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A Comparative Analysis of Simultaneous Nutrient Removal in Two Full-Scale Advanced SBR-based Sewage Treatment Plants

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Abstract: ***Two full-scale pre-anoxic selector-equipped SBR based wastewater treatment systems were analyzed in this study to determine the role of storage products in simultaneous nutrient removal (TN and TP) via simultaneous nitrification and denitrification (SND) and enhanced biological phosphorus removal (EBPR) mechanisms, respectively. For nutrient removal, it was observed that there are specific wastewater parameters that influence the plant's performance considerably, i.e., the concentration of readily biodegradable COD fraction, soluble BOD_5, which can be quickly taken up by the denitrifiers and polyphosphate accumulating organisms (PAOs) for their metabolism and stored in the form of Poly-β-hydroxybutyrates (PHB) in anaerobic phase and subsequent storage of polyphosphates (poly-P) in the aeration phase. The TN removal was ~71% (SND ~78%) and ~85% (SND ~94%), and TP removal was ~41% (EBPR ~18%) and ~68% (EBPR ~51%) in Roorkee, Uttarakhand (India) and Varanasi, Uttar Pradesh (India) SBR plants respectively. Both of them are working well in performing high SND, which requires optimized management of dissolved oxygen (DO) levels and C/N ratios. The Varanasi SBR plant is working more efficiently for the EBPR process because of the several conditions prevailing in the plant. It comprises of anaerobic/ anoxic selector compartments (nine selector compartments per basin, which diminishes the effects of RAS falling in the anoxic zones and maintains ORP < -200 mV to ferment the rbCOD into acetates) along with six aeration basins (ORP > +150 mV and DO ranges from 0-2.4 mg/L for effective SND). It has optimized rbCOD/ TP ratios (10-20) in the raw sewage and good TP storage (as poly-P) in the biological sludge (>3%), leading to effective Bio-P removal.***

Keywords: Enhanced biological phosphorus removal, Poly-β-hydroxybutyrate, Readily biodegradable COD, Simultaneous nitrification and denitrification

1. Introduction

The advanced Sequencing Batch Reactor based plants have been quite useful for decades for treating different quality of wastewater (municipal/ or domestic) based on fill-and-draw batch processes. In the early twentieth century, many full-scale fill-and-draw systems were in operation. The concern in SBRs was stimulated during the end of the 1950s and near the beginning of the 1960s, with the advancement of equipment and technologies [25]. Upgrading in aeration devices and DO/ OUR control has allocated SBRs to successfully contend with conventional activated sludge systems, especially for nutrient removal [25]. Operation of an improved, representative SBR based WWTP is usually controlled by parameters such as flow rate, solids retention times, hydraulic retention times, the concentration of organic matter (chemical oxygen demand (total, fractions), and biochemical oxygen demand (total and soluble)), suspended solids, nutrients (nitrogen and phosphorus), pH, oxidation-reduction potential (ORP) and dissolved oxygen (DO) under anoxic/ anaerobic/ aerobic sequences [5]. COD fractionation has become an important decisive factor for developing efficient nutrient removal SBR systems worldwide [5], [8]. In current conditions, special attention has been given to the readily biodegradable and slowly biodegradable fractions necessary to design a successful technology for wastewater treatment, particularly denitrification and Enhanced Biological Phosphorus Removal (EBPR), because wastewater composition matters a lot to treat the contaminants effectively. The rbCOD fractions are an essential source to increase nitrate reduction rates and are used as a potential substrate during fermentation reactions to form readily available acetates for Bio-P removal [7], [11].

Anoxic/ anaerobic selectors compartments (the zone where return AS and influent wastewater combines at no or low DO conditions) before SBR aeration tanks have been found helpful in augmenting; a) excellent sludge settling characteristics via the development of floc-formers instead of filamentous bacteria, b) denitrification and increment in floc sizes in the aeration phase for SND and c) Bio-P i.e.., biological phosphorus removal (uptake of readily biodegradable substrates in the form of PHBs and simultaneously release of orthophosphate by prevailing PAOs) [6], [9], [10], [21]. Larger-sized flocs development occurs where the floc reaction profile permits for the nitrification in the peripheral parts, and denitrification occurs in the floc's inner parts [14]. Different C/ N and C/ P ratios are significant parameters for nutrient removal [16], [17].

The storage products are the complex composition (slowly biodegradable carbon source) prepared inside the bacterial cell from the hydrolysis and metabolization of simple soluble, the biodegradable substrate including readily

biodegradable COD, soluble BOD_5, soluble COD, and VFAs (readily available fermented carbon source) during the mechanism of biological nutrient removal (simultaneous nitrification and denitrification and enhanced biological phosphorus removal processes). To identify the storage products like Polyhydroxyalkanoates, i.e., PHAs (most commonly PHBs) and polyphosphates, there are specific staining procedures obtained from different research materials/ studies. The formation of these substrates as stored products is thoroughly related to the nutrients' removal efficiencies in the plant. Hence, a qualitative approach (staining-based) was utilized to observe the mechanisms undergoing in the plant with excellent N and P removal with a configuration of anoxic/ anaerobic/ aerobic phases in the SBR plant. Staining procedures using dyes like Sudan Black B and Bismarck brown were used for identifying PHBs and polyphosphates by Sudan Black B staining and Neisser-staining techniques, respectively [6]. Pictures based on Sudan Black B staining of two SBR plants, one with effective SND and EBPR, and the other plant with only SND, not much effective EBPR, has been demonstrated in the study. This research aims to understand why the difference occurred for specially bio-P removal in the same technologically designed plants and the necessary parameters to be satisfied or critical parameters to be included in the design to undergo both TN and TP removal biologically (mainly Bio-P).

2. Plant location and configuration

Two plants were analyzed for this study: (a) 3 MLD SBR plant located in Roorkee, Uttarakhand (India) (29°52'05" N 77°54'06" E) and (b) 120 MLD SBR plant located in Goithaha, Varanasi (India) (25°23'15" N 82°59'52" E) (Figure 1). Both the SBRs have proper automated systems governed by the programmable logic controller (PLC) and DO/ OUR control systems. A thorough study was done, and the results were shown in table 2. Roorkee SBR could remove all the contaminants (ammonia, nitrates, TN, COD, BOD_5, and TSS) except TP to the stringent limits. But wastewater composition, controlled operation, and specific microbial diversity (mainly polyphosphate accumulating organisms (PAOs)) prevalence benefitted the Varanasi SBR in removing the nutrients (TN and TP), suspended solids, and organic matter up to the NGT effluent discharge standards. In both of these plants, the tertiary treatment/ final polishing of the biologically treated effluent was carried out by chlorination and UV Disinfection.

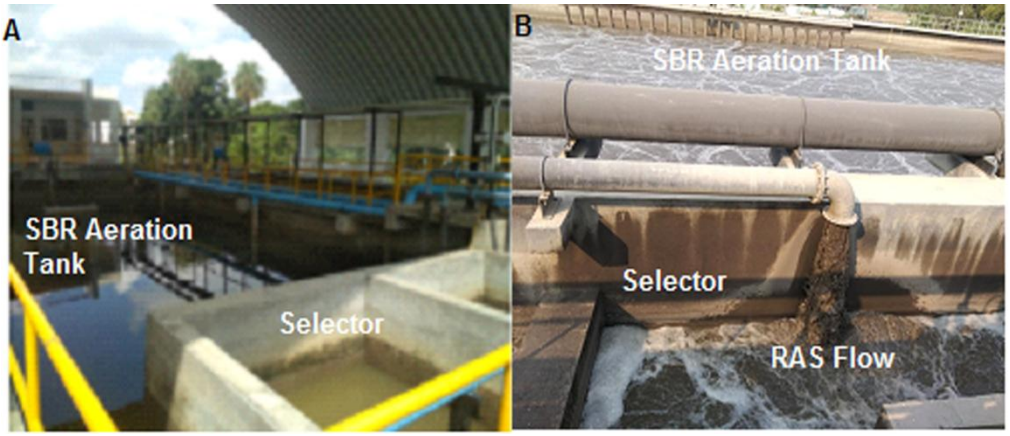

Figure 1: (A) Full-scale 3-MLD SBR plant, Roorkee, and (B) Full-scale 120-MLD SBR plant at Goithaha, Varanasi

3. Physicochemical parameters and wastewater characteristics

The operational parameters like pH, DO, and ORP were observed regularly in the treatment plants along with the parameters like COD (total, filtered, and other fractions), BOD_5 (total and filtered), TN, TKN, Ammonia, Nitrate, Orthophosphate, and Total Phosphorus according to *Standard Methods* [1] and rbCOD by Floc-filtration method [26] (Table 1 and 2). EBPR was calculated as TP removed more than ~2.7% of PO_4-P uptake as VSS in the sludge.

Table 1: Operational parameters in the SBR plants

SBR Plants	Cycle time (h)	Denitrification % in Anoxic/ anaerobic selector	HRT (h)	SRT (d)	Mixing time in selector (min) and RAS %	ORP in A.T. and Anoxic/ Anaerobic Selector (mV)	pH in A.T. and Anoxic/ Anaerobic Selector	D. O. in A.T. and Anoxic/ Anaerobic Selector (mg/L)	rbCOD/TN and rbCOD/ TP	COD/ TN and COD/ TP	BOD_5/ TN and BOD_5/ TP
Roorkee	4	39.7	18	15	(60 min) 25% RAS	+105 ± +45 and -90 ± -24	7.49 ± 0.14 and 7.79 ± 0.2	1.4 ± 0.95 and 0.14± 0.06	1.36 and 7.5	11.8 and 65.7	4.8 and 26.7
Varanasi	3	46.9	14	10	(45 min) 18% RAS	+130 ± 70 and -208 ± - 90	7.38 ± 0.14 and 7.9 ± 0.12	1.4 ±1.2 and 0.12 ± 0.10	0.96 and 12	5.6 and 70.6	3.4 and 42.4

*A.T. corresponds to Aeration Tank

Table 2: Wastewater characteristics and Nutrient removal in the SBR

Parameters (mg/L)	3 MLD SBR (Influent (Raw Sewage))	3 MLD SBR (Final Effluent after disinfection)	120 MLD SBR (Influent (Raw Sewage))	120 MLD SBR (Final Effluent after disinfection)
Total COD	401 ± 130	18 ± 7.7	318 ± 12	24.7 ± 9.1
Soluble COD (sCOD)	139 ± 45	10.4 ± 5.7	111.5 ± 6.4	10.05 ± 4.7
Biodegradable COD (bCOD)	325 ± 105	-	280 ± 43	-
Readily biodegradable COD (rbCOD)	45.7 ± 17.8	-	53.9 ± 1.6	-
Ammonia (NH_4-N)	21.8 ± 6.0	0.7 ± 0.5	41.3 ± 3.4	1.5 ± 0.2
Nitrate (NO_3-N)	0.9 ± 0.8	5.6 ± 1.8	1.3 ± 0.3	3.7 ± 0.1
TKN	33 ± 9	4.2 ± 3.0	55 ± 2.7	4.7 ± 2.6
Total Phosphorus (TP)	6.1 ± 2.5	3.6 ± 1.7	4.5 ± 0.1	1.4 ± 0.5
Ortho-phosphate (PO_4-P)	2.8 ± 1.1	1.8 ± 0.6	3.6 ± 0.5	1.0 ± 0.6
Total BOD_5	163 ± 57	6.0 ± 2.2	191 ± 55	6.3 ± 0.7
Soluble BOD_5	63 ± 28	3.1 ± 1.5	48 ± 11	2.9 ± 0.6
Total Suspended Solids (TSS)	237 ± 79	9.4 ± 2.1	324 ± 18	9.0 ± 1.4
Volatile Suspended Solids (VSS)	128 ± 48	4.9 ± 1.5	137 ± 49	3.6 ± 1.4
Alkalinity (as CaCO3)	350 ± 30	260 ± 20	480 ± 10	340 ± 28
Total Coliforms (average) (MPN/ 100 mL)	$3.6x10^6$	$5.4x10^3$	$3.6x10^6$	Nil
Fecal Coliforms (average) (MPN/ 100 mL)	$1.6x10^3$	45	$7.4x10^4$	Nil

4. Intracellular polymers stored during EBPR and SND

Successful enhanced biological phosphorus removal is explained by the models defined as a consequence of the PAO achieving supremacy under an anaerobic/ aerobic recycling environment by having discerning benefits over the several different bacteria present in their capabilities to produce intracellular storage complexes under the 'feast and famine' provisions which describe EBPR processes [20]. Thus, under anaerobic conditions, the Polyphosphate Accumulating Organisms are assumed to rapidly assimilate organic substrates like acetates (fermented products of rbCOD) and utilize these to synthesize PHA employing stored poly-P as an energy source. The orthophosphates created from the poly-P degradation are discharged into the water (Figure 2). Then, during the deficiency of any exogenous substrates in the aerobic zone, organisms with stored PHA can use these as carbon and energy sources to cultivate and incorporate phosphate to produce poly-P. Thus, PAO attains dominance under the prevailing anaerobic-aerobic conditions because they alone can grow aerobically during the lack of any exogenous source of carbon and energy by using the PHA synthesized anaerobically. Implications were that the electrons needed were obtained from the anaerobic operation of the TCA cycle. Simultaneously, [13] generated substantiation that glycogen, are intracellular storage compounds produced aerobically by the PAO, was catabolized anaerobically to make electrons for PHA synthesis [20].

Similarly, PHAs (most commonly PHBs) are an excellent source of slowly biodegradable substrate for denitrification. They are accumulated by these microbes and denitrifiers in the anaerobic period and are used as an energy source during settling conditions of SBR aeration basins for denitrification. Hence, they are identified as an active substrate for both SND and EBPR processes [12], [19], [23].

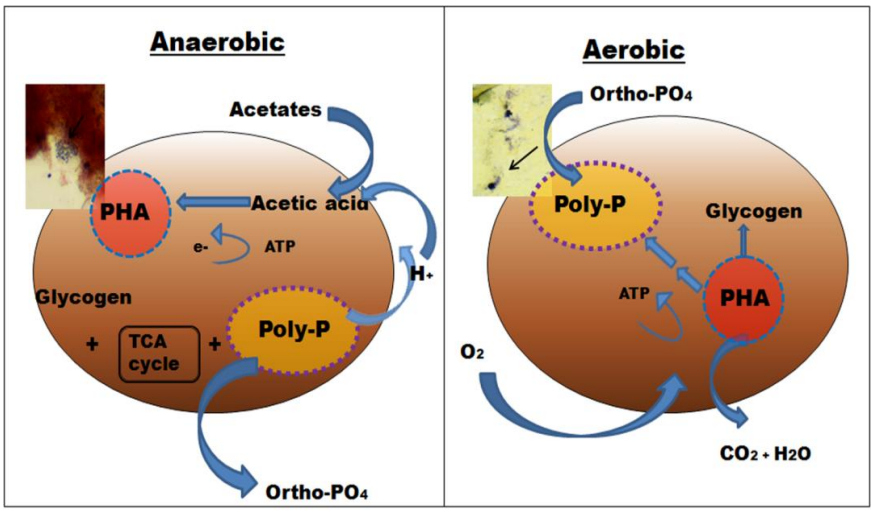

Figure 2: Intracellular storage products (poly-P and PHB) during biological phosphorus removal. A précis of the critical features of the biochemical models for EBPR according to [20]. The changes hypothesized to occur during the aerobic and anaerobic phases are demonstrated. The insert shows the characteristic microscopic manifestation of the biomass taken from the anaerobic zone and aerobic zone, with bunched cells staining purple-black with the Neisser stain for poly-P and granules stained blue-black with the Sudan black B stain for PHA (commonly PHBs).

4.1 Qualitative analysis of PHB granules in the SBR plants

The poly-β- hydroxybutyrate (PHB) qualitative analysis was conducted on both the plants in the SBR aeration tank and anoxic/anaerobic selector tank. The images were obtained at 100X magnification with immersion oil using a light 'Optika microscope (Italy)' and software 'Optika proview.' The bright-field and phase-contrast images were examined by placing 100 µL of sample on the slides, and the procedure followed was according to [22], [24]. PHB granules were present in between the sludge flocs and in the filaments. Sudan Black B dye was used to stain PHB inclusions in samples [blue-black cells: PHB (+), pink cells: PHB (-)] (Figure 3).

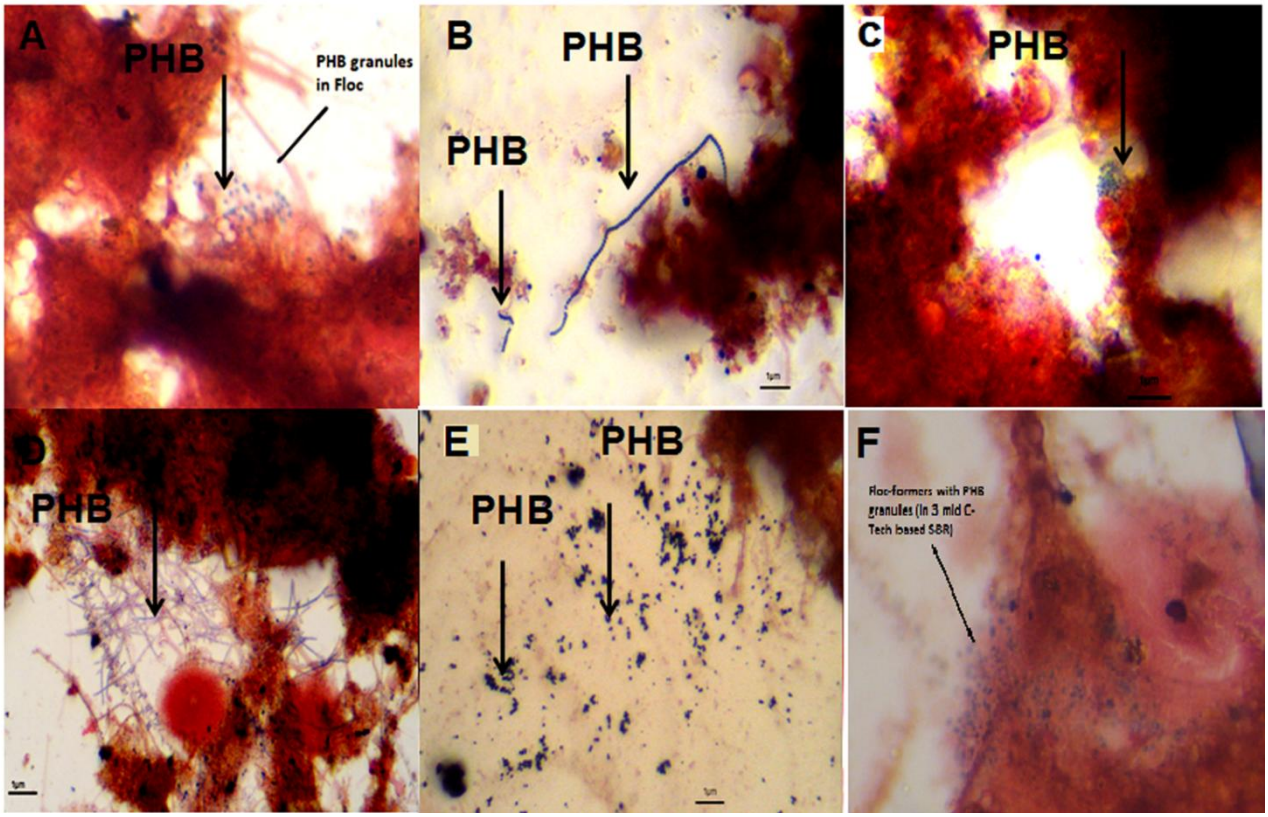

Figure 3: Bright-field micrographs of samples from the two SBR plants biomass: Case I. panels [A], [B], and [C] are from the SBR aeration tank of 120 MLD SBR plant, Goithaha; Panels [D], [E] and [F] are from the aeration tanks of 3 MLD SBR plant and at the end of the anaerobic period. Sudan Black B was used to stain PHB inclusions seen in panels [Blue-black stained cells/ granules are PHB (+), and pink-red cells are PHB (-)]. Panels [A], [C], [E], and [F] denote the PHB granules inside the sludge flocs, and panels [B] and [D] signifies the blue-black colored PHB granules in the filaments observed at a magnification of 100X with immersion oil. All the panels are comprising of the same scale bars of 1 µm.

4.2 Poly-P visualization in the SBR plants

The qualitative analysis was conducted on both the plants in the SBR at the end of the anoxic/ anaerobic period and the aerobic period. The images were obtained at 10X, 20X, and 100 X magnifications with immersion oil using a light optika microscope (Italy) and software 'Optika proview.' The bright-field and phase-contrast images were gathered by placing 100 µL of sample on the slides, and the procedure followed was according to [6], [22], [24]. Polyphosphate globules/ blobs were present in between the sludge flocs. Bismarck brown dye with methylene blue and crystal iodine indicators was used to stain poly-P inclusions in samples [blue-purple-black cells: poly-P (+), yellow-brown cells: poly-P (-)]. Intracellular poly-P inclusions also referred to as volutin or metachromatic granules, are not noticeable unless stained. Methylene Blue is the active constituent of two stains commonly used in EBPR studies, Neisser staining (Figure 4).

EBPR sludge at the end of aerobiosis was examined using Neisser staining (bright field, 10X, and 100X). The arrow shows a typical cluster of possible poly-P accumulating bacteria containing poly-P granules; all cell stains are black-purple due to large polyphosphate content. The main groups of Neisser-positive bacteria can be differentiated into three types [15]. First, 'Filamentous bacteria' which stain completely grey-violet. This approximately at all times applies to *Nostocoida limicola* or Type 0092. The second types are filamentous bacteria, which contains blue-black colored polyphosphate globules. Without staining, these globules cannot be observed with a light microscope. They are undoubtedly detectable if a much higher magnification (electron microscopy) is used but noticeable at 100X magnification using light microscopy. These globules, which are observed in pairs, are an essential identification characteristic for *Microthrix parvicella* [15]. The third one can be the colonies of blue-black colored cells comprising of Bio-P bacteria. There are several differences in the way in

which these kinds of colonies stain through Neisser. The shade is sometimes much lighter, or only a fraction of the cell stains darkly [6].

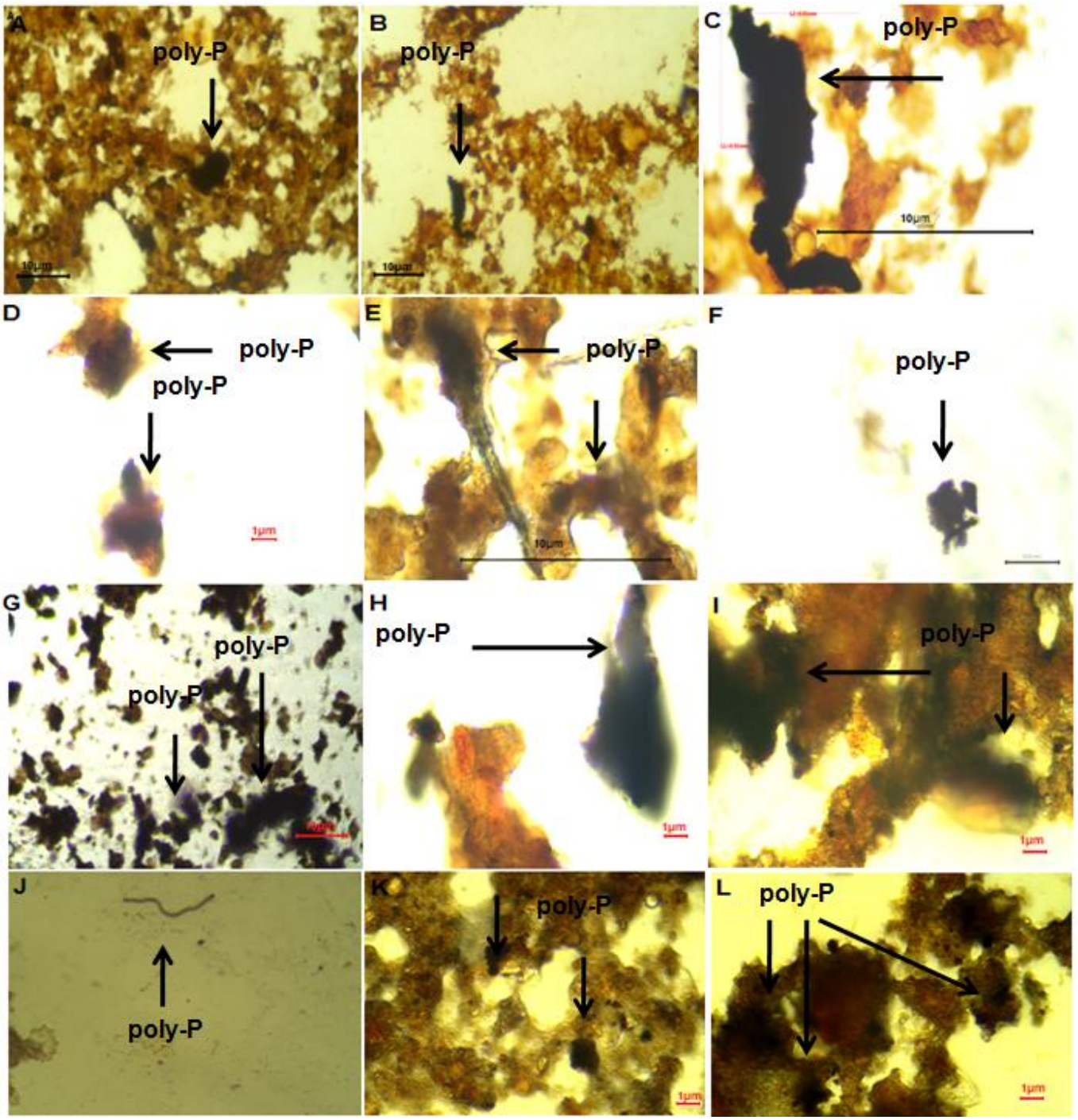

Figure 4: Bright-field micrographs of samples from the two SBR plants biomass: Case I. Panels [A] to [F] are from the aeration tanks of 3 MLD SBR plant and panels [G] to [L] are from the SBR aeration tank of 120 MLD SBR, Goithaha; at the end of the anaerobic period at a magnification of 10X, 20X and 100X (using immersion oil). Neisser staining (traditional dye Bismarck brown) was used to stain poly-P inclusions [Blue-purple-black stained cells/ blobs/ globules are poly-P (+), and yellow-brown cells are poly-P (-)]. Interpretation of these micrographs is summarized in Table 3.

Table 3: Summary of the results for Neisser and PHB staining experiments for SBR biomass

Figure 3 and 4	3 MLD SBR, Roorkee		120 MLD SBR, Goithaha Varanasi	
	End of Aerobic period	End of Anaerobic period	End of Aerobic period	End of Anaerobic period
PHB Staining	PHB (++-)	PHB(++-)	PHB (++-)	PHB(+++)
	(Protocol: Sudan Black B, blue-black granules- PHB(+) and pink-red cells- PHB (-))			
Poly-P Staining	Poly-P (++-)	Poly-P (+--)	Poly-P (++-)	Poly-P (++-)
	(Protocol: Neisser Staining, blue-purple-black cells- poly-P (+) and yellow/ brown cells- poly-P (-))			

*((+++) or (++-) is mentioned to emphasize these were stained strongly, and (---) shows those which stain hardly or not at all)

5. Typical Cycle Profiles of Nutrient Removal in SBR plants

The ammonia (NH4-N) removal was 96.7% (SND- 78%) and 96.4% (SND- 94%) in the Roorkee and Varanasi plants, respectively. The overall TN removal was 14% higher in 120 MLD SBR (85.1%) than 3 MLD SBR (71.1 %); however, both the plants were found successful in eliminating the TN from the effluent to <10 mg/L [18]. The total nitrogen removal was optimized by the DO and ORP levels in the successive anoxic/ anaerobic and aerobic stages in the plants. The C/N ratio also influenced the denitrification (SND) and TN removal in the plants. COD/TN ratio was 12% and 6% in the plants and was enough to provide sufficient TN removal. It has been concluded in researches that complete denitrification is achieved at TCOD: TKN ratio of 7 [16]. It was proposed that the COD: TN ratio for denitrification should be in the range of 3.5-4.5 g COD/ g N [16], [17]. It has also been proposed that the rbCOD content in COD highly governs the denitrification rate and SND [8], which was 12% and 17% in Roorkee and Varanasi plants, respectively. The role of storage products like PHBs also influenced the SND performance as they are the active substrate (slowly biodegradable form) for denitrification [23].

However, total phosphorus removal is quite complicated due to PAO/ GAO dominance competition. Several parameters are to be optimized like C/P ratio (specifically BOD_5/ TP and rbCOD/ TP), oxidation-reduction potential (ORP) in the anaerobic and aerobic zones, sufficient availability/ formation of volatile fatty acids (VFA) in the anaerobic zones or sewer lines, etc. for the proper functioning of PAOs in the plants. The availability of sufficient rbCOD concentration in the COD is essential to form VFAs via fermentation (at ORP <-200 mV) in the anaerobic selector compartments along with the suitable ORP range for fermentation of rbCOD, i.e., -200 to -300 mV [6].

In the Varanasi plant, EBPR% was around 51% compared to 18% in the Roorkee SBR. Excellent Bio-P was observed in the Varanasi plant compared to the Roorkee plant because all the parameters were successfully satisfied by the plant. It can be observed that the release of soluble phosphorus in the anaerobic phase was around 36.9 mg/L from the ~4 mg/L in the influent and subsequently removed to 1.4 mg/L in the effluent (Figure 5). The TP% in the biological sludge was 3% - 5%. The rbCOD/TP ratio was around 12, which was sufficient (10-20 mg rbCOD per mg TP removed) for the prevalence of PAOs [3]. While in Roorkee SBR, the rbCOD/ TP content is limited (~7.5) and has shorter sewer lines to supplement or compensate for the VFA sources. Even ORP has also reached only ~-90 mV in the anoxic/ anaerobic compartment due to continuous Return Activated Sludge mixing with the influent flow; hence lesser fermentation products (VFAs) may have been formed in the anaerobic zone for the PAOs growth. This might be the reason for inferior biological phosphorus removal in the plant. But, biological phosphorus removal has occurred in both the plants, more or less. Polyphosphate globules/ blobs signify the possible poly-P accumulating organisms in the SBR plants.

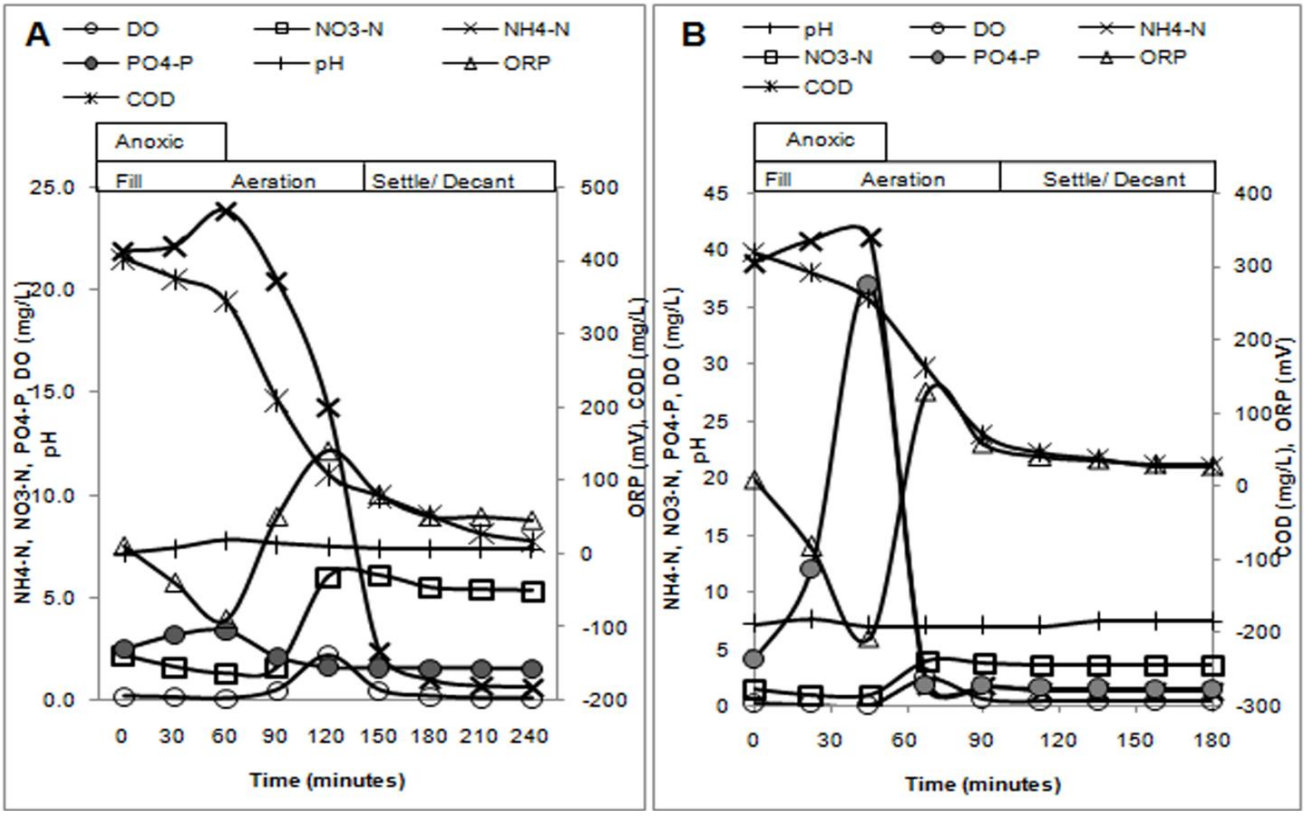

Figure 5: Time phase profiles of COD and nutrient removal with pH, DO, and ORP in SBR plants: (A) 3 MLD SBR plant (B) 120 MLD SBR plant

6. Sludge parameters

The operational sludge parameters are MLSS, MLVSS, and SVI in the SBR aeration tanks analyzed in the SBR plants according to *Standard Methods* [1]. The MLSS and MLVSS concentration in the Roorkee SBR plant are 7177 mg/L and 3527 mg/L, respectively, and 3778 mg/L and 2140 mg/L in the Varanasi SBR plant, respectively. The SVI is ~40.6 mL/g in the Roorkee SBR plant and ~34.4 mL/g in the Varanasi SBR plant. Both the plants were working satisfactorily in controlling filamentous organisms and proliferating floc formers. The protozoan diversity can be observed in table 4.

The microbiota (protozoa, metazoan, rotifers, etc.) represents an excellent sludge formation in the SBR plants. Protozoa are liable for the flocculation development, which outcomes in the biosorption occurrence of organic substances. These processes are essential in the treatment of conventional pollutants and micro-contaminant degradation. Hence, protozoa's presence or absence indicates the number of bacteria in the sludge and the degree of treatment [4]. Counting microbiota includes protozoa, metazoa, and filamentous organisms carried under a phase-contrast microscope at 10 X, 20 X; 40 X magnifications (table 4).

Table 4: Microbiota profile and the sludge conditions in the SBR aeration tanks (interpreted according to [2] and [4])

Protozoa species	Arcella	Vorticella	Paranema	Colpidium	Linototus	Aspidisca	Rotaria	Opercularia	Filamentous
3 MLD SBR	100-1000*	100-1000	Nil	Nil	10-100	10-100	5-10	10-100	10-100
120 MLD SBR	100-1000	10-100	5-10	Nil	Nil	10-100	5-10	10-100	5-10
What they Indicate	Satisfactory water quality and occurrence of nitrification in the aeration tank	Satisfactory floc & water quality	Satisfactory water quality. The population doesn't become large.	The sludge condition is not satisfactory.	It appears from the time the load is high until the condition becomes good.	It disappears when water quality becomes satisfactory.	Nitrification is occurring.	The sludge condition is satisfactory.	It leads to sludge bulking when higher in number.

*****These numbers indicate protozoans/ mL of activated sludge sample

7. Conclusion

The results show that 120 MLD SBR was functioning better in removing the contaminants from the wastewater compared to 3 MLD SBR because most of the conditions regarding SND and EBPR mechanism are prevailing inside the plant to increase the nitrifiers, denitrifiers, possible polyphosphate accumulating organisms. However, microbial studies are essential to confirm and critically evaluate these results effectively. Moreover, specific protozoan, metazoan, and rotifers like arcella, vorticella, and opercularia were identified, responsible for benefitting excellent sludge settling characteristics in the plant. Filamentous bacteria were found lesser, which indicates the prevalence of floc formers and no bulking sludge (SVI< 50 mL/ g). The PHB and poly-P staining show the abundance of the intracellular substrate and PO4-P storage inside the bacterial cells confirming good SND and EBPR potential in the plants. Some other conditions affect bio-P processes, which include optimized rbCOD/ TP, VFA/ TP ratios, and negative ORP (<-150 mV) in the anaerobic zones (i.e., anoxic/ anaerobic selector compartments) of advanced SBR plants.

References

[1] APHA, AWWA, WPCF. "Standard Methods for the Examination of Water and Wastewater." 22nd Edn. American Public Health Association, Washington, DC. 2005.

[2] Bhatia, A., Singh, N. K., Bhando, T., Pathania, R. and Kazmi, A. A. "Effect of intermittent aeration on microbial diversity in an intermittently aerated IFAS reactor treating municipal wastewater: A field study." Journal of Environmental Science and Health, Part A. 52, pp. 440-448. 2017.

[3] Broughton A, Pratt S, Shilton A. Enhanced biological phosphorus removal for high strength wastewater with a low rbCOD:P ratio. Bioresource Technology 99(5): 1236-41.2008. DOI: 10.1016/j.biortech.2007.02.013

[4] Chigusa, K. "Biota in Activated sludge (Maintenance and Control of Activated Sludge by the Biota)." Nishihara Environmental Sanitation Research Corporation, Ltd. 1990.

[5] Drewnowski J, Remiszewska-Skwarek A, Fudala-Ksiazek S, Luczkiewicz A, Kumari S & Bux F. "The evaluation of COD fractionation and modeling as a key factor for appropriate optimization and monitoring of modern cost-effective activated sludge systems." Journal of Environmental Science and Health, Part A, 54:8, 736-744, 2019. DOI: 10.1080/10934529.2019.1592531.

[6] Ge, Y., Zhao, L., and Zhou, Y. "Detection and Analysis of Intracellular Polymers Stored by Polyphosphate-accumulating Microorganisms." Advanced Materials Research Vol. 777. pp 314-317 doi:10.4028/www.scientific.net/AMR.777.314, 2013.

[7] Goronszy, M. C. In Course Notes on "Intermittently operated activated sludge plants." Department of Chemical engineering, University of Queensland, Australia 1992.

[8] Khursheed, A., Gaur, R. Z., Sharma, M. K., Tyagi, V. K., Khan, A. A. and Kazmi, A. A. "Dependence of enhanced biological nitrogen removal on carbon to nitrogen and rbCOD to sbCOD ratios during sewage treatment in sequencing batch reactor." Journal of Cleaner Production, 171, pp. 1244-1254. 2018.

[9] Magdum, S. S., Varigala, S. K., Minde, G. P., Bornare, J. B., Kalyanraman, V. "Evaluation of sequencing batch reactor (SBR) cycle design to observe the advantages of selector phase biology to achieve

maximum nutrient removal." International Journal of Scientific Research in Environmental Sciences, 3(6), pp. 234-238. 2015.
[10] Mangrum, C. R. L., 1998. "The effect of anoxic selectors on the control of activated sludge bulking and foaming." Masters' thesis submitted to the Faculty of Virginia, Polytechnic Institute and University Lancaster, Pa.: Technomic Pub. Co. 1998.
[11] Metcalf and Eddy, "Wastewater Engineering Treatment, Disposal and Reuse", 4th ed. McGraw-Hill, Indian Edition. 2003.
[12] Miao, L., Wang, S., Zhu, R., Cao, T. and Peng, Y. "The effect of oxygen supply on nitrogen removal via nitrite using stored substrate (PHB) as the electron donor in SBRs." Biochemical Engineering Journal, 103, pp. 130-137, 2015.
[13] Mino, T., Tsuzuki, Y. and Matsuo, T. "Effect of phosphorus accumulation on acetate metabolism in the biological phosphorus removal process." In: Biological Phosphate Removal from Wastewaters (Ramadori, R., Ed.), pp. 27-38. Pergamon Press, Oxford. 1987.
[14] Mustafa, I. H., Ibrahim, G., Elkamel, A. and Elahwany, A. H. "Modeling of Activated Sludge Floc Characteristics." American Journal of Environmental Sciences, 5 (1), pp. 69-79, 2009.
[15] Neisser and Gram Staining. https://www.asissludge.com/NeisGram.htm
[16] Pochana, K. and Keller, J. "Study of factors affecting simultaneous nitrification and denitrification." Wat. Sci. Tech., 39 (6), pp. 61-68. https://doi.org/10.1016/S0273-1223(99)00123-7. 1999a.
[17] Pochana, K., Keller, J. and Lant, P. "Model development for simultaneous nitrification and denitrification." Wat Sci Tech., 39(1), pp. 235-243. 1999.
[18] Recent Notified Effluent standards of National Green Tribunal (NGT). 2019
[19] Sathasivan, A. "Biological phosphorus removal processes for wastewater treatment." Water and wastewater treatment technologies. Encyclopedia of Life Sciences. 2009
[20] Seviour R. J., Mino T., and Onuki M. "The microbiology of biological phosphorus removal in activated sludge systems." FEMS Microbiology Reviews 27 pp. 99-127. 2003
[21] SFC Environmental Technologies Pvt Ltd. 2020. http://www.ctechsbr.com/c-tech/process_1.php
[22] Sharma, M. and Dhingra, H. K. "Isolation and Culture Conditions Optimization For PHB Production by Pseudochrobactrum asaccharolyticum." International Journal of Science and Research (IJSR) ISSN (Online): 2319-7064, 4(10), pp. 1895-1901. 2015.
[23] Third, K. A., Burnett, N. and Cord-Ruwisch, R. "Simultaneous Nitrification and Denitrification Using Stored Substrate (PHB) as the Electron Donor in an SBR." Wiley Periodicals, Inc., Biotechnology and bioengineering, 83(6), pp. 706-720. 2003.
[24] USEPA "Summary Report- The causes and control of Activated Sludge bulking and foaming." (1987)
[25] USEPA "Wastewater Technology Fact Sheet Sequencing Batch Reactors." (1999)
[26] Wentzel, M.C., Mbewe, A., Lakay, M.T., and Ekama, G.A. "Evaluation of a modified flocculation filtration method to determine wastewater readily biodegradable COD." WISA, Biennial Conference, Sun City, South Africa. 2000. http://citeseerx.ist.psu.edu/viewdoc/summary?doi=10.1.1.517.5571

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