denitrification rate values in comparison to other heterotrophs, also supported enrichment of denitrifying phosphate accumulating organisms (DNPAOs) in the anoxic–aerobic sequencing batch reactor. Diverse micro flora ensured robustness and performance stability in high strength wastewater.

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## 1. Introduction

Enhanced Biological Phosphorus Removal (EBPR) processes enriched with DNPAOs have been under investigation as these microorganisms possess equal potential for simultaneous phosphate uptake and nitrate removal from wastewater [1–3]. DNPAOs are capable of utilizing nitrate as electron acceptor instead of oxygen hence the former have been enriched by introducing an anoxic

*Abbreviations:* COD, chemical oxygen demand; DNPAO, denitrifying phosphate accumulating organisms; OHO, other denitrifying organisms; HRT, hydraulic retention time; N<sub>2</sub>O, nitrous oxide; OTU, operational taxonomic unit; PHB, poly hydroxy butyrate; PolyP, polyphosphate; RDP, ribosomal database project; SBR, sequencing batch reactor; SDNR, specific denitrification rate; TSS, total suspended solid; VSS, volatile suspended solid; y<sub>HD</sub>, anoxic yield; MLSS, mixed liquor suspended solid.

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phase between the anaerobic–aerobic cycles of the conventional EBPR system [4]. In general, DNPAOs uptake short chain fatty acid in anaerobic condition to store it as polyhydroxyalkanoates (PHA) during which Poly-P mutilates to supply the required ATP. The reduction equivalents are provided by glycogen through glycolytic pathway. In the following anoxic condition microbes utilize the internally stored PHA to uptake phosphate, generally termed as luxury phosphate uptake, with nitrate as an electron acceptor [1,2,5,6].

Various combinations of operational phases like anaerobic–anoxic–aerobic (AAO), anaerobic–aerobic–anoxic (AOA) [7], aerobic–anoxic condition [8] have been evaluated for the treatment of municipal wastewater [9]. However, application of anoxic–aerobic treatment process in SBR systems for simultaneous removal of nitrate, phosphate and COD, is a less discussed phenomenon. Presence of electron acceptor in both the phases leads to elimination of the fasting/starvation phase (anaerobic) from the system which might alter the entire process mecha-

nism. Therefore, the question is could treatment of wastewater containing nitrate, phosphate and COD be possible in the absence of an anaerobic phase? Can PAO population be enriched without the presence of an anaerobic cycle? There are few reports which have discussed that presence of nitrate in aerobic phase might disturb the phosphorus removal efficiency [7,10]. Casey et al. [11] found that under anoxic–aerobic condition nitrate did not inhibit phosphate uptake and the kinetic model of Kuba et al. [12] indirectly supported phosphate uptake in presence of nitrate by reporting that exhaustion of nitrate under anoxic condition inhibits cell growth, poly-p uptake and glycogen synthesis. Anoxic phosphorus uptake by denitrifying PAOs is feasible when the anoxic zone was supplied with excess nitrate load so as to exceed the denitrifying potential of other heterotrophic organisms [13]. Kim et al. [14] reported that *Ca. accumulibacter* clades could successfully uptake phosphorus in the presence of nitrate. There are also other reports on successful EBPR using nitrate as terminal electron acceptor, in SBR systems using seed sludge from sewage treatment plants [15,16]. However, performance studies using high strength wastewater in biological SBR systems is still an untapped area of research, that needs attention, pertaining to the exponential growth of industries generating huge amount of nutrient rich effluents [17]. Though municipal wastewater treatment processes are well established, specific strategies have to be adapted to develop a sustainable method for treatment of high strength wastewater from pulp and paper, pharmaceutical, leather, steel, fertilizer and dairy industries. Vibrant growth of such industries in a rapidly developing country like India, demands the implication of advanced treatment processes for safe disposal of industrial effluents.

Most of the conventional nutrient removal processes operating under anaerobic–anoxic conditions have usually dealt with the internal storage compounds such as glycogen and PHB, accumulated by the microorganisms during the anaerobic phase, to act as electron donors. However, the real wastewater being a more complex formulation will have both carbon and nutrient load in the effluent. When such effluents are exposed to treatment processes it is indeed difficult to provide a condition devoid of electron acceptor (anaerobic) prior to the anoxic phase as in the conventional EBPR systems. Therefore, the current anoxic–aerobic configuration will be very crucial to evaluate the SBR performance, designed for simultaneous nitrate and phosphate removal in the presence of both electron acceptor and donor at the same time.

Presence of both carbon source and nitrate will facilitate denitrification. During complete denitrification of  $\text{NO}_3/\text{NO}_2$ , intracellular NO accumulation is prevented. This in turn facilitates the substrate utilization rate in the subsequent aerobic period [11]. Complete denitrification depends on various factors, like availability of optimum substrate, population density of denitrifying bacteria in the consortium and length of anoxic phase. Critical anoxic phase length is considered to be an important aspect as anoxic phase of very short or very long time period impedes the reactor performance [18,19] and denitrification by normal heterotrophs may fail to provide the required advantage of phosphate removal in the SBR. Therefore, population dynamics of the microbial consortium and presence of phosphate in waste effluent exposed to treatment in SBR system, will evaluate the adaptability and efficiency of the mixed consortium to achieve simultaneous phosphate removal and denitrification.

The experimental plan for this study was designed to evaluate the performance of SBR under anoxic–aerobic process in terms of simultaneous nitrate, phosphate and COD removal from high strength synthetic wastewater representing the effluents from pharmaceutical as well as dairy industries. The main objectives are:

- i To determine whether replacement of anaerobic phase with anoxic–oxic cycling can help in enriching Denitrifying Phosphate Accumulating Organisms (DNPAOs) in a SBR system for simultaneous removal of nitrate, phosphate and COD from high strength wastewater, exposed to anoxic–aerobic treatment.
- ii To evaluate the effect of high COD/phosphate ratio on DNPAO population in the SBR.
- iii To evaluate the SBR performance and reaction dynamics in the presence of both electron donor and acceptor in the anoxic and aerobic cycles.
- iv To study the microbial diversity in the SBR to identify the dominant microbial species.

## 2. Materials and methods

### 2.1. Wastewater and seed sludge

The synthetic feeding medium used as influent in the reactor contained 6 g/L  $\text{CH}_3\text{COONa}$ , 1.63 g/L  $\text{KNO}_3$ , 0.043 g/L  $\text{KH}_2\text{PO}_4$ , 1.86 g/L  $\text{MgSO}_4$ , 0.38 g/L peptone and 0.3 mL of trace element solution (0.15 g/L  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ , 0.15 g/L  $\text{H}_3\text{BO}_3$ , 0.03 g/L  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 0.18 g/L KI, 0.12 g/L  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ , 0.06 g/L  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ , 0.12 g/L  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.15 g/L  $\text{CoCl}_2 \cdot \text{H}_2\text{O}$ , 10 g/L EDTA).

The seed MLSS was collected from the anaerobic phase of a bench scale 2 L SBR performing nutrient removal under anaerobic (1 h)—aerobic(4 h)—settle/decant/refill (1 h) process for more than 2 months prior to the set-up of the current anoxic–aerobic process.

### 2.2. SBR set up

Experiments were performed in a lab scale SBR with a working volume of 2 L with a cycle time of 24 h under ambient temperature. Keeping in view the high initial nitrate (1000 mg/L) and COD (4000 mg/L) load in the influent, each cycle consisted 18 h anoxic followed by 5 h aerobic period and 1 h settle/decant/refill. Pertaining to the long anoxic phase followed by the short aerobic phase, the current SBR is termed as the LASA (Long Anoxic Short Aerobic)-SBR. The main purpose of the long anoxic phase length was:

- i To provide sufficient time for complete exhaustion of the high nitrate load from the system.
- ii To study the effect of a long anoxic phase on the overall reactor performance.
- iii To enrich the denitrifying population in the sludge.

Anoxic period in the LASA–SBR was maintained by purging of Nitrogen gas (purity 99.99%) and air was provided during the aerobic stage through aquarium air bubbler. After settling period, 1 L of the supernatant was removed, resulting in a HRT of 24 h followed by addition of 1 L synthetic wastewater to the reactor by two different peristaltic pumps connected to a timer. The wasting rate was 50 mL/day to keep the solid retention time (SRT) at about 20 days. Synthetic wastewater and MLSS were constantly mixed with a magnetic stirrer except for settling/decanting period. Initial pH was maintained at 7.0 (adding 1 N  $\text{H}_2\text{SO}_4$ ) and during operation pH was recorded but not controlled.

### 2.3. Physicochemical analysis

Nitrate, phosphate, nitrite, ammonia, COD, pH, TSS and VSS was measured according to the standard method [20].

### 2.4. Glycogen and PHB analysis

Activated MLSS was drawn from the SBR and analyzed by phenol–sulphuric acid technique to determine the concentration

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