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Enhanced combined assimilative and bound phosphorus uptake in concurrence with nitrate removal in pre-anoxic cyclic sequencing batch reactor

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ABSTRACT

Needless to specify, controlling nitrogen and phosphorus discharge from wastewater treatment plants is synonymous with the prevention of eutrophication of surface waters, as one of the major issues related to water security. The present study investigates the performance of a pre-anoxic sequencing batch reactor (SBR) working on the basis of intermittent aeration, operated at varied carbon (bCOD) to nitrogen (C/N) ratio of 3, 7.5, and 10, and readily biodegradable (rbCOD) to slowly biodegradable (sbCOD) ratio of 0.1, 0.25, and 0.5. The findings revealed that an enhanced nitrogen removal was observed, together with higher C/N and rbCOD to sbCOD ratios. The results also show a consistent increase in total phosphorus removal with an increase in nitrogen removal. The phosphorus uptake of sludge varied from 0.02 – 0.045 mgP/mgVSS (avg. 0.031 ± 0.004), which resulted in enrichment levels of 0.88 – 1.68 times the stoichiometric value of 0.0267 mgP/mgVSS (avg. 1.45 ± 0.14). On an average basis, the assimilative total phosphate (TP) content was increased by 0.008 gTP/gNO$_3^-$-N removal rate. The excess phosphorus removal was due to the formation of poorly soluble polyvalent phosphate compounds, which was found based on dry analysis, which persisted as bound phosphate in the sludge.

1. Introduction

Eutrophication due to nitrogen and phosphorus disposal restricts the available sources of freshwater due to secondary pollution and benthic loads (Gillefalk et al., 2019). It, therefore, causes severe issues related to water security. A very

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Abbreviations

ADP	Adenosine diphosphate
ANOVA	Analysis of variance
bCOD	Biodegradable Chemical Oxygen Demand
BOD	Biochemical Oxygen Demand
BNR	Biological nutrient removal
C/N	Carbon/Nitrogen
C/P	Carbon/Phosphorus
COD	Chemical Oxygen Demand
DPAO	Denitrifying Phosphate Accumulating Organisms
DO	Dissolved Oxygen
EBPR	Enhanced Biological Phosphate Removal
HRT	Hydraulic Retention Time
MLSS	Mixed Liquor Suspended Solids
MLVSS	Mixed Liquor Volatile Suspended Solids
TKN	Total Kjeldahl Nitrogen
ORP	Oxidation–Reduction Potential
PAO	Phosphate Accumulating Organisms
PHA	Polyhydroxy Alkaonate
Qr	Recirculation flow
rbCOD	Readily Biodegradable COD
SBR	Sequential Batch Reactor
sbCOD	Slowly biodegradable
SRT	Solid Retention Time
TN	Total Nitrogen
TP	Total Phosphorus
TSS	Total Suspended Solids
VSS	Volatile Suspended Solids

pertinent question concerning biological nutrient removal (BNR) was raised by Barnard (2006) that where we have been and where we are going? If one gram of phosphorus is discharged and presumed as completely assimilated by algae (composition as $C_{106}H_{263}O_{110}N_{16}P$) for the production of new biomass from photosynthesis, an amount of 114.5 g biomass with an equivalent COD of 109.4 g would be produced. Thus, a discharge of 7 mg/L phosphorus (typical average value in medium strength sewage) could potentially generate 765.8 mg/L COD (Metcalf and Eddy, 2003), which amounts to much more than the COD in untreated wastewater.

Similarly, a gram of nitrogen could produce 16 g of algae, which would be equivalent to 15.5 g of COD. Thus, every discharge of 30 mg/L of nitrogen (typical average value in sewage) can produce 465 mg/L COD in equivalent terms (Randall et al., 1992). A variety of biological process configurations removes nitrogen in varying degrees of success. However, the presence of phosphorus is more critical because nitrogen is in abundance in the freshwater environment. The cyanobacteria can fix the nitrogen from the ambient atmosphere (Conley et al., 2009). Thus, Phosphorus removal is key to the maintenance of the sustainability of water resources. Phosphorus can be removed either by chemical processes or through the assimilative process through cell growth. The biological treatment can remove phosphorus from medium to low levels, which may result in reduction of the amount of precipitating chemicals required to obtain further lower desired levels (Barnard, 1974, 2006). Saxena et al. (2022) in a recent study observed aerobic granular reactor (AGR) was used to treat landfill leachate by varying C/N and C/P ratio using varying concentration of COD of landfill leachate. The results are expectedly reduction in phosphorus removal due to lower C/P ratio when phosphorus concentration was high due to limitation of assimilation of phosphorus into cell.

The phosphorus content of synthesized biomass ($C_5H_7O_2NP_{0.1}$) typically varies from 2%–3% by its dry weight; typically, 2.67% phosphorus stoichiometrically. The phosphorus accumulating organisms (PAO) could assimilate up to 2 to 5 times the above value through enhanced biological phosphate removal (EBPR) process (Seviour et al., 2003; Oehmen et al., 2007). EBPR essentially requires the combination of anaerobic followed by aerobic/anoxic conditions to facilitate PAOs so that they can impound the electrons, especially in the form of acetic acid into storage products Poly-β-HydroxyAlkaonate (PHA) within the cells (Mino et al., 1998; van Loosdrecht et al., 1997). This reaction takes place in the absence of oxygen and utilize nitrate as electron acceptor. Chemical precipitation is mainly used to remove the inorganic forms of phosphate by adding calcium, aluminium, or iron to precipitate phosphates as hydroxyapatite (Metcalf and Eddy, 2003).

The anaerobic–anoxic alternating phosphorus accumulating phenotype termed as denitrifying phosphate accumulating organisms (DPAOs) use nitrate as sole electron acceptor for the oxidation of PHA under anoxic condition in place of oxygen (Kuba et al., 1993, 1996a,b; Zeng et al., 2003). The energy production efficiency of PAOs with nitrate seems to be about 40% lower than with oxygen, and the lower energy generation efficiency of DPAOs leads to a 20%–30% and produce less sludge (Kuba et al., 1993).

The metallic precipitates phosphorus in the wastewater and removed by settling within the process. Whilst these chemically-bonded precipitates may be difficult in efficient phosphorus recovery for further use (Oleszkiewicz et al., 2015). The grape-biochar was used to remove phosphorus through the formation of biochar/struvite precipitates. However, the process was chemical (Piri and Sepehr, 2022). The simultaneous phosphorus precipitation with biological uptake causes extra sludge production with enhanced maturity due to high inert content. This nevertheless reduce the potential for biogas production through anaerobic digestion (Parsons and Smith, 2008).

Intermittent aeration and/or internal circulation to the pre-anoxic reactor is practiced in most of the process configurations for the purpose of simultaneous nitrification and denitrification (Demuynck et al., 1994). However, lower carbon to nitrogen ratio (C/N ratio) due to insufficient biodegradable COD (bCOD) in the raw wastewater is cited as limiting factor in the nitrogen removal (Kuba et al., 1996a,b). Other studies where high C/N ratio was resulted in efficient nitrogen removal (Sobieszuk and Szewczyk, 2006; Khursheed et al., 2012, 2018). Demuynck et al. (1994) showed that shorter alternating aerobic and anoxic phases reduce the readily biodegradable COD (rbCOD) dependence and oxygen consumption to achieve higher nitrogen removal. The enhanced denitrification could be achieved by maximizing the use of rbCOD and recirculation of the nitrate to inlet (Yuan and Keller, 2003; Bernardes and Klapwijk, 1996).

An attempt was made to study the effect of availability of biodegradable COD and more specifically readily biodegradable COD in the form of carbon to nitrogen ratio and readily biodegradable COD to slowly biodegradable COD ratio (C/N and rbCOD/sbCOD) ratios on nitrate and phosphorus removal in an SBR operated at variable mixed liquor suspended solids (MLSS) recirculation ratio to the pre-denitrification tank. The recirculation and pre-anoxic chamber allowed consumption of most of the readily biodegradable substrate native to wastewater for denitrification before its aerobic oxidation by dissolved oxygen (DO). Khursheed et al. (2018) demonstrated that maximum total nitrogen (TN) removal of 90.7% was achieved at high C/N ratio of 10 and rbCOD/sbCOD ratio of 0.5. However, the average sewage quality consisting of C/N ratio of 7.5 and rbCOD/sbCOD ratio of 0.25; the TN removal was not more than 83%. The specific nitrate removal rate varied from 0.015, 0.017 and 0.014 gNO3-/gVSS.d at C/N ratio of 3, 7.5 and 10, respectively.

2. Materials and methods

The pre-anoxic fitted SBR consisted of 8 L effective volume of the main aeration chamber and 2 L pre-denitrification anoxic chamber (Figs. 1 and 2). The SBR operating parameters consisted of 1.0 d hydraulic retention time (HRT), four operation cycles a day (4 cycle/d) of six hours consisting of 3 h filling-aeration, and 3 h of settling and decantation. The synthetic sewage was fed to pre-anoxic tank along with recirculation flow (Qr) during filling hours (180 min) (Fig. 1(b)). Fig. 1(a) shows all SBR operations, including cycle time maintained by timers and solenoid valves.

The reactor was inoculated with active sludge collected from a nearby sewage treatment plant. Its characteristics consisted of a mixed culture of microorganisms namely; SV30 220, MLSS 2687; SVI 82; MLVSS 2127.6 ± 193.4 mg/L; and MLSS 2820.3 ± 367.2 mg/L.

The composition of synthetic wastewater used is summarized in Table 1.

The biodegradable COD to ammonia nitrogen content of synthetic sewage (bCOD/NH_4^+-N) was taken as C/N ratio. The bCOD was the sum of rbCOD and sbCOD. The substrate C/N ratio was designed to keep at 3.0, 7.5, 10, and rbCOD/sbCOD ratios of 0.1, 0.25, and 0.5 values considering the prevailing municipal sewage constitution. Similarly, the carbon to phosphorus ratio (C/P) varied from 38.8–58.2 (46.4 ± 4.23). The bCOD was kept fixed at around 300 mg/L (Khursheed et al., 2015) in all the above-mentioned cases. Thus, the process variables of denitrification were MLSS internal recirculation ratio (Qr/Q) to pre-anoxic tank, C/N ratio, and rbCOD/sbCOD ratio for the pre-denitrification process. Other operating parameters such as temperature, solid retention time (SRT), and DO, etc. were maintained within limits as given in Table 2. The nitrate, TN, bCOD, COD, and phosphorus removal were the main parameters to assess varied from 0.015, 0.017 and 0.014 gNO_3^-/gVSS.d at C/N ratio of 3, 7.5 and 10, respectively.

The reactor performance was monitored by collecting samples of influent, effluent and reactor sludge at the end of each cycle. The major characteristics, namely. COD, BOD, TKN, ammonia nitrogen, TN, ortho-P, poly-P and TP of the synthetic sewage and TSS, VSS of sludge were performed according to Standard Methods (APHA, 2017). Readily biodegradable COD (rbCOD) was determined by the method evolved by Mamais et al. (1993) and modified by Wentzel et al. (2000).

3. Results and discussion

3.1. Effect of C/N and rbCOD/sbCOD ratio on total nitrogen removal

In presence of consistently high ammonia nitrogen conversion from 80.2–85.6% by virtue of controlled operating DO level and SRT (Khursheed et al., 2018), the total nitrogen remained the function of nitrate removal during denitrification. This is shown in Fig. 3(a) depicting an increasing trend in nitrate removal (the error varies nominally from 1.21–4.13%.).

Table 1
Synthetic wastewater component (Khursheed et al., 2018).

Component[c]	Parameter	Parameter per unit component	Component per unit parameter
Glucose	sbCOD	1.06 mgCOD/mg	0.94 mg/mgCOD
Na-acetate	rbCOD	1.06 mgCOD/mg	0.94 mg/mgCOD
Starch	sbCOD	1.12-1.38 mgCOD/mg	0.9-0.72 mg/mgCOD
NH_4Cl[a]	Ammonia -N	0.26 mgN/mg	3.82 mg/mgN
Urea	Ammonia -N	0.47 mgN/mg	2.14 mg/mgN
KH_2PO_4	Ortho-P	0.23 mgP/mg	4.39 mg/mgP
$(NaPO_3)_6$	Poly-P	0.30 mgP/mg	3.3 mg/mgP
$Na_4P_2O_7$	Poly-P	0.23 mgP/mg	4.3 mg/mgP
$MgSO_4.7H_2O$	Trace minerals		
$NaHCO_3$[b]	Alkalinity		
NaCl	Trace minerals		
KCl	Trace minerals		
$FeCl_3.6H_2O$	Trace minerals		
$CaCl_2.2H_2O$	Trace minerals		

[a] NH_4Cl was added separately for stability.
[b] $NaHCO_3$ was added separately for stability and alkalinity requirement.
[c] Subject to purity correction.

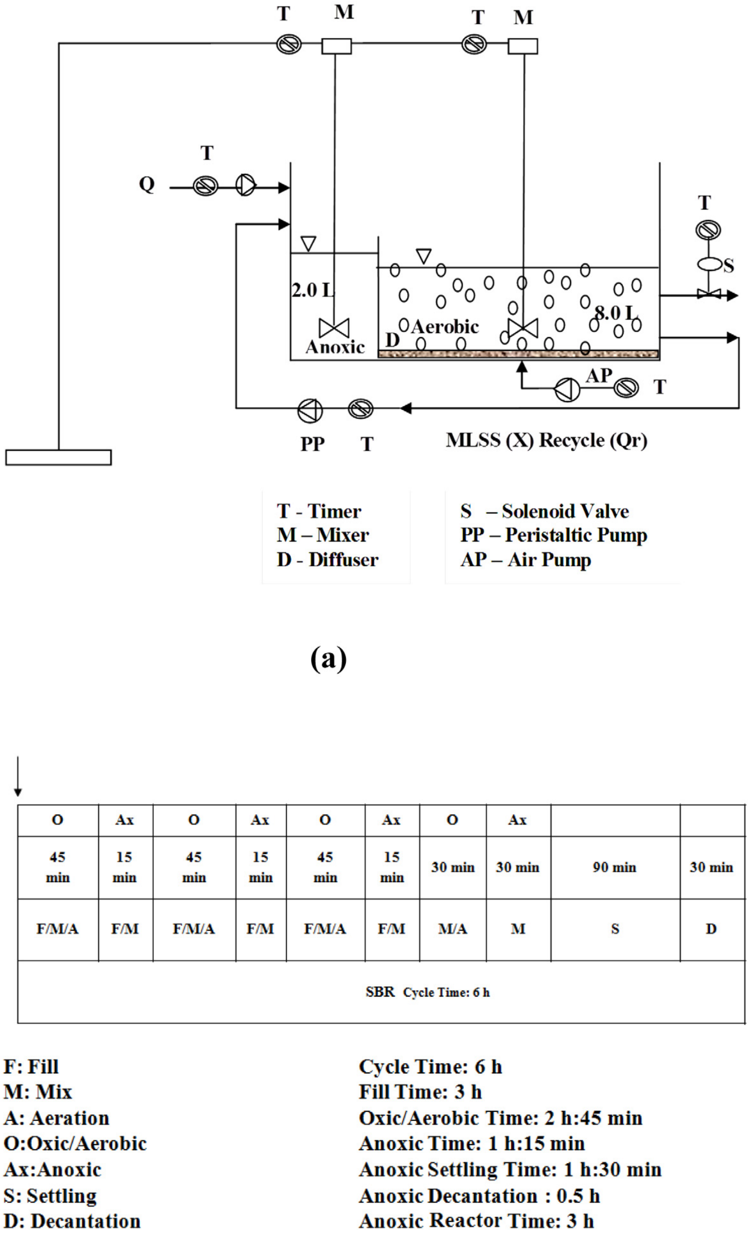
Table 2
Scheme of SBR operation at steady-state condition.

C/N	C/P	rbCOD/ sbCOD	Optimum (Qr/Q)	No of operating days	Temperature (°C)	DO (mg/L)	SRT (d) Calculated
					Range (Avg. ± σ)	Range (Avg. ± σ)	Range (Avg. ± σ)
3	50	0.1	0.5	23	23.2–31.1 (27.0 ± 1.8)	1.67–2.36 (2.14 ± 0.18)	18.53–19.73 (19.18 ± 0.33)
		0.25	1	16	22.0–27.5 (25.5 ± 1.2)	1.76–3.00 (2.37 ± 0.46)	18.25–19.64 (18.94 ± 0.44)
		0.5	1.5	15	22.0–27.5 (25.3 ± 1.2)	1.87- 2.75 (2.27 ± 0.26)	18.35–19.60 (19.03 ± 0.37)
7.5	50	0.1	1.5	25	24.0–30.5 (27.5 ± 1.7)	1.89–3.03 (2.45 ± 0.26)	17.26–19.68 (19.17 ± 0.49)
		0.25	4	20	22.5–28.4 (25.8 ± 1.2)	1.84–2.96 (2.54 ± 0.33)	18.93–19.66 (19.26 ± 0.23)
		0.5	4	23	24.2–30.1 (27.6 ± 1.9)	2.22–2.62 (2.50 ± 0.10)	18.09–19.76 (19.17 ± 0.49)
10	50	0.1	2	18	22.0–26.2 (24.7 ± 1.3)	1.60–2.60 (2.13 ± 0.26)	17.39–19.63 (18.79 ± 0.55)
		0.25	4	15	22.0–27.5 (25.2 ± 1.6)	1.76–2.75 (2.26 ± 0.24)	17.23–19.39 (18.58 ± 0.59)
		0.5	4	16	24.0–27.5 (25.8 ± 10.9)	1.83–2.81 (2.21 ± 0.23)	17.94–19.37 (18.62 ± 0.39)

At the C/N ratio of 10 and rbCOD/sbCOD ratio of 0.1, the nitrate removal was 74.8% at the recirculation ratio of 2 but this increased to 85% once the recirculation ratio increased to 4. However, decreasing the C/N ratio to 7.5 and increasing the rbCOD/sbCOD ratio of 0.25 at the same recirculation ratio of 4 resulted in almost close nitrate removal of 83%. The trend could not remain continued at decreasing C/N ratio and increasing relevance of rbCOD/sbCOD diminished as the nitrate removal at much lower C/N ratio dropped to 14.4–35.2% only. Thus, the increase in nitrate removal rate was consistent to the high rbCOD/sbCOD ratio, but as the C/N increased to 10, this effect was annulled. Khursheed et al. (2018) also mentioned that higher rbCOD demand at lower rbCOD/sbCOD ratios was met through solubilization of bCOD.

3.2. Effect of C/N and rbCOD/sbCOD ratio on phosphorous removal

Influent and effluent TP varied from 4.56–6.18 mg/L (average 6.01 ± 0.29) and 3.02–4.66 mg/L (average 3.92 ± 0.31). Influent TP concentration varied in a precisely close range as C/P ratio was attempted to be maintained close to 50, as mentioned above. Variations of Phosphate-P removal for different Qr/Q stages were distinctly observed from 24.6–40.5% (average 34.8 ± 3.96). Since C/P ratio was in a close range, it was expected that phosphate removal would also vary. The average increase in phosphate removal with an increase in Qr/Q varied from 16.9–22.7%, concerning the base value in the absence of any recirculation. The results indicated that the increased recirculation was more advantageous in terms of



Ax	O	Ax	O	Ax	O	Ax	O	Ax		
180 min	45 min	15 min	45 min	15 min	45 min	15 min	30 min	30 min	90 min	30 min
M	F/M/A	F/M	F/M/A	F/M	F/M/A	F/M	M/A	M	S	D
Anoxic Reactor	SBR Cycle Time: 6 h									

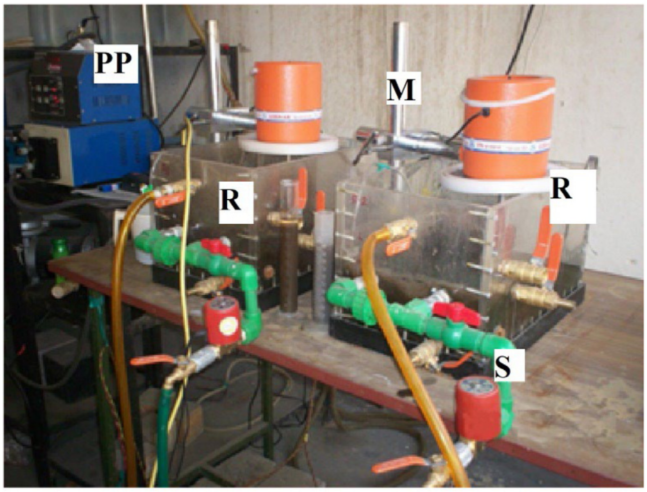
F: Fill
M: Mix
A: Aeration
O:Oxic/Aerobic
Ax:Anoxic
S: Settling
D: Decantation

Cycle Time: 6 h
Fill Time: 3 h
Oxic/Aerobic Time: 2 h:45 min
Anoxic Time: 1 h:15 min
Anoxic Settling Time: 1 h:30 min
Anoxic Decantation : 0.5 h
Anoxic Reactor Time: 3 h

Fig. 1. (a) Schematic diagram of reactor set up (b) Reaction cycle in sequencing batch reactor with Pre-anoxic tank.

percent phosphate removal owing to higher nitrate removal. Fig. 3(b) also reveals that the increased TP uptake was not only due to a high C/N ratio, but the high rbCOD was equally pivotal. As a result, TP uptake at a lower C/N ratio with a high rbCOD/sbCOD ratio was almost the same as that of a higher C/N ratio with lower rbCOD/sbCOD ratio. The more than stoichiometric nitrate removal at lower C/N ratios and rbCOD/sbCOD ratios demands increased availability of rbCOD. This could be presumed due to hydrolysis of particulate or slow fraction of bCOD. However, it was impossible to assess the hydrolyzed sbCOD, as some fraction was oxidized during aerobic decimation.

During reactor data analysis it was observed that the phosphorus removal increased with increase in nitrate (NO_3^- -N) removal. The average phosphate content assimilation increased by 0.008 gTP/gNO_3^- -N removal rate, which in terms of percent amounted to 0.8% increased TP assimilation. The increase in phosphorus content was due to the formation of poorly soluble polyvalent phosphates compounds, which persisted as bound phosphate in the sludge. This consistent increase in phosphorus removal in conjunction with an increase in nitrate removal was a subject of interest. Therefore, the average phosphate content of the sludge in every stage of reactor operation was plotted against nitrate removal rate, and the R^2 value was 0.793. The plotted trend-line was then rationalized at 0.0267 gTP/gVSS at zero nitrate removal rate,



S – Solenoid Valve with Timer
R – Reactors SBR with air supply & Diffusers
PP – Peristaltic Pump
M – Mixer

Fig. 2. Picture of experimental set up.

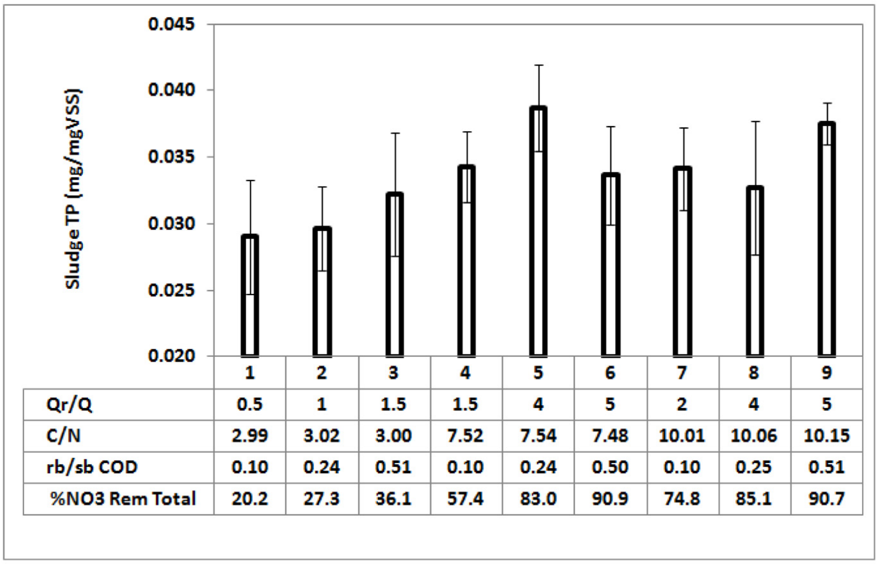
on the bases of unenhanced theoretical phosphate content. However, in doing so, the R^2 value was compromised, and the adjusted R^2 value was reduced to 0.502. Fig. 4 shows the linear empirical relationship between TP content and nitrate removal rate.

The increase may not continue linearly for long, and in this study, it was confined to the daily maximum nitrate removal rate of 0.91 gNO_3^--N removed/gNO_3^--N resulting in maximum TP content of 0.039 gTP/gVSS which amounted to a 1.46-fold increase from theoretical cellular uptake of 0.0267 gTP/gVSS (2.67%). It seems unlikely that the same increasing trend would continue further since this increase was most likely a result of the formation of polyvalent phosphates compounds, which are generally very poorly soluble and persisted as bound phosphate in the sludge. Usually, any phosphorus uptake above the stoichiometric value of 2.67% is taken as enhanced phosphate uptake. The assimilation into the biomass is the main mechanism of phosphate removal during biological processes. However, bound uptake through chemical precipitation during denitrification has also been reported (Oehmen et al., 2007; Ryu and Lee, 2010; Quintana et al., 2005). In the absence of any condition of growth of phosphate accumulation microorganisms (PAO) essential for enhanced biological uptake, this study distinguishes the extent of bound uptake from it. The phosphorus content in the sludge was verified by drying and measurement of the waste sludge samples. The results are shown in Table 3. The measured values of P content in dry samples were enriched from 0.94–1.65 times compared to experimentally observed in SBR 1.024–1.55 times the stoichiometric value of 0.0267 mgP/mgVSS (2.67%) respectively are generally in agreement with each other.

3.3. Alkalinity and pH

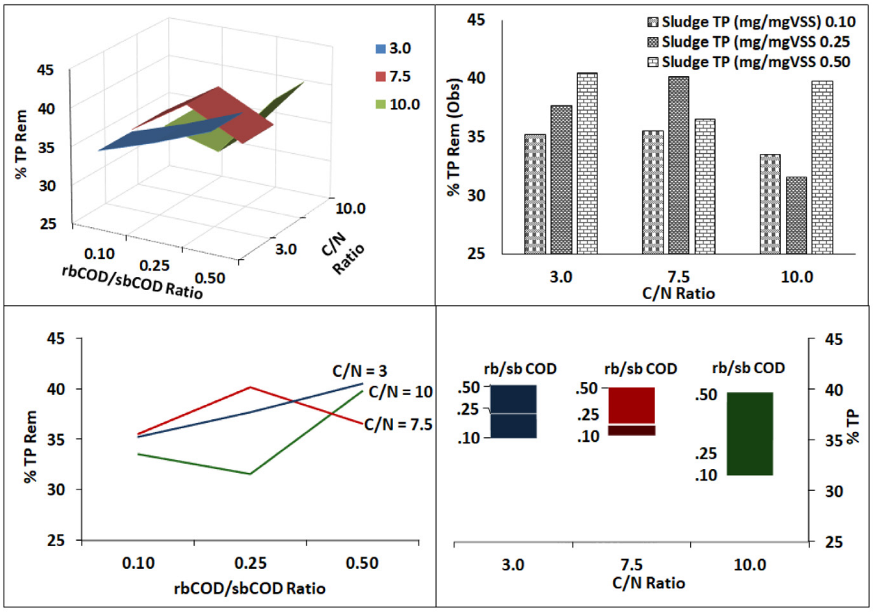
The increase in pH in the effluent in each operation stage substantiated that enhanced phosphorus removal is mainly derived from the formation of bound phosphate and the increase in nitrate removal. Table 4 shows an increase in pH due to enhanced nitrate removal coupled with enhanced TP removal. However, this phenomenon is pH and alkalinity dependent, which may unlikely be increased to very high values-a rudimentary factor for phosphate precipitation. It was observed that in each phase of the study, the total nitrogen increased with an increase in bCOD to nitrogen ratio and rbCOD/sbCOD ratio. Nitrification consumes alkalinity, but it recovers to a significant extent during denitrification (Metcalf and Eddy, 2003). Based on its two-step energy-yielding oxidation and the stoichiometry of nitrification and denitrification, the net change in alkalinity is given in Eqs. (1)–(3) below (Khursheed et al., 2012, 2018);

$$\text{Change in Alkalinity} = \frac{\left(\left(\frac{\text{fsn}}{20}\right)\text{HCO3} - {+0.25\text{H}+}\right) * 50 \text{ as CaCO3}}{\left(\frac{1}{8} + \frac{\text{fsn}}{20}\right) * 14 \text{ mg NH4} - \text{N}} - \frac{\left(\left(\frac{1}{50} - \frac{\text{fsn}}{50}\right)\text{HCO3} + \left(\frac{1-\text{fsd}}{5}\right)\text{H}+\right) * 50 \text{ as CaCO3}}{\left(\frac{1-\text{fsd}}{5}\right) * 14 \text{ mg NO3} - \text{N}} \tag{1}$$



	1	2	3	4	5	6	7	8	9
Qr/Q	0.5	1	1.5	1.5	4	5	2	4	5
C/N	2.99	3.02	3.00	7.52	7.54	7.48	10.01	10.06	10.15
rb/sb COD	0.10	0.24	0.51	0.10	0.24	0.50	0.10	0.25	0.51
%NO3 Rem Total	20.2	27.3	36.1	57.4	83.0	90.9	74.8	85.1	90.7

(a)



(b)

Fig. 3. (a) Summarized phosphorus uptake at enhanced nitrate removal under different conditions (b) Effect of C/N and rbCOD/sbCOD ratios on NO_3^- removal.

where;

$$\text{Net fraction of electron donor used in nitrifiers' synthesis; fsn} = 0.14\,\frac{(1+0.022\theta_x)}{(1+0.11\theta_x)}. \tag{2}$$

$$\text{Net fraction of electron donor used in denitrifiers' synthesis; fsd} = 0.52\,\frac{(1+0.01\theta_x)}{(1+0.05\theta_x)} \tag{3}$$

$\theta_x = \text{SRT}$

Table 3
Calculated and measured phosphorus in sludge on dry basis.

Phase	Day of operation	Qr/Q	rbCOD/sbCOD Ratio	C/N Ratio	% TN Remove	Sludge TP content gP/gVSS			TP enrichment gP(Obs)/gP(S)	
						Obs	Stoich[a]	Obs (Dry)	Obs	Obs (Dry)
1	7	1	0.1	3	25.5	0.029	0.027	0.026	1.11	0.97
	7	0.5	0.25	3.03	30.9	0.028	0.027	0.025	1.03	0.94
	6	0	0.5	3	31.0	0.027	0.027	0.028	1.02	1.05
	10	1.5	0.51	2.96	41.7	0.029	0.027	0.026	1.10	0.97
2	9	1	0.1	7.31	59.5	0.031	0.027	0.03	1.18	1.12
	6	3	0.1	7.37	60.2	0.03	0.027	0.027	1.13	1.01
	6	4	0.26	7.65	85.7	0.039	0.027	0.042	1.46	1.57
	7	5	0.51	7.43	88.6	0.038	0.027	0.042	1.42	1.57
	8	6	0.5	7.63	86.9	0.03	0.027	0.025	1.13	1.27
3	8	0	0.1	10.45	66.4	0.03	0.027	0.029	1.12	1.09
	9	2	0.1	10.36	80.0	0.039	0.027	0.038	1.47	1.42
	8	4	0.1	9.14	80.2	0.035	0.027	0.04	1.33	1.50
	10	0	0.25	10.12	59.6	0.027	0.027	0.025	1.03	0.94
	3	0	0.5	9.89	64.5	0.03	0.027	0.027	1.12	1.01
	15	5	0.51	10.34	89.9	0.041	0.027	0.044	1.55	1.65

[a] Stoichiometric P-Content = 2.67%.

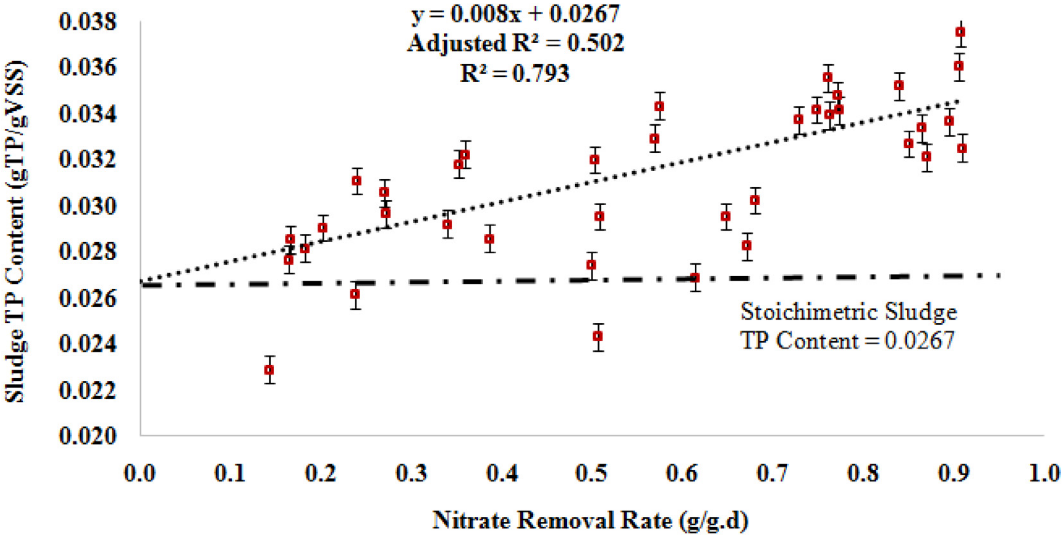


Fig. 4. Sludge phosphorus content in relation to nitrate removal rate.

The high variation in alkalinity is caused by high nitrification, less denitrification and, overall low TN removals and normal TP removal. However, alkalinity recovers with higher denitrification and TN removal. The results showed that the alkalinity remained further below the stoichiometric values, which exhibited that factors other than nitrification/denitrification also acted during the treatment process. The reduction in alkalinity change below the stoichiometric value varies from 5%–28.2% (Fig. 5). The lowering of alkalinity is synonymous with chemical precipitation of phosphorus; thus, the same could be the reason. This phenomenon is less visible at a lower C/N ratio of 3, as shown in Fig. 5(a) as compared to higher C/N ratios shown in Fig. 5(b) and (c). As mentioned above, the biomass uptake (assimilation) is the major mechanism of phosphate removal, although concurrent chemical precipitation during denitrification in the form of Hydroxyapatite $Ca_5(OH)(PO_4)_3$ also took place. Similarly, Struvite ($MgNH_4PO_4$) formation at 7.5 pH has also been reported, but the mechanism is not well understood (Quintana et al., 2005).

3.4. Statistical analysis

The influence of C/N ratio on bound phosphorus uptake was examined statistically using analysis of variance (ANOVA). At 95% confidence level, p-values smaller than 0.05 indicate statistically significant difference (Gao et al., 2019; Sokkanathan et al., 2018). Bound phosphorus uptake percentage recorded for three groups of different C/N ratio viz. 3 ± .10, 7.5 ± .45 and 10 ± .40 was subjected to one factor ANOVA to determine the statistical significance of the

Table 4
Summary of pH variation in conjunction with Nitrate and TP removal[b].

C/N ratio	rbCOD/ sbCOD ratio	IR[a] (Qr/Q)	Inf. pH	Eff. pH	NO_3^- removal (%)	TP removal (%)
			Avg ± σ	Avg ± σ	Avg ± σ	Avg ± σ
3.0	0.10	0	7.4 ± 0.28	8.2 ± 0.30	14.4 ± 1.91	31.0 ± 4.08
		0.25	7.4 ± 0.39	8.2 ± 0.40	16.5 ± 1.45	34.5 ± 2.39
		0.5	7.4 ± 0.43	8.2 ± 0.32	20.2 ± 2.02	35.2 ± 2.64
		1	7.5 ± 0.33	8.3 ± 0.36	18.3 ± 0.54	35.9 ± 2.53
	0.25	0	7.8 ± 0.44	7.6 ± 0.45	16.7 ± 1.01	36.1 ± 0.86
		0.25	7.6 ± 0.40	7.6 ± 0.42	23.9 ± 0.57	39.4 ± 1.27
		1	7.7 ± 0.36	7.9 ± 0.50	27.3 ± 1.82	37.7 ± 3.14
		2	8.1 ± 0.45	7.8 ± 0.33	27.0 ± 0.45	39.4 ± 1.37
	0.50	0	7.4 ± 0.69	7.7 ± 0.47	23.8 ± 1.36	33.7 ± 3.85
		1	7.5 ± 0.35	8.0 ± 0.53	34.0 ± 1.10	37.9 ± 2.42
		1.5	7.8 ± 0.46	7.8 ± 0.41	36.1 ± 1.21	40.5 ± 3.02
		2	7.3 ± 0.58	7.8 ± 0.28	35.2 ± 1.65	40.1 ± 2.72
7.5	0.10	0	7.4 ± 0.2	8.0 ± 0.4	38.6 ± 2.07	29.0 ± 2.86
		1	7.6 ± 0.3	7.7 ± 0.5	50.2 ± 3.47	31.9 ± 3.02
		1.5	7.4 ± 0.5	8.0 ± 0.5	57.4 ± 1.96	35.5 ± 3.36
		3	7.4 ± 0.5	7.8 ± 0.3	56.8 ± 2.17	35.6 ± 1.97
	0.25	0	7.5 ± 0.37	8.0 ± 0.49	50.9 ± 2.16	30.2 ± 2.79
		2	6.2 ± 0.36	6.6 ± 0.58	64.8 ± 2.10	34.7 ± 2.91
		3	7.5 ± 0.69	8.3 ± 0.29	77.1 ± 2.01	37.8 ± 4.00
		4	7.4 ± 0.35	7.9 ± 0.30	83.0 ± 2.91	40.1 ± 3.97
		5	7.8 ± 0.54	8.1 ± 0.20	77.4 ± 1.68	35.0 ± 1.97
	0.50	0	7.5 ± 0.33	8.2 ± 0.34	49.9 ± 2.63	30.3 ± 1.86
		3	7.5 ± 0.34	8.2 ± 0.36	86.4 ± 2.70	38.1 ± 0.71
		4	7.6 ± 0.30	8.1 ± 0.20	90.9 ± 2.29	36.5 ± 2.93
		5	7.5 ± 0.34	8.2 ± 0.36	89.5 ± 3.33	39.2 ± 1.25
		7	7.4 ± 0.36	8.1 ± 0.38	87.1 ± 4.95	38.1 ± 1.33
10	0.10	0	8.0 ± 0.38	8.4 ± 0.20	61.4 ± 4.40	27.0 ± 3.51
		1	7.4 ± 0.39	7.9 ± 0.35	68.1 ± 1.23	30.8 ± 2.09
		2	7.6 ± 0.49	7.9 ± 0.25	74.8 ± 4.13	33.5 ± 3.03
		4	7.5 ± 0.36	7.7 ± 0.32	76.0 ± 1.87	34.4 ± 1.27
	0.25	0	7.7 ± 0.20	8.2 ± 0.36	50.7 ± 2.64	24.6 ± 3.32
		2	7.4 ± 0.30	8.2 ± 0.09	67.1 ± 3.55	28.9 ± 3.42
		3	7.4 ± 0.51	7.9 ± 0.25	72.9 ± 2.43	30.6 ± 2.07
		4	7.9 ± 0.39	8.0 ± 0.37	85.1 ± 3.45	31.5 ± 2.52
		5	7.9 ± 0.42	8.1 ± 0.20	84.0 ± 3.49	34.0 ± 2.46
	0.50	0	6.2 ± 0.16	6.8 ± 0.33	76.2 ± 3.50	33.9 ± 2.30
		4	7.5 ± 0.22	8.0 ± 0.29	88.6 ± 3.18	39.8 ± 1.44
		5	7.5 ± 0.42	8.0 ± 0.14	90.7 ± 1.69	38.9 ± 1.15
		6	7.6 ± 0.32	7.9 ± 0.46	90.6 ± 2.50	36.4 ± 1.50

[a] Internal MLSS recycle ratio.
[b] C/P ratio = 50.

Table 5
One way ANOVA test: mean and variance of the individual groups of C/N ratios.

Summary				
Groups	Count	Sum	Average	Variance
3	9	246.8121	27.42356	16.59194
7.5	9	586.2233	65.13592	130.3954
10	9	736.112	81.79023	44.17587

difference between their mean values. The results from the statistical analysis have been compiled in tabular form as shown in Tables 5 and 6. Table 5 shows the mean and variance of the individual groups. Table 6 shows the ANOVA results, the F-Value was obtained as 109.58 which is far greater that the F-Critical value of 3.40. The corresponding p-Value was obtained as 8.54×10^{-13}($\ll$.05), hence null hypothesis can be rejected implying that the difference between the means of these groups is statistically significant (Karim et al., 2005). These results suggest that the samples for each group i.e., bound phosphorus uptake for different C/N ratios represent different populations which implies the dependence of former on the later.

Therefore, phosphorus removal other than assimilation into the cell and without chemical precipitation is also a possibility and needs to be investigated. In summary, it could be a subject of further investigation.

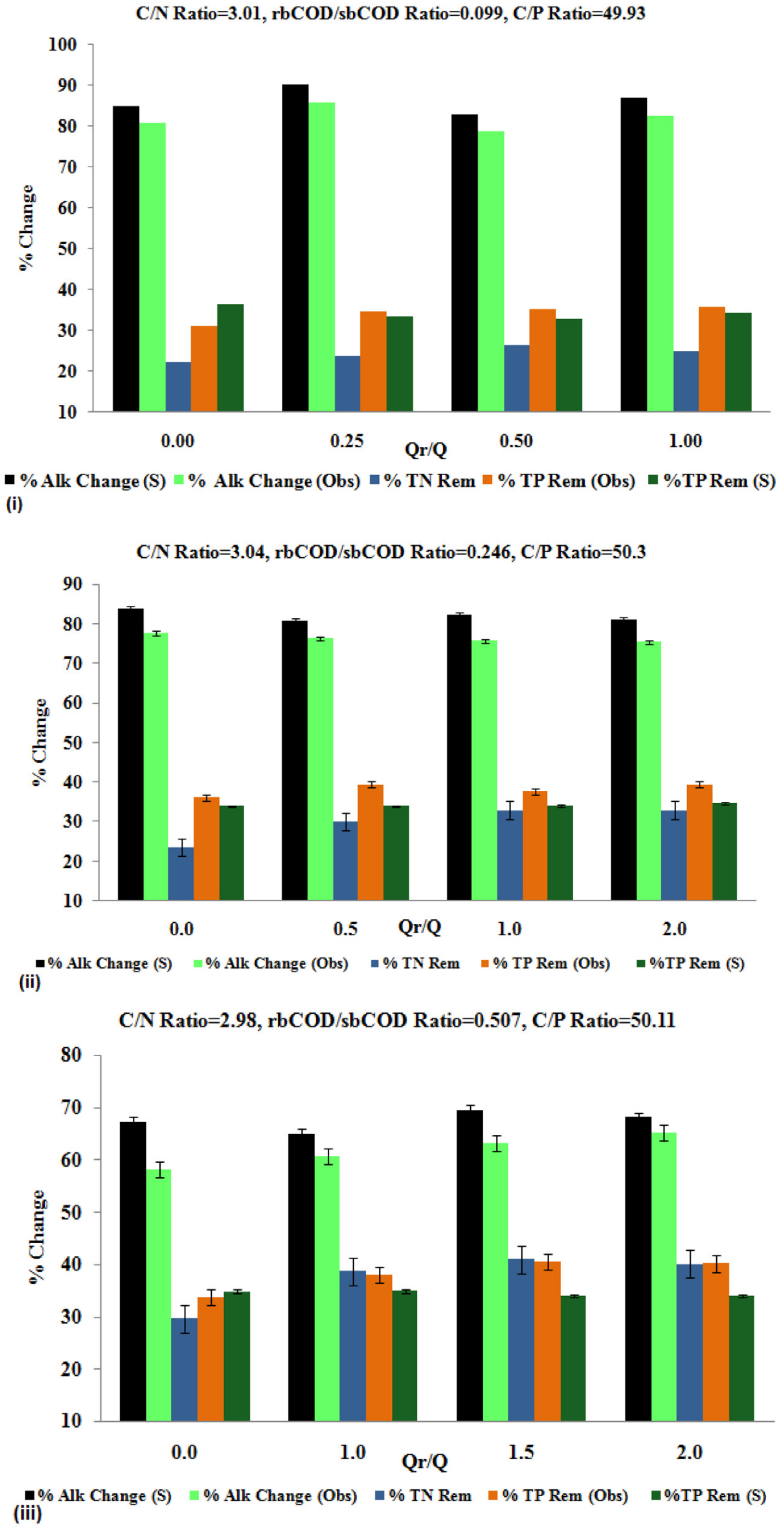


Fig. 5. Observed alkalinity change with reference to TN removal and TP uptake in comparison to stoichiometric alkalinity change at variable sludge recirculation rates under C/N and rbCOD/sbCOD ratios of (a) C/N = 3 (b) C/N = 7.5 and (c) C/N = 10 and (i) rbCOD/sbCOD ratio = 3, (ii) rbCOD/sbCOD ratio = 7.5, and (iii) rbCOD/sbCOD ratio = 10.

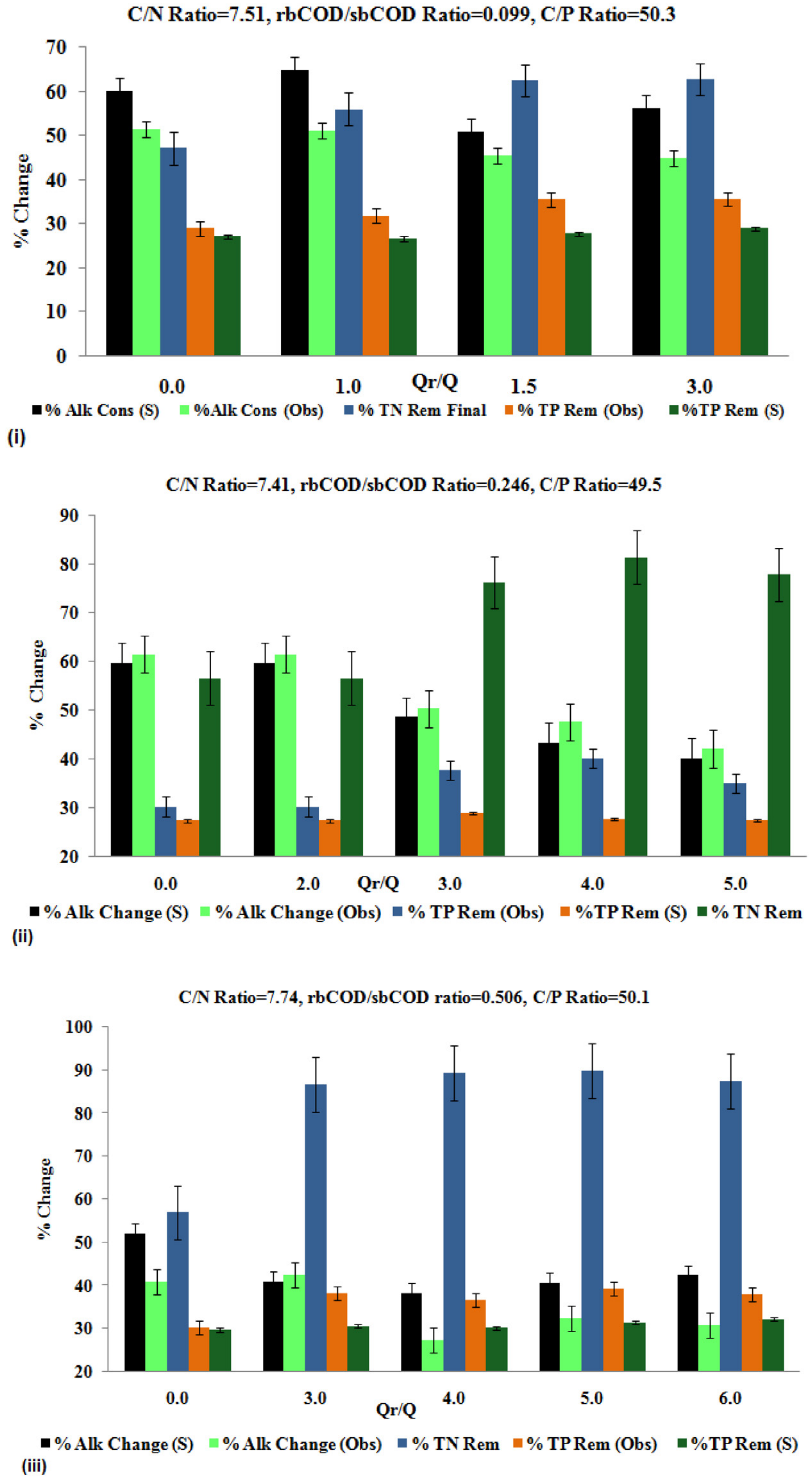


Fig. 5. (*continued*).

4. Conclusions

This study demonstrates a consistent increase in phosphorus removal with an increase in nitrate removal. The maximum nitrate removal of 90.7% was observed at the highest C/N ratio and rbCOD/sbCOD ratio of 10 and 0.5.

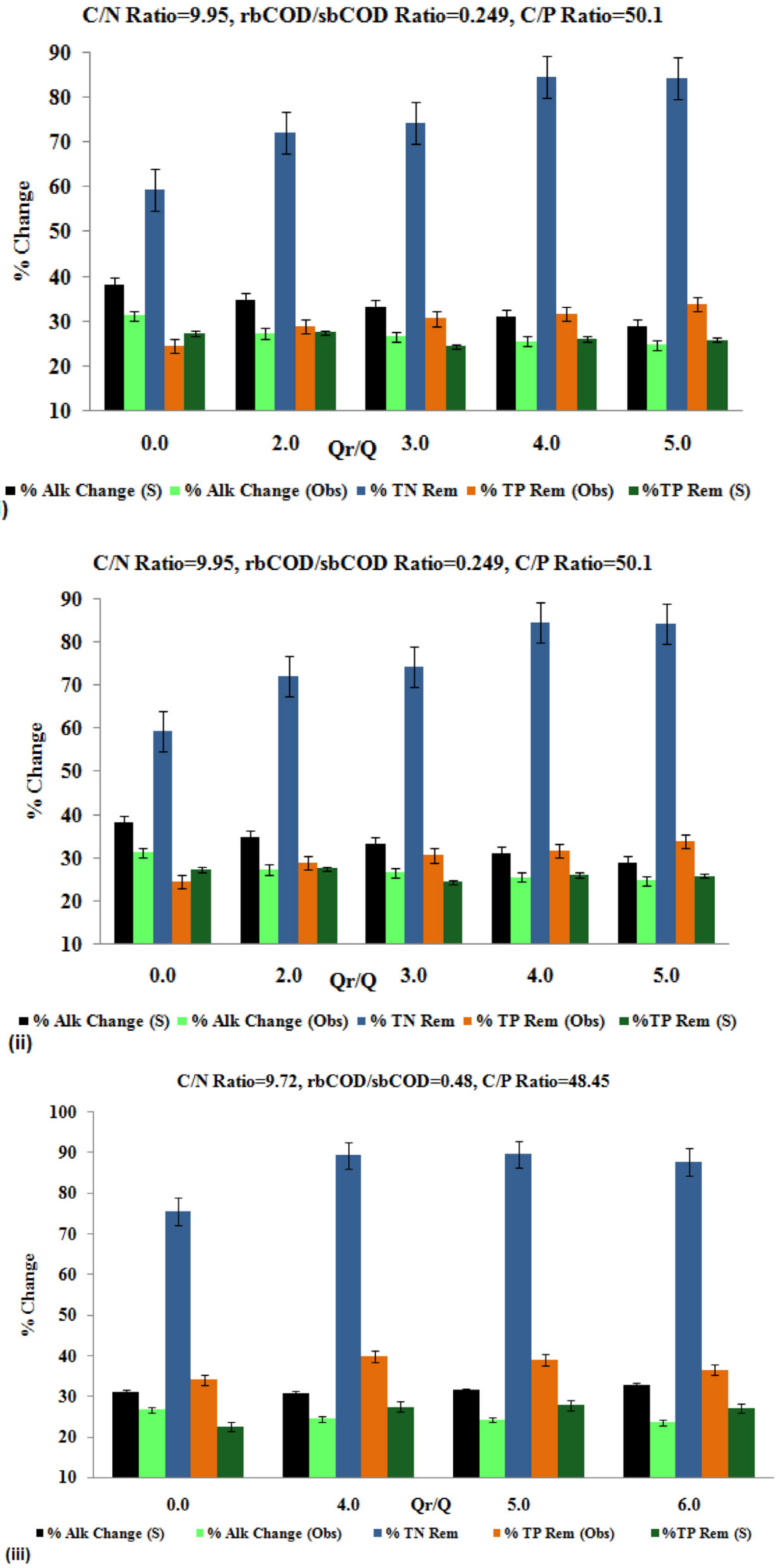


Fig. 5. (*continued*).

Table 6
One way ANOVA results.

Source of variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between groups	13 965.96	2	6982.982	109.5867	8.54 E−13	3.402826
Within groups	1529.305	24	63.72105			
Total	15 495.27	26				

However, it could not be more than 83% at average sewage quality of C/N and rbCOD/sbCOD ratio of 7.5 and 0.25. Since ammonia removal was consistent and independent of C/N ratio, rbCOD/sbCOD ratio, and sludge recirculation, phosphorus enrichment has concurred with nitrate alone. On average basis, the phosphate content increased by 0.008 gTP/gNO_3^--N removal rate, which amounts to 0.8% enhanced TP in VSS/mgNO_3^--N removal rate (1.46-fold increase than theoretical value). In the absence of EBPR, the increase in phosphorus uptake was due to the formation of very poorly soluble polyvalent phosphates compounds, which persisted as bound phosphate in the sludge. The extent of concurrent enhanced phosphorus assimilation and precipitation would expectedly give a clear picture of actual enhanced biological phosphorus uptake. This study identifies that some fraction of additional phosphorus is also removed in conjunction with an increase in nitrate removal. Moreover, even in case of EBPR, the whole phosphorus uptake is not biological, but some complex formation forces phosphates to precipitate if sufficient alkalinity is also innate to wastewater.

CRediT authorship contribution statement

Anwar Khursheed: Conceptualization, Methodology, Experimentation, Writing – original draft, Writing – review & editing. **Muntjeer Ali:** Conceptualization, Formal analysis, Writing – original draft. **Faris Mohammad A. Munshi:** Conceptualization, Writing – original draft. **Abdulrhman Fahmi Alali:** Conceptualization, Writing – original draft. **Mohab Amin Kamal:** Conceptualization, Writing – original draft. **Abdulaziz Ibrahim Almohana:** Conceptualization, Writing – original draft. **Omar Alrehaili:** Conceptualization, Writing – original draft. **Rubia Z. Gaur:** Conceptualization, Writing – original draft. **Vinay Kumar Tyagi:** Conceptualization, Writing – original draft. **Abid Ali Khan:** Conceptualization, Writing – original draft. **Gaurav Goel:** Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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