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Enhanced biological phosphorus removal in aerated stirred tank reactor using aerobic bacterial consortium

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ABSTRACT

Recently, some studies in the field of enhanced biological phosphorus removal (EBPR) have reported that anaerobic zone is not mandatory and EBPR could be accomplished under fully aerobic conditions. This study investigated the EBPR performance of a bacterial consortium in a fully aerobic continuous stirred tank reactor (CSTR) fed with synthetic wastewater containing molasses as the sole carbon source. Total phosphorus (TP) concentration in the feed was varied between 1.4 and 40 mg/L. The results showed that TP removal efficiency was over 90% up to 20 mg/L of influent TP concentration but decreased to 62% and 30% at 30 and 40 mg/L of influent TP concentrations, respectively. TP removal efficiency was affected more by its own concentration in the influent. COD and NH_4^+-N removal efficiencies largely remained unaffected by different TP concentrations. Further investigations showed that TP was transformed to intracellular storage of polyphosphate (poly-P) with single stage aerobic process. The primarily reason for TP removal was the synergistic activity among the individual strains of phosphate solubilizing and accumulating microbes. Based on tracer studies, dead space in CSTR was found to range from 1.5–2.5%. The flow pattern in the CSTR was classified as completely mixed.

1. Introduction

Efficient and reliable phosphorus removal methods are required in order to control eutrophication in water bodies. Enhanced biological phosphorus removal (EBPR) and chemical precipitation are the two major approaches to phosphorus removal from wastewater. Because of the lower operation costs and lower environmental impacts, the importance of biological phosphorus removal is increasing.

EBPR is conventionally achieved by an anaerobic-aerobic process. The alternating anaerobic and aerobic process can exploit the ability of phosphorus accumulating organisms (PAOs) to consume phosphorus in excess of their normal metabolic necessity and to store this as the intracellular biopolymer polyphosphate (poly-P) [1]. However, some studies have reported that excess phosphorus removal could be achieved under strictly aerobic conditions in laboratory scale reactors and anaerobic zone was not mandatory for EBPR [2–4]. Ghigliazza et al. [5] found that anaerobiosis did not have any effect if EBPR system was cultured by *Acinetobacter lwoffi*. They further showed the feasibility to obtain phosphorus removal without the anaerobic phase in continuous stirred tank reactor (CSTR) inoculated by the pure culture [6]. More than 90% phosphorus removal was observed in a sequencing batch reactor (SBR) with single stage aerobic process using glucose as a carbon source [4]. High aerobic EBPR capacity was only observed during the first four days of operation using acetate as sole carbon source [7]; while Vargas et al. [8] observed that aerobic EBPR performance persisted for 46 days when propionate was used as the sole carbon source. However, aerobic EBPR performance was not observed in full scale wastewater treatment plants without providing an anaerobic phase [7].

The importance of bacterial population in EBPR process has been reported by different researchers [9–12]. Molecular ecology studies indicate that EBPR performing activated sludge is a microbial consortia consisting of phylogenetically and morphologically differing populations with broadly varying metabolic capabilities. This mixed microbial population exists in a dynamic environment influenced by different environmental and/or operational factors, such as organic loading rate, anaerobic–aerobic contact time, pH, dissolved oxygen (DO) and temperature [13,14]. Thus, phosphorus removal from wastewater, using activated sludge, is among the most significant biotechnological processes in a wastewater treatment plant.

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There is dearth of studies focusing on addition of pure cultures or bacterial consortium for the development of aerobic EBPR performing sludge. Therefore, in the present study, we investigated the EBPR potential of a bacterial consortium in an aerobic CSTR operating under varying influent total phosphorus (TP) concentrations. The use of molasses as the sole carbon source for EBPR and hydrodynamics of CSTR has also been discussed.

2. Materials and methods

2.1. Experimental setup

The experimental setup consisted of the following components: one feeding tank, one lab-scale aerobic CSTR, one settling tank, fine bubble diffusers and two peristaltic pumps.

A lab-scale CSTR (Fig. 1) having effective capacity of 35 L was fabricated using transparent plexi glass sheets. The reactor was 495 mm long, 300 mm wide and 270 mm high. Aeration and stirring inside the reactor were respectively carried out with fine bubble diffusers. The feeding tank used was of 250 L capacity. A hopper bottom type secondary settling tank was used to settle the sludge, desired amount of which was recycled to the reactor. Clear effluent was wasted with the help of a pipe connected near the top of settling tank, while excess sludge was wasted out manually keeping sludge retention time (SRT) equal to 10 days. Two peristaltic pumps were used. First pump maintained the desired influent flow rate to the reactor while second pump regulated the sludge recycling to the reactor.

2.2. Feed composition and seed sludge

Synthetic wastewater composed of molasses (carbon source), urea, KH_2PO_4, $CaCl_2$ and $MgSO_4$ was used as the feed. COD/N/P ratio in the influent was kept as 300/30/1 [15] such that it simulated actual medium strength domestic wastewater [16]. The influent TP concentration was maintained at about 1.4 mg/L during startup of the reactor. It was then increased to 10, 20, 30 and 40 mg/L at higher phosphorus loading conditions while keeping all other concentrations unchanged.

The reactor was initially seeded with 20 L of activated sludge obtained from recycling unit of activated sludge process (ASP) at municipal wastewater treatment plant located at Kankhal (Haridwar), Uttarakhand, India. The main physical and chemical characteristics of the seed sludge are as follow: color grey to light brown, temperature 24 °C, pH 7.78, mixed liquor suspended solid (MLSS) 8436 mg/L, mixed liquor volatile suspended solid (MLVSS) 6919 mg/L, sludge volume index (SVI) 114 mL/g, total nitrogen (% on VSS basis) 6.1, TP (% on VSS basis) 3.2, potassium (% on VSS basis) 0.06.

2.3. Startup and operation of the reactor

After seeding with activated sludge, continuous feeding of the reactor was started. The reactor was started with 12 h hydraulic retention time (HRT) which was reduced to 10 and 8 h on day 22 and 60, respectively, while maintaining a constant influent substrate concentration. Pseudo steady state (PSS) was achieved at every HRT. The reactor was stated for activated sludge process. Carbon removal from wastewater being the main emphasis in these reactors, COD was decided as the parameter to be monitored. However, the main aim of this study was TP removal from wastewater. So in the context of this work, PSS refers to a period of reactor performance where variation in both COD and TP effluent values was found to be insignificant (<1 mg/L) for 3–4 consecutive days.

During startup, the TP concentration in the influent was kept equal to 1.4 mg/L which was increased to 10, 20, 30 and 40 mg/L on days 76, 97, 115 and 128, respectively. The reactor was again brought back to normal TP loading condition (1.4 mg/L) on day 145 in order to study the recovery of the reactor. The influent synthetic wastewater had COD of 400–500 mg/L, NH_4^+-N of 42–50 mg/L and pH of 7.1–7.7. DO, MLSS and temperature inside the reactor was observed between 2 and 3 mg/L, 1963–3527 mg/L and 22–33 °C, respectively, throughout the study. The reactor was operated for 173 days.

Table 1
Bacteria used for the development of aerobic bacterial consortium.

Bacteria	MTCC Code	Activity		References
		PA	PS	
Acinetobacter calcoaceticus	2291	+	+	[6,17]
Pseudomonas fluorescens	2421	+	+	[18,19]
Staphylococcus aureus	9886	+	–	[18]
Staphylococcus epidermidis	6810	+	–	[18]
Bacillus cereus	6629	+	+	[18,20]
Pseudomonas mendocina	11808	+	+	[18,21]
Bacillus thuringiensis	6941	–	+	[22]
Pseudomonas putida	2475	+	+	[23,24]
Bacillus subtilis	6710	–	+	[25]
Pseudomonas fragi	10212	–	+	[26]
Burkholderia gladioli	10242	–	+	[27]
Serratia marcescens	97	+	+	[28,29]

'+' Proved, '–' not proved, PA phosphate accumulation, and PS phosphate solubilization.

2.4. Selection and development of bacterial consortium

Twelve different types of bacterial strain were selected from literature keeping in mind their availability with Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbiology, Chandigarh, India, optimum condition required for their growth and their phosphorus accumulation and solubilization efficiency (Table 1).

Freeze-dried samples of twelve different types of bacteria procured from MTCC were resuscitated in suitable growth media in the laboratory for the development of the pure cultures (see Supplementary material Fig. S1 in the online version, at http://dx.doi.org/10.1016/j.jwpe.2016.08.005). After incubation, the colony of each bacteria were transferred to sterile liquid mediums, which contained the same synthetic feed elements as the ones used in the CSTR (pH 7). The suspension of each bacterial strain was adjusted to an optical density at 600 nm (OD_{600}) of 0.1 to provide final bacteria concentration of approximately 10^8 organisms per mL (according to 0.5 McFarland standards). The cells were recovered by centrifugation at 10,000 rpm for 15 min, washed and used as activated bacterial consortium in CSTR.

The CSTR was first seeded with activated sludge from a municipal wastewater treatment plant with no phosphorus removing capabilities. Afterwards, activated bacterial consortium consisting of all twelve different bacterial strains was augmented to the reactor and was allowed to acclimatize to activated sludge, as the biomass also adapted to the fully aerobic conditions and the synthetic substrate.

2.5. Analyses

The reactor was monitored daily for pH, temperature, DO, COD and TP. MLSS, MLVSS, SVI and NH_4^+-N were analyzed weekly. Biomass TP content was analyzed at PSS conditions. All the parameters, except biomass TP content, were determined according to the *Standard Methods for the Examination of Water and Wastewater* [30]. The biomass TP content was determined by the acid digestion of the biomass [6]. Sludge samples were examined using scanning electron microscope (SEM). Poly-P staining was carried out with