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ORIGINAL PAPER

Influence of variations in wastewater on simultaneous nutrient removal in pre-anoxic selector attached full-scale sequencing batch reactor

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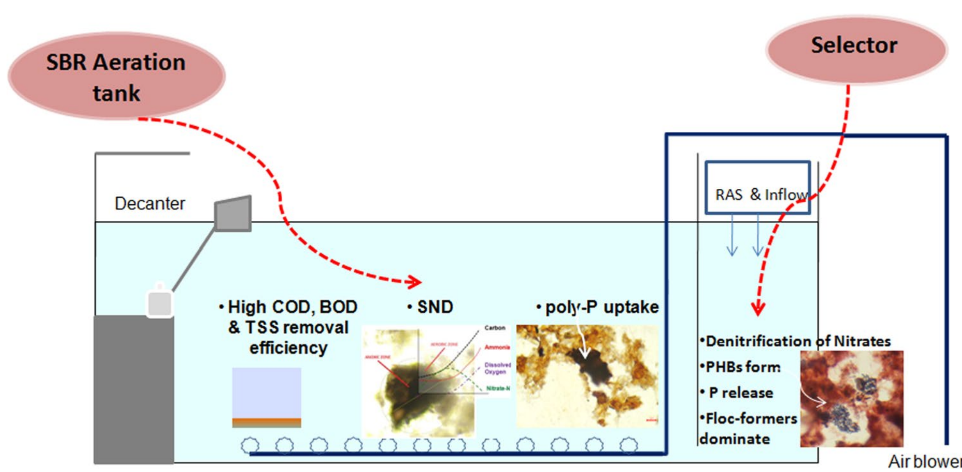
Abstract
In addition to many other well-documented factors, local conditions are rudimentary conditions of sharp change observed in wastewater characteristics from place to place. The monitoring of 3 million liters per day-capacity full-scale Sequencing Batch Reactor (SBR) at Roorkee (India) drew attention to the processes involving simultaneous nitrification and denitrification (SND) and biological phosphorous removal (BPR) undergoing with the variations in influent wastewater, particularly the readily biodegradable chemical oxygen demand (rbCOD). Regular monitoring of all the units revealed that the nutrient removal efficiencies were 94.9 ± 3.6% Chemical Oxygen Demand (COD) (17.9 ± 7.7 mg/L in effluent), 95.4 ± 2.7% Biochemical Oxygen Demand (BOD_5) (6.0 ± 2.2 mg/L in effluent), 95.4 ± 1.6% Total Suspended Solids (TSS) (9.4 ± 2.1 mg/L in effluent), 96.7 ± 2.6% Ammonia-N (0.7 ± 0.5 mg/L in effluent), 69.1 ± 11.5% Total Nitrogen (TN) (9.7 ± 3.0 mg/L in effluent), 31.3 ± 24.9% orthophosphate (1.8 ± 0.7 mg/L in effluent) and 42.0 ± 15.3% Total Phosphorus (TP) (3.6 ± 1.8 mg/L in effluent) and achieved < 50 MPN/100 mL fecal coliform in the final effluent after disinfection. Anoxic tri-sectional selector and an aeration tank constituted one SBR followed by the other, availed 76.4 ± 9.2% SND at rbCOD/COD of 0.12 ± 0.04 and showed linear relationship at $R^2 > 0.8$, and COD/TN of 12.3 ± 4.7. The study clarifies the degree of variations in key factors included in design guidelines for laying out an optimized treatment system for COD, Nitrogen, and Phosphorus removal in the Indian scenario.

Graphical abstract



Keywords Biological phosphorus removal · Oxidation–reduction potential · Readily biodegradable chemical oxygen demand · Simultaneous nitrification and denitrification · Total chemical oxygen demand

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Abbreviations

ATP	Adenosine triphosphate
bCOD	Biodegradable chemical oxygen demand
BOD_5	Biochemical oxygen demand
bpCOD	Biodegradable particulate chemical oxygen demand
BPR	Biological phosphorus removal
C/N	Carbon to nitrogen ratio
C/P	Carbon to phosphorus ratio
DO	Dissolved oxygen
EBPR	Enhanced biological phosphorus removal
FC	Fecal coliforms
GAOs	Glycogen accumulating organisms
HRT	Hydraulic retention time
MLD	Million liters per day
MPN	Most probable number
nbCOD	Non-biodegradable chemical oxygen demand
nbpCOD	Non-biodegradable particulate chemical oxygen demand
nbsCOD	Non-biodegradable soluble chemical oxygen demand
ORP	Oxidation–reduction potential
PAOs	Polyphosphate accumulating organisms
PCOD	Particulate chemical oxygen demand
PHA	Polyhydroxyalkanoates
PHB	Poly-β-hydroxy butyrates or polyhydroxy butyrates
Poly-P	Polyphosphates
rbCOD	Readily biodegradable chemical oxygen demand
sbCOD	Slowly biodegradable chemical oxygen demand
sBOD	Soluble biochemical oxygen demand (filtered from 0.45 µm sieve)
SBR	Sequential batch reactors
sCOD	Soluble chemical oxygen demand (filtered from 0.45 µm sieve)
SND	Simultaneous nitrification and denitrification
SRT	Solid retention time
STP	Sewage treatment plant
SV_{30}	Sludge volume in mL/L (after 30 min)
TC	Total coliforms
TCOD/or COD	Chemical oxygen demand
TKN	Total kjeldahl nitrogen
TN	Total nitrogen
TP	Total phosphorus
TSS	Total suspended solids
UBOD	Ultimate biochemical oxygen demand
VFA	Volatile fatty acids
VSS	Volatile suspended solids

Introduction

A comprehensive data of total organic matter present in the wastewater can be achieved by characterizing TCOD into various fractions. Additionally, the major characteristics of wastewater can be studied based on COD fractionations following ATV-A. (2000) guidelines, and subsequent modifications (Płuciennik-Koropczuk and Myszograj 2019). The TCOD of wastewater, segregated in fractions, can be calculated as the sum of rbCOD (metabolism), nbsCOD (observed in the treated effluent), bpCOD (i.e., sbCOD) (adsorption, hydrolysis, and metabolism) and nbpCOD (regarded in the sludge production) as g O_2 m^{-3} (Choi et al. 2017; Płuciennik-Koropczuk and Myszograj 2019). The substantial the amount of rbCOD, the faster the nitrate reduction rate (Metcalf and Eddy 2003; Khursheed et al. 2018). It has been investigated in different studies that C/N is an essential factor in biologically removing the nutrients (N and P) from domestic wastewaters, however, readily biodegradable content in the TCOD, in particular, directly affects the nutrient removal efficiency (Khursheed et al. 2018). Denitrifying bacteria require an optimum carbon source for succeeding in excellent denitrification, and therefore, they have to contend with other heterotrophs. Lesser C/N ratio in the influent effects in a rapid carbon discrepancy and consequences in unstable SND (Zhao et al. 2008; Phanwilai et al. 2020).

EBPR governs the prominent characteristic of uptake of organic matter and release of phosphorus in anaerobic states, and uptake of excess phosphorus under subsequent aerobic conditions (Seviour et al. 2003). High phosphorus is accumulated in the sludge by PAOs. Polyphosphates are reduced to supply ATP obligatory for the formation of PHB, and the degradation of polyphosphates is achieved by the discharge of PO_4-P, Mg, Ca, and K. (Davis 2013; Rosigkeit et al. 2021). The rbCOD concentration in the influent predicts more accurately the performance of biological nutrient removal, consists of complex soluble COD that can be fermented to VFA; therefore, initial rbCOD/TP ratio is a better indication of the EBPR's process and performance than the total COD to P ratio (Barnard et al. 2017). Hence, influent parameters like C/N, rbCOD/TCOD (/or rbCOD/sCOD), and rbCOD/TP play an essential role in enhancing the SND and BPR as observed in the study (Sager 2016; Majed and Gu 2019).

Limited literature is available based on investigating the influence of wastewater characteristics on the nutrient removal process's efficiency in SBRs, so novel findings in the present study may be helpful for further researches in this field (Gajewska et al. 2015). Even the effect of an anoxic selector on the SND and EBPR process has been lesser explored to date (Albertson 2002). As the main features concerning the activated sludge microbial diversity are the possible substrate composition of the incoming sewage and the ongoing significant operational variations in the treatment plant (Mielczarek 2012; Shchegolkova et al. 2016; Xu et al. 2019), therefore, this study aims to observe the influence of wastewater characteristics on Nitrification, Denitrification, and Biological Phosphorus removal in SBR and the role of internal storage products during these mechanisms. Concomitantly, the sludge biomass and wastewater microbiome were considered critically important.

The main objectives of this study include detailed performance evaluation of 3 MLD full-scale pre-anoxic selector attached sequencing batch reactor installed near IIT, Roorkee, and observing the role of influent wastewater parameters, i.e., rbCOD/TCOD, rbCOD/sCOD, COD/TN, BOD/TKN or sBOD/TKN, rbCOD/TN on SND efficiency and TN Removal and rbCOD/TCOD, rbCOD/sCOD, rbCOD/TP, COD/TP, sBOD/TP, and BOD/TP on TP and orthophosphate removal (biological phosphorus removal) in the plant. Regular monitoring of all the units in the SBR plant was performed from 13th August to 29th January (170 days). The study focused on identifying wastewater characteristics as an essential key factor/parameter for designing an optimized advanced SBR technology concerning biological nutrients (N and P) removal.

Materials and methods

This 3-MLD SBR has been set up in close vicinity to the residential area near the IIT, Roorkee campus, Uttarakhand (India). The essential features of this institutional STP are the deodorization system's additional odor control for sump well, pre-treatment units, and advanced tertiary treatment facility (Fibre Disc filtration and ultraviolet radiations and chlorine dosing for disinfection) [Fig. 1, Table S2 (Supplementary Material)]. The onsite monitoring of various parameters was performed in the bio-selectors and aeration tanks of the 3-MLD SBR Plant.

Physicochemical parameters'analysis

Onsite monitoring of DO, pH, ORP, and SV_{30} are executed regularly in the bio-selectors and aeration tanks of the 3 MLD SBR Plant. To determine the DO, temperature, and pH in the aeration tanks and selectors, a portable DO meter (Hach 110Q multimeter, Hach, USA) and pH meter (HQ11d pH Meter, Hach) was used (Srivastava et al. 2021b). ORP was measured by the convenient ORP meter (HQ11d ORP Meter, Hach). Complete performance evaluation of the plants for COD, sCOD, BOD_5, $sBOD_5$, TSS, VSS, NH_4-N, NO_3-N, TN, PO_4-P, TP, Total Coliforms, Fecal Coliforms, and Sludge operational parameters were performed according to Standard Methods (APHA 2005). The rbCOD was determined using the modified flocculation filtration method prescribed by Wentzel et al. (2000). SV_{30} was measured using the measuring cylinder and timer (Srivastava et al. 2021a). Grab samples of 0.5L were used for analyzing the parameters mentioned above, according to *Standard Methods* (APHA 2005). Regression analysis of all the variables was performed using the Microsoft Excel spreadsheet application.

Wastewater characteristics

The design quality of raw sewage for the SBR plant is shown in Table 1. The ratio between VSS to TSS was found around 0.53 ± 0.05. The overall SRT of the plant was approximately 15 days. The designed flow rate and HRT were 3.2 MLD and 18.11 h. The raw wastewater pH was 7.2 ± 0.3, and finally, treated effluent after disinfection was 7.4 ± 0.2 (Table S1, Supplementary Material). Regular sampling and analysis were conducted for five and a half months in this plant. The average daily flow rate was 1877 ± 573 m^3/d.

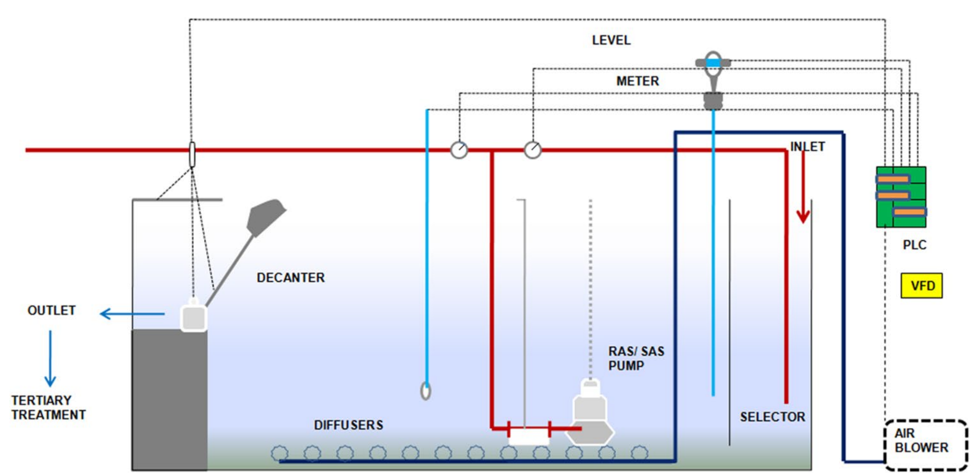

Fig. 1 The layout of the full-scale 3 MLD SBR STP comprising all units

Table 1 General designed and actual wastewater characteristics in 3 MLD full-scale SBR plant

Parameters	Designed quality	Actual quality
pH	5.5–9.0	7.2 ± 0.3
Total COD (mg/L)	450	401 ± 129
BOD_5 (mg/L)	200	163 ± 57
TSS (mg/L)	407	237 ± 79
TKN (as N) (mg/L)	34	33 ± 9
TP (as P) (mg/L)	7	6.1 ± 2.4

Design of aeration tank and multi-cell selector

In the full-scale SBR, there are two aeration basins, and each basin consists of a tri-compartmented anoxic selector. The design parameters of the aeration tank and selector are given in Table 2. The wastewater from the sump well (collection well) joins the first compartment of the pre-anoxic selector, where it mixes with the RAS. Then, after passing from the other two selector zones/compartments (partially anaerobic cum anoxic), the wastewater moves towards the SBR aeration basin. The water remains in the selector for 60 min as per the design conditions. The filling and the aeration process lasts for 120 min, and settling (45 min) and decanting (75 min) occur in the next 120 min of a cycle.

Total nitrogen balance

Mass balance calculations to be carried out mathematically for nitrogen balance in the plant. The following equation is being used:

$$\text{Mass of Influent TN} = \text{Mass of Effluent TN} + \text{Mass of TN denitrified} + \text{Mass of TN wasted with sludge} \quad (1)$$

where

$$\text{Total Nitrogen} = \text{Ammoniacal - N} + \text{Nitrites} + \text{Nitrates} + \text{Organic - N.} \quad (2)$$

The mass of total nitrogen in the dissipated sludge (kg/d) was computed by the product of daily sludge wasted in L/d

Table 2 (A) SBR Aeration Tanks design; (B) Selector Design

S. no	Parameters	Values (Unit)
(A) SBR aeration tanks design		
a	Number of basins	2 (numbers)
b	Average flow rate	133.33 (m^3/hr)
c	Total average flow = average flow + recycle flow	3.2 (MLD) = 3 + 0.2 (MLD)
d	Number of basins receiving flow simultaneously	1 (numbers)
e	Provided volume of SBR	2414.448 m^3 (length = 24.3 m width = 9.2 m depth = 5.4 m)
f	Total hydraulic retention time (HRT)	18.11 (hours)
g	Solids retention time provided (SRT)	15.18 (days)
h	Food/microorganisms (F/M)	0.089 (d^{-1})
(B) Selector design		
a	Recirculation flow	25%
b	Capacity of return activated sludge (RAS) and surplus activated sludge (SAS) pumps	35 (m^3/hr)
c	Design flow	168.33 (m^3/hr)
d	Volume provided	168.91 m^3 (side water depth = 5.4 m length = 9.2 m width = 3.4 m)
e	Number of sub-compartments per basin	3 (numbers)
f	Hydraulic retention time provided at design flow	60.0 (minutes)

(q wasted), MLSS of the wasted sludge, and a fraction (%) of total nitrogen contained in the sludge wasted. The fraction was around 1.6–2.7% in the sludge.

To estimate the denitrified part of TN, an indirect way was applied by deducting the Mass of TN in wasted sludge and the Mass of TN in the effluent from the Mass of TN in the influent wastewater. The incoming wastewater's flow rate 'Q' is 1.88 ± 0.6 MLD, while 'q waste' is a wastage flow rate of approximately 5.84×10^3 L/d with six cycles per day in the two SBR tanks. All the masses are taken as Kg/d, and mixed liquor suspended solids (MLSS) concentration in g/L.

Microscopic analysis for identification of protozoa, PHBs, and polyphosphates

Microscopic analysis for protozoa, metazoan, filamentous and foaming organisms, bacteria, sludge floc morphology was observed at 10, 20, 40, and 100× magnifications. Qualitative microscopic observations were carried out in a mixed liquor sample of the aeration tank. Aliquots of 25 µL sludge were examined under phase contrast (Radison RXLr5) illumination at 40× and 100× magnifications (Bhatia et al. 2017). Brightfield microscopic observations for PHBs and polyphosphates were obtained after staining the samples (USEPA 1987; Dulekgurgen et al. 2003a, b; Ong et al. 2014; Sharma and Dhingra 2015). PHB granules and polyphosphates globules identification is being carried out using Sudan Black B dye (Sudan Black B for staining PHB and Safranin O for counter-staining) and Neisser staining (Methylene blue and crystal violet for staining poly-P and Bismark Brown for counter-staining), respectively, at 100× magnification with immersion oil on the sludge samples from aeration tank and anoxic selector compartments. Brightfield micrographs/images were captured using a Light microscope (Optika microscope, Italy) connected with a personal computer containing software, 'PROVIEW.'

Results and discussion

Observation-based on the influence of wastewater and quantitative analysis

The overall performance evaluation of STP is presented in Table 3. The plant was efficient in removing COD, BOD, NH_4-N, TN, TSS, and Fecal coliform and achieved the latest effluent standards requirements (NGT 2019). The nutrient removal performance is described in detail in Sects. 3.2, 3.3, and 3.7, corresponding to the variations in readily biodegradable COD demand of bacteria.

Table 3 Summary of the performance evaluation of the SBR plant

S. No	Parameters	Influent wastewater	Finally treated effluent	Units
1	pH	7.2 ± 0.3	7.4 ± 0.2	
2	Alkalinity	350 ± 30	260 ± 20	mg/L
3	Color	Grey	Colorless	
4	Odor	Foul	Odorless	
5	COD	401 ± 129	17.9 ± 7.7	mg/L
6	sCOD	140 ± 45	10 ± 6	mg/L
7	BOD	163 ± 57	6 ± 2	mg/L
8	sBOD	63 ± 28	3.1 ± 1.5	mg/L
9	NH_4-N	21.8 ± 5.8	0.7 ± 0.5	mg/L
10	NO_3-N	0.8 ± 0.8	5.6 ± 1.8	mg/L
11	TKN	32.8 ± 9	4.2 ± 2.9	mg/L
12	TN	33.6 ± 9	9.7 ± 3.0	mg/L
13	PO_4-P	2.7 ± 1.0	1.8 ± 0.7	mg/L
14	TP	6.1 ± 2.4	3.6 ± 1.8	mg/L
15	TSS	237 ± 79	9.4 ± 2.1	mg/L
16	VSS	127 ± 5	4.9 ± 1.5	mg/L
17	TC	3,600,000 ± 80	5400 ± 10	MPN/100 mL
18	FC	160,000 ± 13	35 ± 9	MPN/100 mL

Advances in the aeration equipment and oxygen uptake rate controls have facilitated SBRs to successfully contend with conventional activated sludge processes (USEPA 1999). The enhanced SBR technologies have gained attention globally for biological nutrients (N and P) removal (Dutta and Sarkar 2015). A comparative description among different sewage treatment plants with similar treatment processes is presented in Table S6 (Supplementary material). Ghehi et al. 2014 evaluated a lab-scale enhanced SBR performance on synthetic wastewater. At C:N:P of 100:5:1, 50:5:1, and 25:5:1, they observed > 89% COD removal, > 59% TN removal, and 22–98% TP removal that can be compared with this study at an average COD:TN:TP of 65.7:5.5:1. Showkat and Najar (2019) have performed their analysis on 16.1 MLD full-scale SBR over domestic wastewater, and 63.8% COD, 64.9% NO_3-N, and 68.4% TP removal were observed. Mahvi et al. 2004 worked on continuous flow SBR in Tehran, Iran, and observed 93.0–94.9% COD, 96.8–97.7% BOD, 57.9–71.4% TN, and 38.5–55.9% TP removal. Their results were quite similar to this study (pre-anoxic selector attached SBR) on domestic wastewater. In the pilot-scale SBR plant of Magdum et al. 2015, the selector-phase biological study was carried out, and excellent TP removal (97.6%) was observed along with > 90% COD and > 88% TN removal in their plant.

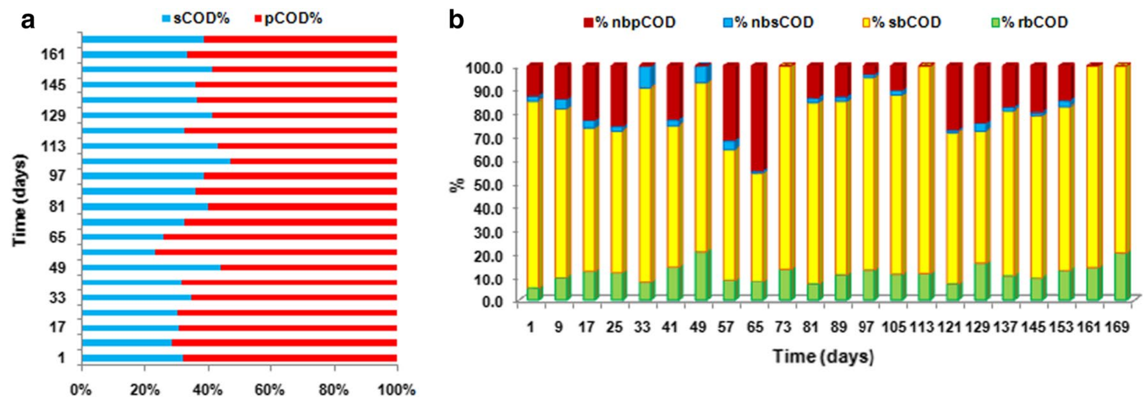

Fig. 2 Wastewater characterization of 3 MLD SBR- (**a**) soluble and particulate fractions of COD, **b** bars showing different COD fractions analyzed in the SBR plant

According to Magdum et al. 2015, selecting the phases as anoxic, anaerobic, and aerobic in a single tank reactor for a definite time interval helps the biology achieve efficient nutrient removal.

After the basic wastewater characteristics and analysis, thorough wastewater characterization was performed to estimate the various portions (fractions) of total COD in the 3 MLD SBR plant (Fig. 2). Table S3 (Supplementary Material) shows the methodology used to determine different COD fractions in the plant. Wastewater characterization of 3 MLD SBR, IITR, resembles the wastewater characteristics reported by Rossle and Pretorius (2001). Table 4 gives us an estimated range of different COD fractions in reported studies.

COD, BOD_5, and TSS removal

The full-scale SBR plant designed for 3.2 MLD flow (average flow of 3 MLD and Recycle discharge of 0.2 MLD) was operated under HRT of 18.1 h for BOD_5, COD, and TSS removal during the study period. The plant achieved the targeted design quality of treated wastewater for BOD and TSS $\leq$ 10 mg/L and COD $\leq$ 50 mg/L. There was no chemical addition except chlorine (bleaching powder) for disinfection. The plant has shown excellent results since commissioning and has attained reasonably low values of operating parameters in the effluent (Figure S1, Supplementary Material). COD, BOD_5 and TSS removal were 94.9 $\pm$ 3.6%, 95.4 $\pm$ 2.7%, and 95.4 $\pm$ 1.6%, respectively, in the 3-MLD SBR plant.

Nitrogen removal, the effect of C/N, and the effect of variations on the denitrification rate and TN removal

The overall nitrification of ammonia was 96.7 $\pm$ 2.6%; total nitrogen removal was 69.1 $\pm$ 11.5%. During the sampling period, the plant runs efficiently with total nitrogen in effluent achieved $\leq$ 10 mg/L. Figure S2 (Supplementary Material) shows the temporal variation in ammonia, nitrate, and total nitrogen (TN) in the influent and effluent of the plant. Figure 3 illustrates the effect of the C/N ratio on the total nitrogen removal and denitrification rate of the plant.

The average TN in the effluent was 9.7 $\pm$ 3.0 mg/L. It was observed that the COD:TN ratio was independent with TN Removal % and effluent TN values and represented

Table 4 COD fractions in municipal raw wastewater

Location of incoming wastewater	rbCOD (Ss) %	nbsCOD (Si) %	bpCOD (sbCOD) (Xs) %	nbpCOD (Xi) %	Reference
Flawil, Switzerland	10–20	7–11	53–60	7–15	Kappeler and Gujer (1992)
Istanbul, Turkey	9	4	77	10	Sozen et al. (1998)
Zielona Góra, Poland	50.0–61.7	2.2–6.0	22.0–34.4	8.0–16.2	Płuciennik-Koropczuk et al. (2017)
South Africa	20–25	8–10	60–65	5–7	Ekama et al. (1986)
Kielce, Poland	24–32	8–11	43–49	11–20	Henze et al. (2002)
South Africa	8–25	4–10	50–77	7–20	Rossle and Pretorius (2001)
India (3 MLD SBR STP, IIT Roorkee)	11.7 $\pm$ 4.0	3.2 $\pm$ 3.1	70.6 $\pm$ 10.5	14.6 $\pm$ 12.4	Present study

*Municipal wastewater (primary effluent of domestic and industrial origin) statistically insignificant linear decreasing functions ($p > 0.05$). However, at COD:TN ratios < 11, the TN in the effluent was < 10 mg/L. According to Pochana and Keller (1999); Ge et al. 2010 and Sadowski (2015), entire denitrification can be attained at a COD:TKN ratio of 7, which is also observed in the present study. Generally, at least a value of 9 is obligatory for accomplishing biological nutrient removal (Pochana and Keller 1999; Curtin et al. 2011). The optimized COD:TN range in which the best TN removal of ~ 82.5% was observed at COD:TN of 9.13.

To carry out the denitrification process during biological treatment, the presence of readily biodegradable organic carbon is an indispensable factor (Randall et al. 1992; Gajewska et al. 2015). Under anaerobic/anoxic conditions, the denitrification capacity of nitrates is evaluated by the requirement of available carbon source, which is managed by the readily biodegradable fraction of COD (Tas et al. 2009). Operation data from 3 MLD SBR showed the effects of the influent rbCOD:TN ratios on the effluent Nitrate concentrations operating in SND mode (Fig. 3), and the relationship showed a decreasing linear trend. However, it lacked the statistical significance as $p \geq 0.05$. But, it can be observed that higher rbCOD specifically is needed to achieve denitrification and which strongly influences SND performance (Pochana and Keller 1999; Jimenez et al. 2010). In this STP, also higher rbCOD:TN ratio above 2.0 showed higher SND (> 80%). Like rbCOD:TN ratio, the BOD_5:TKN and $sBOD_5$:TKN ratio also showed effects on denitrification rates and TN removal in the plant, but the relationships lacked statistical significance ($p \geq 0.05$). Still, it was interpreted that at a higher BOD_5:TKN ratio above 6.0, more than 80% denitrification was achieved, and when the BOD_5:TKN fraction dropped below 2, lesser denitrification was attained (Jimenez et al. 2010). Even at higher values of $sBOD_5$:TKN > 3.0, better and consistent TN removal was observed in the plant, and effluent TN reached the stabilized results of 8.1 ± 2.2 mg/L, while at lower values of $sBOD_5$:TKN < 1.6 showed higher TN in effluent 11.1 ± 3.9 mg/L (Fig. 3).

Relationship with rbCOD (readily biodegradable COD) and simultaneous nitrification and denitrification undergoing in the plant

Denitrifiers are recognized to struggle in search of carbon supply amid other heterotrophs; a lesser C/N ratio in the incoming wastewater outcomes in a quick carbon shortage, originating unstabilized SND (Kim et al. 2008; Zhao et al. 2008). SND in the SBR plant was 76.4 ± 9.2%, where average ammonia from the influent wastewater was removed from ~ 21.8 to ~ 0.7 mg/L in the effluent. At the same time, nitrate observed in the influent and effluent was ~ 0.9 mg/L and ~ 5.6 mg/L, respectively. Detailed analysis of 3 MLD SBR, IIT Roorkee, was performed to analyze the relationship between rbCOD/TCOD (%) and SND (%) (Fig. 4). Figure 4a can be considered as statistically significant ($p < 0.05$ and $R^2 > 0.8$, Table 5). This signifies that there is a dependency between rbCOD fraction in total COD of influent wastewater and simultaneous nitrification and denitrification (%).

Total nitrogen balance

In the SBR, a typical total nitrogen (TN) balance has been observed (Fig. S3, Supplementary Material). The TN in the inlet 79.4 ± 26.1 kg/d gets distributed in three parts during biological treatment; (a) some quantity of it went to the dissipated sludge: 13.5 ± 7.3% (9.7 ± 4.1 kg/d) (b) some part left untreated in the effluent: 30.2 ± 11.3% (22.4 ± 7.3 kg/d), and (c) the remaining (most significant part) found is released as N_2 gas via denitrification: 56.3 ± 14.7% (47.3 ± 24.5 kg/d) (Srivastava and Kazmi 2020). According to primary treated water, the removal of nitrogen by incorporation in sludge during domestic wastewater treatment varies from 8 to 20%, and noticeable outcomes were observed in the study attributed to ~ 13.5% removal by assimilation (Srivastava and Kazmi 2020). After biological treatment, the quality of the sludge showed excellent settling features, i.e., SVI < 50 mL/g and SV_{30} of 250–350 mL/L.

Phosphorus removal, the effect of C/P, and the impact of variations

TP and PO_4-P in influent were 6.1 ± 2.4 mg/L and 2.7 ± 1.0 mg/L and TP and PO_4-P in effluent were 3.6 ± 1.8 mg/L (removal 42.0 ± 15.3%) and 1.8 ± 0.7 mg/L (removal 31.3 ± 24.9%), respectively (Fig. S4, Supplementary Material). In biological phosphorus removal systems, PAOs uptake organic substrate, PHB formation occurs by sequestering rbCOD and exogenous BOD, and PO_4-P is released in anaerobic conditions. PAOs take up rbCOD in the form of VFA in the anaerobic phase, VFAs got converted into PHA (or PHB) through hydrolysis of glycogen, which is the only means of energy for PAOs, intended for this mechanism (Mino et al. 1998). At the same time, PHB degradation occurs in the aerobic zone; PAOs uptake phosphate and form polyphosphates in cells during the oxic condition, and biological phosphorus removal occurs. Therefore, the C/P ratio in the wastewater is considered an important parameter regarding biological phosphorus removal in the treatment plants (Isaacs and Henze (1995)). The rbCOD concentration in the influent COD predicts the biological phosphorus removal process's performance more accurately than the complex soluble COD that can be fermented to VFA (Broughton et al. 2008). The variations in different C/P ratios and effluent TP and PO_4-P in the effluent are shown

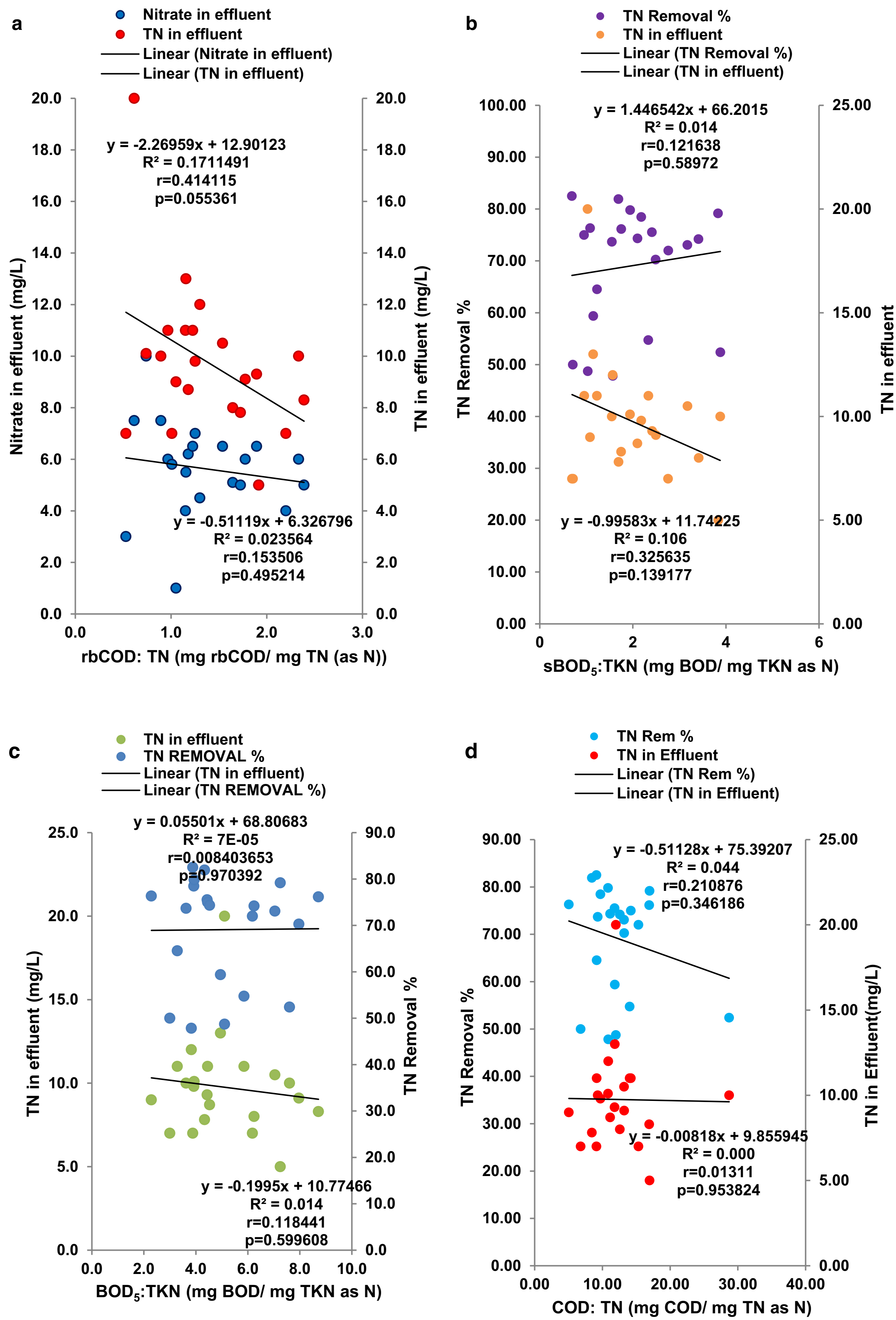

Fig. 3 Effects of (**a**) rbCOD/TN on the effluent NO_3-N and effluent TN, and **b** $sBOD_5$/TN, **c** BOD_5/TN, and **d** COD/TN on the effluent TN and TN Removal% during SND activity in SBR

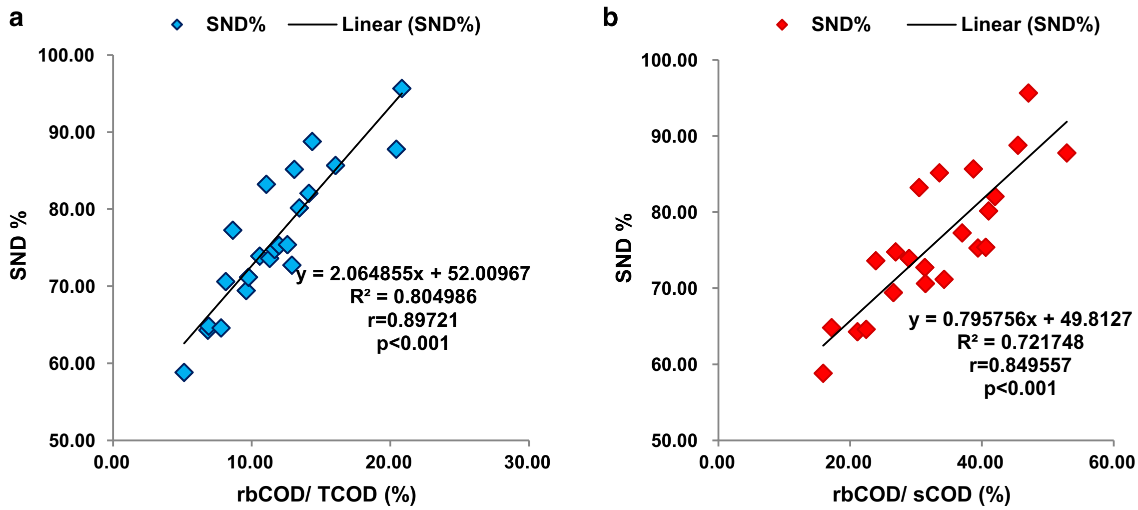

Fig. 4 Relationships between (**a**) SND% with rbCOD/TCOD (%), and **b** SND% with rbCOD/sCOD (%) in 3 MLD SBR plant

in Fig. 5. The total and soluble phosphorus removal can be found dependent on rbCOD/TP, and BOD/TP ratios of the influent ($p < 0.05$) but statistically insignificant ($R^2 < 0.8$).

Relationship with readily biodegradable COD and biological phosphorus removal undergoing in the plant

The fraction of rbCOD to TP is an improved implication of the biological phosphorus removal process performance besides the total COD:TP ratio suggested in classic models (Broughton et al. 2008). In the EBPR process, the soluble readily biodegradable fraction of COD gets fermented to VFA in the anaerobic zone (Majed and Gu 2019). The examined stoichiometry requisite of carbon for an elemental quantity of phosphorus to be removed has subsisted in the range of 10–20 mg rbCOD/mg P eliminated (Barnard et al. 2017). Elevated rbCOD/P ratios, i.e., 40–50 mg rbCOD/mg P, have been perceived to be related to GAO-controlled diversity, and smaller ratios < 10–20 mg rbCOD/mg P have been related with PAO led-community (Broughton et al. 2008). In 3 MLD SBR plant mg rbCOD/mg TP ratio was on an average 9.6 ± 4.8 and mg COD/mg TP and mg BOD_5/mg TP ratio were observed as 81.8 ± 37.2 and 33.3 ± 14.5, respectively. Because of the rbCOD/TP ratio's unfulfilled requirement of 10–20, TP Removal was 42.0 ± 15.3%, and EBPR was only 17.8 ± 17.3%. Enhanced uptake is being ascertained after excluding the 1/100th part of mg BOD/L from Total Phosphorus removed, i.e., exceeded from PO_4-P uptake by ~ 2.67% of cell biomass. Figure 6 exhibits the effect of rbCOD/TCOD (%) and rbCOD/sCOD (%) on the total phosphorus and orthophosphate removal of the plant and showed dependency based on statistical analysis, but R^2 was obtained as < 0.8.

Zone-wise, PO_4-P removal is illustrated in figure S7 (Supplementary Material). The release of phosphate is observed as 26.4% in the anoxic selectors, and then a reduction in the aeration tanks was observed as 52.9%, which showed uptake of 26.5%. Return Activated Sludge (RAS) in nitrifying processes planned to eliminate ammonia includes considerable nitrate concentrations that are not suited to two-stage (anoxic-aerobic) EBPR systems. In the following circumstances, prerequisites must be taken care of for denitrifying the return solids to circumvent negotiating the anaerobic zone's integrity, which might be fulfilled by having one or more anoxic phases (Minnesota Pollution Control Agency 2006). Other than the requirements of rbCOD:TP ratio, the necessary conditions of EBPR are VFA to TP ratio should be more than 7, and pH supposed to be between 8.0–8.5 and 7.0–7.5, for anaerobic and aerobic zones, respectively, for efficient EBPR process (Mino et al. 1998). ORP in the anaerobic, anoxic, and aerobic zones should cover the range of − 100 mV to − 200 mV, − 50 mV to + 50 mV, and + 100 to + 300 mV, respectively (Burkhardt 2012). Acid formation from the fermentation of rbCOD occurs at an ORP of − 100 to − 250 mV (Goronszy 1992; Goronszy et al., 1996). Even the sludge's phosphorous content should be reasonably more significant than the stoichiometric value, and P content in

Table 5 Summary of statistical analyses

S. no	Regression equations with std err. for coefficient	Factor x	Factor y	F value	p value of F test	R^2	Adjusted R^2	p value of t test for intercept	p value of t test for x
1	$y=-2.26959*x+12.90123$ (1.115, 1.654)	RBCOD/TN	TN in effluent	F (1, 20) = 4.139	0.055361	0.1710	0.130	*1.72E-07*	0.055361
2	$y=-0.51119*x+6.326796$ (0.736, 1.091)	RBCOD/TN	Nitrate in effluent	F (1, 20) = 0.4826	0.495214	0.0235	−0.02526	*1.13E-05*	0.495214
3	$y=1.446542*x+66.2015$ (2.639, 5.824)	sBOD/TKN	%TN Removal	F (1, 20) = 0.3003	0.58972	0.0148	−0.03446	*3.52E-10*	0.589721
4	$y=-0.99583*x+11.74225$ (0.646, 1.427)	sBOD/TKN	TN in effluent	F (1, 20) = 2.372	0.139177	0.1060	0.06134	*7.5E-08*	0.139177
5	$y=0.055019*x+68.80684$ (1.464, 7.889)	BOD/TKN	%TN Removal	F (1, 20) = 0.001413	0.970392	7.06214E-05	−0.04992	*2.99E-08*	0.970392
6	$y=-0.1995*x+10.77466$ (0.374, 2.015)	BOD/TKN	TN in effluent	F (1, 20) = 0.284558	0.599608	0.014028	−0.03527	*3.12E-05*	0.599608
7	$y=-0.51128*x+75.39207$ (0.5299, 6.977)	CODTN	%TN Removal	F (1, 20) = 0.930762	0.346187	0.044469	−0.00331	*8.48E-10*	0.346187
8	$y=-0.008*x+9.855$ (0.1395, 1.8363)	COD/TN	TN in effluent	F (1, 20) = 0.003438	0.953824	0.000172	−0.04982	*2.97E-05*	0.953824
9	$y=2.064855*x+52.00967$ (0.2272, 2.800459)	rbCOD/COD	SND%	F (1, 20) = 82.5568	1.55E-08	0.804986	0.795235	*4.42E-14*	*1.55E-08*
10	$y=0.795756*x+49.8127$ (0.110482, 3.806287)	rbCOD/sCOD	SND%	F (1, 20) = 51.87718	5.67E-07	0.721748	0.707835	*2.89E-11*	*5.67E-07*
11	$y=-0.26999*x+6.1833499$ (0.06309, 0.673584)	rbCOD/TP	TP in effluent	F (1, 15) = 18.31442	0.000658	0.549745	0.519728	*1.52E-07*	*0.000658*
12	$y=-0.09968*x+2.758515$ (0.027232, 2.758515)	rbCOD/TP	PO_4-P in effluent	F (1, 15) = 13.39813	0.00232	0.471796	0.436583	*9.94E-08*	*0.00232*
13	$y=-0.09423*x+6.726807$ (0.019791, 0.714422)	BOD/TP	TP in effluent	F (1, 15) = 22.67038	0.000252	0.601809	0.575263	*1.1E-07*	*0.000252*
14	$y=-0.02951*x+2.783449$ (0.009909, 0.357682)	BOD/TP	PO_4-P in effluent	F (1, 15) = 8.868409	0.009384	0.371554	0.329658	*1.21E-06*	*0.009384*
15	$y=-0.18275*x+6.165551$	sBOD/TP	TP in effluent	F (1, 15) = 20.88007	0.000369	0.581941	0.55407	*6.75E-08*	*0.000369*
16	$y=-0.03881*x+2.34843$	sBOD/TP	PO_4-P in effluent	F (1, 15) = 2.968921	0.105424	0.165225	0.10957	8.29E-06	0.105424
17	$y=-0.03309*x+6.301693$ (0.0087, 0.778351)	COD/TP	TP in effluent	F (1, 15) = 14.4674	0.00173	0.490963	0.45703	*7.43E-07*	*0.001730*
18	$y=-0.0085*x+2.497601$ (0.004336, 0.387946)	COD/TP	PO_4-P in effluent	F (1, 15) = 3.839045	0.068927	0.203781	0.15070	*1.12E-05*	0.068927
19	$y=1.821276*x+19.54589$ (0.848926, 11.00557)	rbCOD/COD	TP removal%	F (1, 15) = 4.602687	0.048699	0.234799	0.183785	0.096017	*0.048699*
20	$y=3.637007*x-13.6104$ (1.270361, 1.4691)	rbCOD/COD	PO_4-P removal%	F (1,15) = 8.196607	0.011851	0.353354	0.310244	0.421518	*0.011851*
21	$y=0.87139*x+12.5395$ (0.33902, 11.91141)	rbCOD/sCOD	TP removal%	F (1, 15) = 6.606549	0.021321	0.305766	0.259484	0.309129	*0.021321*
22	$y=1.493902*x-19.2681$ (0.53844, 18.918)	rbCOD/sCOD	PO_4-P removal%	F (1, 15) = 7.697851	0.01417	0.339144	0.295087	*0.021321*	*0.014170*
23	$y=42.50351*x+51.60973$ (34.821, 14.52146)	BOD/COD	TN removal %	F (1, 20) = 1.489934	0.236423	0.069332	0.022798	*0.00199*	0.236423
24	$y=70.09236*x+12.83828$ (47.16244, 19.96748)	BOD/COD	TP removal %	F (1, 15) = 2.208762	0.157943	0.128351	0.070241	0.529957	0.157943

The significance of values in italic is p level = 0.05

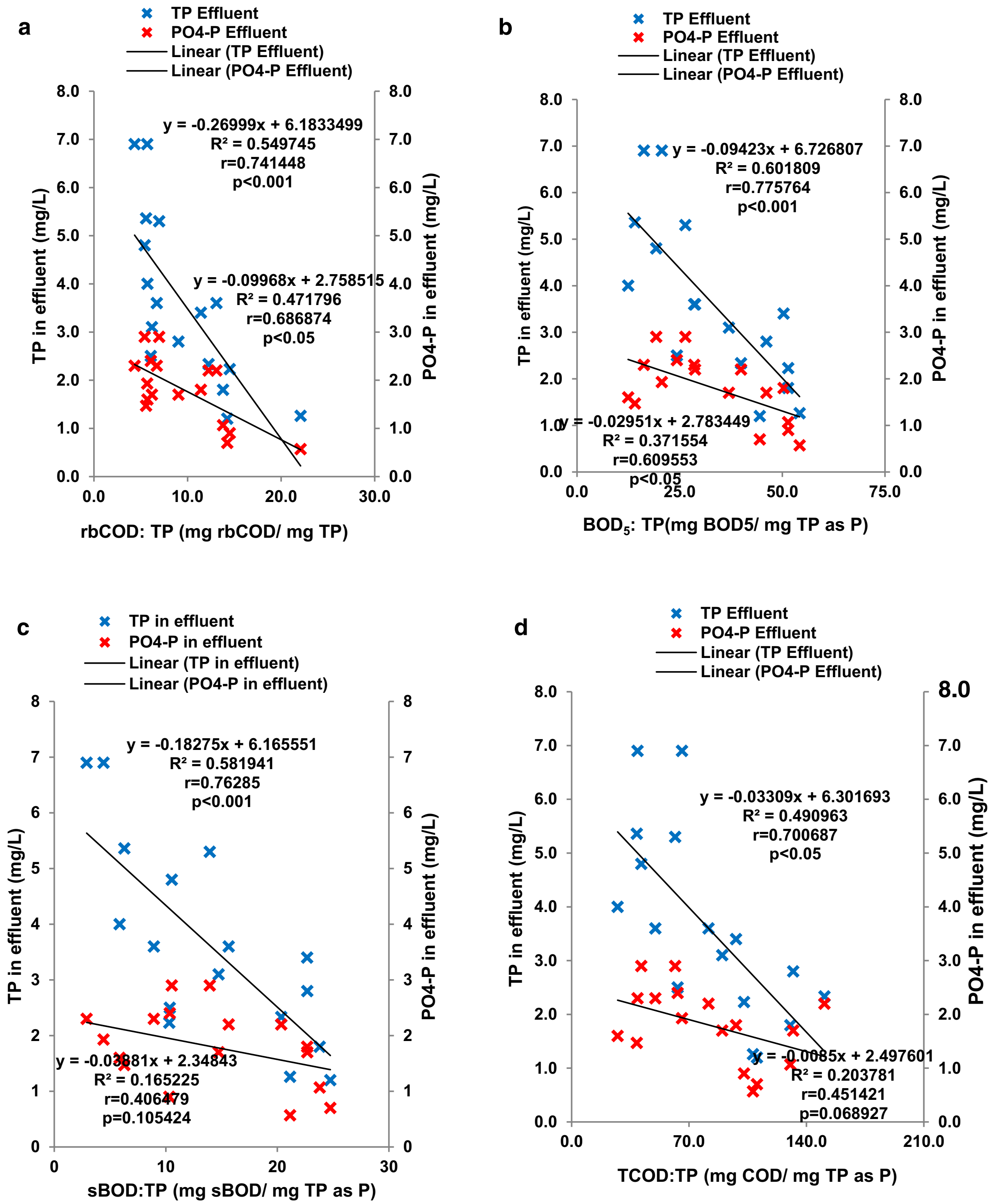

Fig. 5 Relationship between **a** rbCOD: TP, **b** BOD_5: TP, **c** $sBOD_5$: TP, **d** TCOD: TP and effluent TP and effluent PO_4-P in 3 MLD SBR plant

the 3 MLD SBR sludge was only 1.95 ± 0.80% of MLSS in the plant.

In the anaerobic zone, VFAs are stored inside the bacterial cell. PAOs use PHAs and PHBs in the aerobic process during a lack of exogenous substrates sequestering soluble phosphorus as polyphosphates (known as P uptake). This uptake is greater than the P released in anaerobic processes since substantial additional energy is generated by aerobic oxidation of the accumulated carbon compounds than used to conserve them in an anaerobic environment (Oehmen et al. 2007). Anaerobic long-covered sewer lines contain a high amount of VFAs and compensate for the need for a complete anaerobic chamber before SBR basins. Wastewaters that are more septic, from collection systems in warm

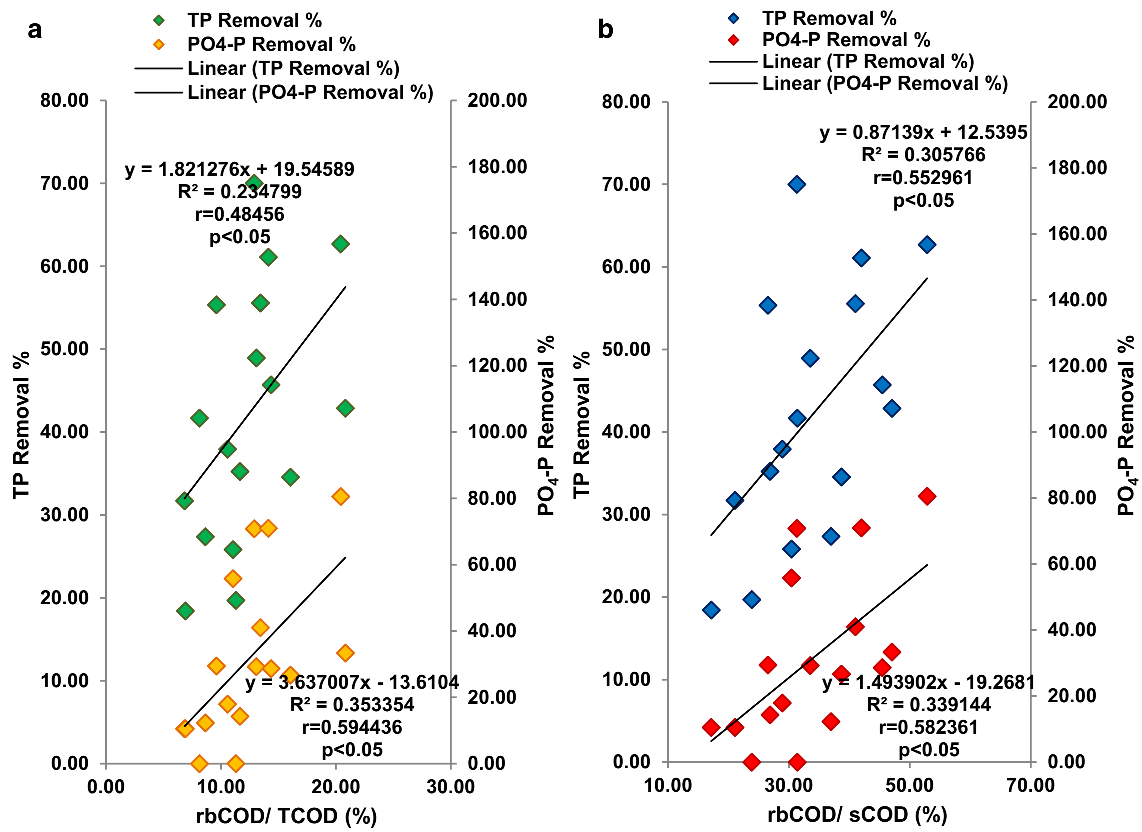

Fig. 6 Relationship between **a** TP, PO_4-P removal with rbCOD/TCOD (%) and **b** TP, PO_4-P removal with rbCOD/sCOD (%)

climates and minimal slope, will contain a high concentration of VFAs (Broughton et al. 2008). But, if fermentation could not happen in the collection system, it must occur in the anaerobic region so that EBPR can work sound. The hydraulic retention times of the anaerobic zone must fulfill the limit between 0.5 and 2.0 h (Burkhardt 2012). However, RAS's falling in the anoxic chamber of selectors dampens plants' productivity in removing TP biologically. If the anoxic selectors could not reach the particular requirement, sufficient formation of VFAs might not occur. VFAs' sources were observed inadequate for proper conditions of PAOs' growth and effective EBPR in the 3 MLD SBR plant. Although, some fermentation of rbCOD occurred at an ORP of -90 ± -24 mV in the third compartment of anoxic selectors of the SBR plant, and ~ 18% EBPR occurred.

Intracellular storage products formation during SND and EBPR

The prospectives for PHB to supply electrons for an efficient SND process can be observed in SBR plants (Miao et al. 2015). The slow degradation characteristics of PHB clarify that it is a deserving active substrate for the SND process (Third et al. 2003; Miao et al. 2015). Internally stored PHBs are removed much slowly than the soluble substrate and, therefore, can be employed as an electron donor for denitrification when exogenous carbon sources are not present (Table S4, Supplementary Material) (Third et al. 2003). The capacity of heterotrophs to quickly sequester the soluble substrate and conserve it as a slowly biodegradable polymer signifies expedient chances in preserving reducing power for SND. PHBs were found sufficient as granules within a filamentous sludge or inside the large flocs governing SND (Fig. 7).

During BPR, PAOs uptake readily degradable organic substrate, and PHB formation occurs by sequestering rbCOD, PO_4-P is released, and exogenous BOD is consumed in anaerobic conditions (takes place inside the anaerobic zone of the selector) (Mino et al. 1998). Then, the PAOs accumulate those released orthophosphates in their cell as poly-P and get energy from stored PHBs during the bio-P removal process occurring in aeration tanks (Figure S5, Supplementary Material). PHA, glycogen, and poly-P are the storage products for PAOs. PHAs are 0.2–0.5 µm sized granules that are present in the cytoplasm of the cell enclosed by a film (membrane). Frequent PHA preserved by bacteria

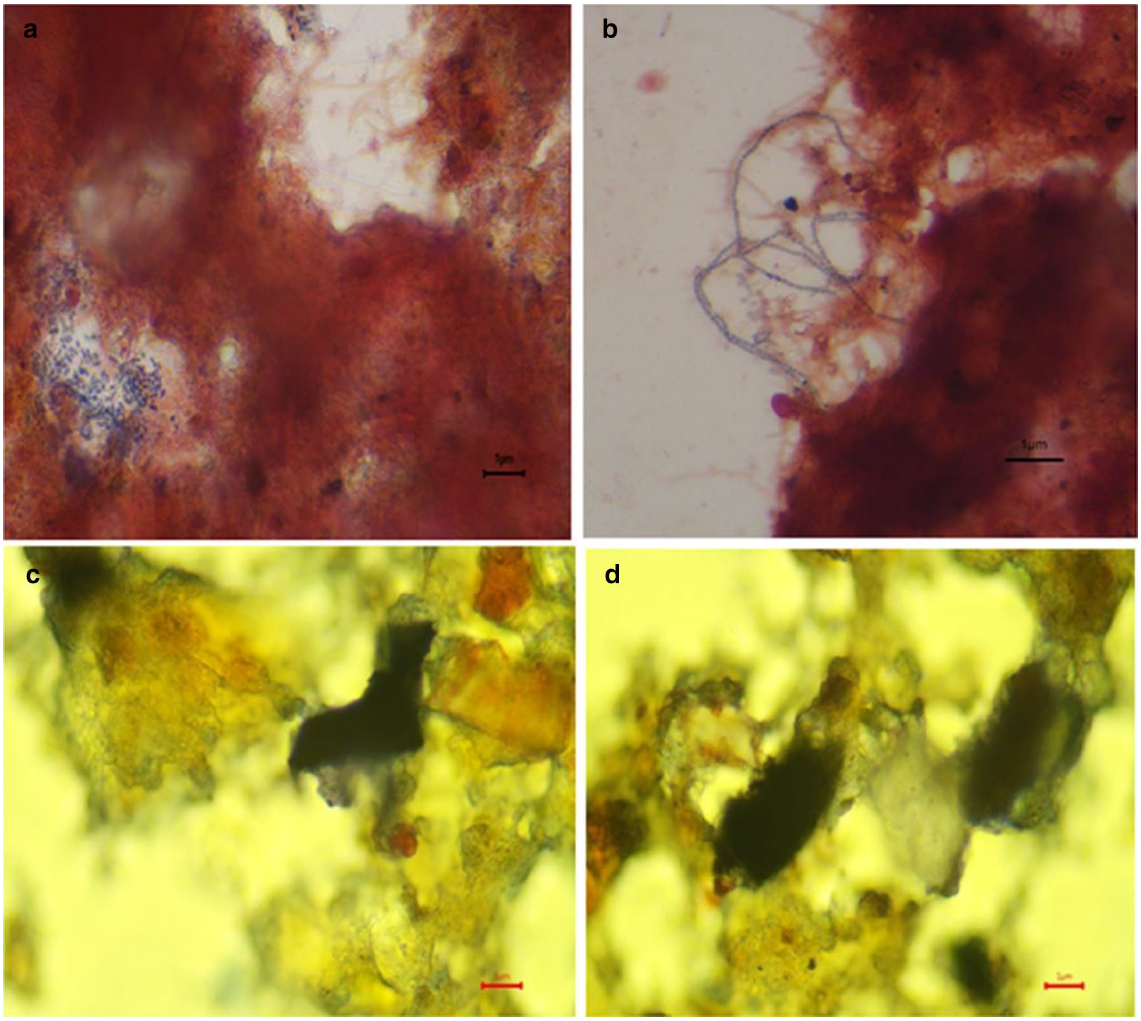

Fig. 7 Brightfield micrographs of the biomass samples: panels (**A**) and (**B**) are showing PHB granules present inside the sludge flocs and filaments, respectively [blue-black cells: PHB (+); pink cells: PHB (−)]. Panels (**C**), and (**D**) are showing poly-P globules [purple-black cells: Poly-P (+); yellow–brown cells: Poly-P (−)]

is Poly-β-hydroxybutyrate (PHB), a lipid-resembling polymer of 3-hydroxybutyrate. However, there are some poly-P collecting bacteria (e.g., M. phosphovorus), which do not accumulate PHA but preserve trehalose, poly-P, and glycogen (Sathasivan 2009).

The literature suggests that PHB formation has a significant role during the processes of SND (a potential substrate for denitrification) and EBPR (a potential substrate for excess phosphorus uptake by PAOs in the aerobic phase). In the 3 MLD SBR plant, some PHBs and poly-P were observed in anoxic selectors and aeration tanks' sludge samples. Qualitative microscopic observations were carried out in mixed liquor samples of aeration tanks and selectors (Fig. 7). 100 µL sub-samples of sludge were examined under 100× magnifications (with immersion oil) as per the prescribed Protocol (USEPA 1987).

Protozoa identification

This study substantiated the prospects of attaining granular sludge in an anaerobic/aerobic sequencing batch reactor with high SND and biological phosphorus removal performance. Operational litheness of the SBR (capability to lessen settling time, initial reactor volume, etc.) played a pivotal role in promoting compact granular biomass formation and maintenance. After staining the samples for PHB, microscopic observations revealed that the biomass consisted of a microbial community diverse in terms of morphology, physiology, and anaerobic PHB storage.

Several protozoa species were identified in the sludge samples (Table S5, Supplementary Material). Protozoa species like arcella, vorticella, and opercularia were dominant, while filamentous species were lesser in the plant's sludge. Lower SVIs, good microbiota (rich in floc-formers), and

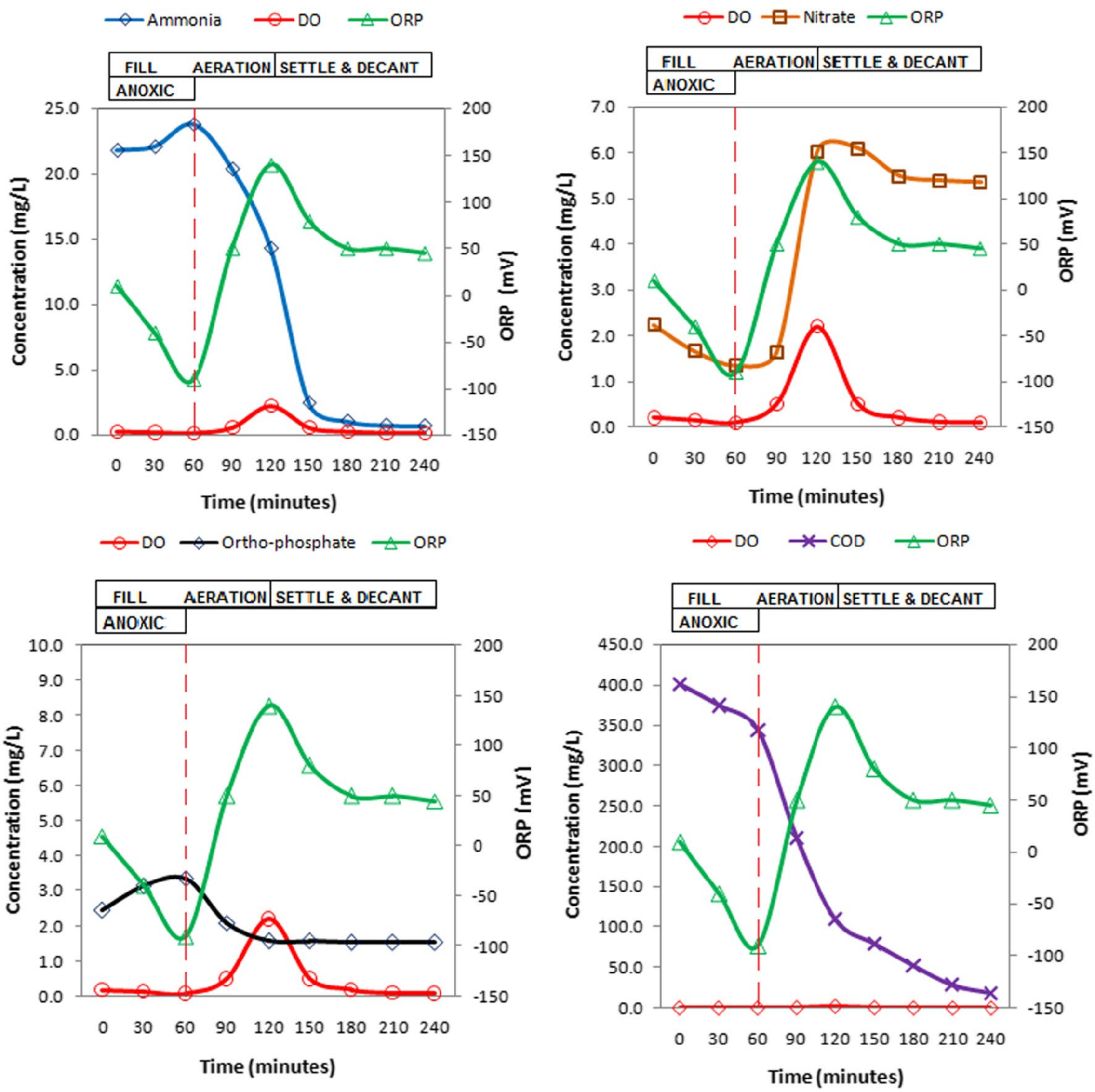

Fig. 8 Cycle wise profiles of DO, ORP, nitrate, ammonia oxidation, COD, and orthophosphate removal

excellent effluent characteristics are interrelated (Albertson 2002). The elemental source for the natural selection of non-filamentous organisms is the management of the surrounding during the primary contact of the influent wastewater, where a large amount of $sBOD_5$ or sCOD is eliminated from the solution to the biomass in the midst of or devoid of limited oxidation (Albertson 2002). The critical situation depends upon the occurrence of DO and the food to microorganisms (F/M) ratio in the anoxic selector compartments. The accumulation of nitrates through return activated sludge or internal recycling from the nitrifying region (aeration tanks) can also contribute constructively in restraining the growth of filamentous organisms (Albertson 2002).

Effect of BOD_5/COD on nutrient removal

The ratio of influent BOD_5 to COD impacts nutrient removal performance. BOD_5:total COD signifies the biodegradable carbon content in wastewater (biochemical oxygen demand) from the whole organic matter (chemical oxygen demand) in wastewater, which is quite imperative for efficient nutrient removal in wastewater treatment plants. Therefore, the evaluation of wastewater propensity for biological treatment is widely revolving around BOD_5/COD (Gajewska et al. 2015). An increasing linear trend but statistically insignificant relationship was observed between BOD_5 to total COD ratio and TN and TP removal in the SBR (Fig. S6, Supplementary Material).

The overall effect of qualitative and quantitative variations on plant performance

The temperature varied from 10 °C to 30 °C during the study. The average MLSS and MLVSS in the aeration tanks were 7189 and 3087 mg/L (Aeration tank 1) and 7518 and 3740 mg/L (Aeration tank 2). Average influent NH_4-N decreased from 21.8 ± 5.8 mg/L to 0.7 ± 0.5 mg/L (96.7% removal), and TN removal was ~ 69.1%. DO concentration varied up to 2.48 mg/L during aeration and from 0.02 to 0.22 mg/L during settling/decanting. ORP fluctuated between − 66 mV and − 114 mV in the anoxic selector compartments and reached ~ 140 mV in the peak hours during the aeration phase in the SBR. It ultimately attained ≤ 50 mV during the settling and decanting phases (Table S1, Supplementary Material). In the third compartment of the selector, ORP reduced to < − 90 mV, contributing to 39.7% denitrification and orthophosphate release of 26.4%. The DO and ORP profiles with the COD and nutrient removal during one cycle of SBR can be observed in Fig. 8. Figure S7 (Supplementary Material) illustrates the profiles in the compartments of the anoxic selector.

The summary of the results of the carried out analysis of statistical significance is presented in Table 5. For the analyses, the *F* test for dependent samples with the significance level $p = 0.05$ was used. Based on the statistical results, the relationships between rbCOD/COD% and SND% can be considered as statistically significant ($R^2 > 0.8$ and $p < 0.05$). Though, relationship between rbCOD/sCOD% and SND%, rbCOD/TP and effluent TP, rbCOD/TP and effluent PO_4-P, BOD/TP and effluent TP, BOD/TP and effluent PO_4-P, sBOD/TP and effluent TP, COD/TP and effluent TP, rbCOD/COD (or rbCOD/sCOD) and TP removal (or PO_4-P removal) can also be expected as dependent functions but statistically insignificant ($p < 0.05$ and $R^2 < 0.8$). So, it can be interpreted as, when RAS (15–30 min contact time) from aeration tanks goes to these selectors, the microorganisms meet a greater amount of substrate (rbCOD) and limited DO concentration in the anoxic selector, natural selection of foremost floc-formers occurs. Large flocs produce, which strengthens the SND efficiency of the plant.

Conclusion

This study concludes four significant contributions of an optimized SBR system regarding the variations in the quality of municipal wastewater, including (i) pre-anoxic selector attached SBR removed > 95% organic matter, > 96% ammonia, and > 96% TSS and performed well to achieve > 76% SND. (ii) A statistically significant result ($R^2 > 0.8$ and $p < 0.001$) was observed between rbCOD/COD (%) and SND (%), which showed a dependence of SND on a readily biodegradable fraction of COD. (iii) The rbCOD/TP affects phosphorus removal, and it is an active substrate (amid total COD) taken up quickly by bio-P removing organisms and stored as PHBs. (iv) PHBs and polyphosphates are the slow degrading polymers stored within the cells of denitrifiers and some PAOs during SND and biological phosphorus removal processes, respectively. (v) The pre-anoxic selector intensified the sludge settling characteristics, and the presence of protozoa indicated excellent biomass rich in floc-formers. Overall, 3 MLD SBR opens many dimensions regarding the dependency of nutrient removal on wastewater quality via SND and BPR approaches and the advantages of selector-based systems. It demands further research over its modifications to achieve better phosphorus removal (EBPR) together with nitrogen.

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Authors Contribution All the authors have participated in: conception and design, or analysis and interpretation of the data. Drafting the article or revising it critically for important intellectual content. Approval of the final version. GS: experiments, data collection, formal and statistical analysis, and writing—original draft, reviewing and editing. AR: writing—reviewing, and editing. AK: writing—reviewing, and editing. AKN: reviewing and editing. VT: reviewing and editing. AAK: supervision, validation, and resources.

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Availability of data and materials For the data available with the paper and Supplementary files: the authors confirm that all the data underlying the findings are fully available without restriction.

Code availability Not applicable.

Declarations

Conflict of interest The authors declare the following financial interests/personal relationships, which may be considered as potential competing interests:

Ethical approval This article does not contain any studies with human participants performed by any of the authors. This article does not contain any studies with animals performed by any of the authors. This article does not contain any studies with human participants or animals performed by any of the authors.

Consent to participate The authors have consented to participate in the study.

Consent to publish All the authors have consented to publish the study.

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