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Resolution of conflict of reduced sludge production with EBPR by coupling OSA to A^2/O process in a pilot scale SBR

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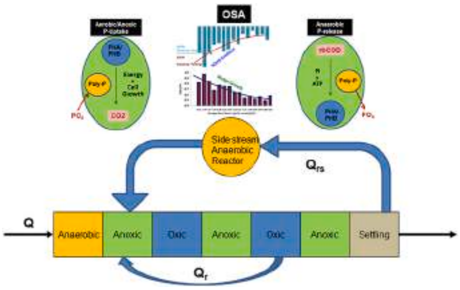
HIGHLIGHTS

- Conflict resolution of simultaneous sludge production with enhanced P-uptake.
- A^2/O and OSA process integration for nutrient removal and sludge reduction.
- Novel configuration resulted in 25% less Sludge and 3.46 times P-enrichment.

GRAPHICAL ABSTRACT



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ABSTRACT

The pinnacle of all the efforts of nutrient removal is practically put-down the moment biological cells are lysed, hydrolyzed or digested causing subsequent reappearance of assimilated nitrogen and phosphorus in any biological process. While sludge reduction requires high SRT, the enhanced phosphorus assimilative uptake demands low SRT. A novel reactor configuration for enhanced sludge and phosphorus removal was put to test by incorporating a side stream anaerobic reactor to an Anaerobic-Anoxic-Aerobic (A^2O) SBR with a pre-anoxic chamber and an influent receiving inlet anaerobic reactor. The reactor was operated at the average and lowest range of prevailing carbon/phosphorus (C/P) ratio of 50 and 15 in the sewage. The phosphorus enrichment was 0.0469–0.135 mgTP/mgVSS resulting in 1.76–5.05-fold increase from cellular content by virtue of maintaining sludge recycle from SBR aeration tank to side stream anaerobic reactor from 3.78 to 9.78 (average 4.4–8.2) gVSS/gVSS present in the reactor. However, the sludge was also reduced from 3% to 51% on an average

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basis during the same recirculation regime. This novel configuration consists of an inlet anaerobic reactor, one pre-anoxic chamber and one intermittent oxic anoxic reaction SBR and a side stream anaerobic reactor. The first anaerobic reactor at inlet followed by pre-anoxic chamber was provided for increased ortho-p released and nitrification respectively and a side stream anaerobic reactor for sludge reduction through sludge fasting mechanism. The EBPR and lesser sludge growth were two conflicting parameters reconciled to the extent that if sludge recycled up to 6.41 gVSS/gVSS the sludge growth would be reduced by 25% and phosphorus enrichment could be attained up to 3.46 times the stoichiometric value. Any further recirculation would reduce the sludge further but at the expense of enhanced phosphorus uptake as released phosphorus from side stream anaerobic reactor also recycled back to main SBR causing looping and at more than 6.41gVSSrecycled/gVSS it nullified the enhanced effect.

Abbreviations[1]

A^2/O	Anaerobic-anoxic-aerobic[1]
ATP	Adenosine triphosphate
bCOD	Biodegradable Chemical Oxygen Demand
BOD	Biochemical Oxygen Demand
CAS	Conventional Activated Sludge
C/N	Carbon/Nitrogen
C/P	Carbon/Phosphorus
COD	Chemical Oxygen Demand
DPAO	Denitrifying Phosphate Accumulating Organisms
EBPR	Enhanced Biological Phosphate Removal
GAO	Glycogen-Accumulating Organisms
HRT	Hydraulic Retention Time
MBR	Membrane Bioreactor
MLSS	Mixed Liquor Suspended Solids
MLVSS	Mixed Liquor Volatile Suspended Solids
ORP	Oxidation-Reduction Potential
OSA	Oxi-Settling Anaerobic
OUR	Oxygen Uptake Rate
PAO	Phosphate Accumulating Organisms
PHA	Polyhydroxy Alkenoate
PHB	Polyhydroxy butyrate
rbCOD	Readily Biodegradable COD
SBR	Sequential Batch Reactor
SOUR	Specific Oxygen Uptake Rate
Sp. SUR	Specific Substrate Utilization Rate
SRT	Solid Retention Time
TN	Total Nitrogen
TP	Total Phosphorus
TSS	Total Suspended Solids
UCT	University of Cape Town
VFA	Volatile Fatty Acid
VSS	Volatile Suspended Solids

1. Introduction

Phosphorous is the major nutrient contributing to the increased eutrophication of lakes and streams. The assimilative phosphorous removal through increased sludge production is the sustainable process of phosphorous removal. Together with the concern towards phosphorus, sludge generation is one of the major challenges of wastewater management (Khursheed and Kazmi, 2011). The efforts of nutrient removal are practically put-down the moment biological cells are lysed, hydrolyzed or digested during sludge reduction within the reactor; causing reappearance of assimilated nitrogen and phosphorus as mentioned above. Therefore, the phenomenon of in-place sludge reduction along with assimilative phosphorus removal is contradictory. High SRT required for carbonaceous oxidation, ammonia oxidation and sludge reduction, while low SRT for enhanced phosphorus assimilative uptake (Ge et al., 2015; Li et al., 2016; Wang and Zhen, 2019).

Further, there is requirement of carbon source for denitrification from the same pool; while PAOs and aerobic heterotrophs also compete for same substrate in order to produce PHA/PHB in enhanced phosphate uptake and aerobic oxidation. Therefore, emphasis on in-place sludge reduction creates a unique or rather conflicting situation in simultaneous removal of organics, nitrogen, phosphorus, and biomass. Nonetheless, simultaneous removal of organics, nitrogen, and phosphorus depends on extent of reconciliation of above-mentioned conflicts.

In the Anaerobic-Anoxic-Aerobic (A^{2}O) process the MLSS during aeration is recycled into the anoxic reactor, causing minimal amount of nitrate fed to the inlet anaerobic reactor. It eliminates any chance of the oxidation of generated VFA by denitrifiers and competes with PAOs (Henze, 2008). The University of Cape Town (UCT) process and its modified form has a common inlet anaerobic reactor and the entering substrate interferes with the absolute fasting conditions. The Phostrip process consists of a side stream anaerobic rector where phosphorus is released in solution and recovered through chemical precipitation (Metcalf and Eddy, 2003). The oxic settling anaerobic (OSA) process is similar as in the form of cyclic anaerobic treatment given to the activated sludge in a side stream anaerobic reactor, however the settled sludge from this anaerobic reactor is returned to pre-anoxic chamber for uncoupling of metabolism resulting in less sludge growth (Chudoba et al., 1992a, 1992b).

Therefore, the present reactor arrangement consisting of incorporation of OSA to A^2/O process creates a new configuration "**Reactor for Enhanced Sludge and Phosphorus Removal**"; which is tested to find the extent to resolve the conflict of "Simultaneous in-place sludge reduction with EBPR".

The synthesized biomass normally contains 2–3% phosphorus (by its dry weight). However, in case of enhanced biological phosphate removal (EBPR) it could be 2 to 5 times over the normal assimilation (Seviour et al., 2003; Oehmen et al., 2007). EBPR is achieved through the growth of phosphorus accumulating organisms (PAO). Since, phosphate can only be taken by microbes in ortho form therefore incorporation of anaerobic treatment is essential within the aerobic treatment sequence. Moreover, conversion of substrate to volatile fatty acids (VFA) is also essential for storage of Poly-β-Hydroxy Alkenoate (PHA) products including polyhydroxy butyrate (PHB) and polyhydroxy valerate (PHV) (Oehmen et al., 2007). The anaerobic–anoxic phosphorus removal phenotype termed as denitrifying phosphate accumulating organisms (DPAOs) is another microorganism mediated form of EBPR which consume less oxygen and produce less sludge (Kim et al., 2013). As reported approximately 6–9 mg of readily biodegradable COD (rbCOD) preferably in the form of volatile fatty acids (VFAs), is required for biological removal of 1 mg of phosphorus (Mulkerrins et al., 2004). Municipal wastewaters are seldom contained with required amount of rbCOD in the form of VFAs. Due to increasing cost of chemicals, their supplementation is uneconomical and the focus has now been shifted to on-site VFA production through waste activated sludges (WAS) acidogenic fermentation (Zhang et al., 2013, Gao et al., 2011). The production of VFAs and soluble COD (sCOD) under alkaline conditions was significantly higher than those at other pH values. Under anaerobic conditions bacteria release phosphate and synthesize PHB by using carbonaceous compounds; COD removal, nitrification, and phosphate uptake take place in the aerobic step (Seviour et al., 2003) and denitrification occurs under anoxic conditions (Metcalf and Eddy, 2003).

Oxic settling anaerobic (OSA) is the cyclic treatment of anaerobic followed by aerobic process which ensures minimum sludge generation/reduction through biological uncoupling of metabolism by virtue of reduced anabolism and enhanced catabolism. The (Chen et al., 2003; Chen et al. 2001a; Chen et al. 2001b; Chudoba et al., 1992a, 1992b; Guo, et al.2020; Semblante et al., 2016). The uncoupling results in restricted ATP formation consequently reduced biomass growth, during simultaneous substrate oxidation (Guo, et al. 2020). The bacterial growth depends on availability of energy after its utilization for different requirement of a living cell including cell maintenance. The high SRT causing long sludge age primarily results in more energy consumption for maintenance, which leaves less energy for cell synthesis (Semblante et al., 2014; Khursheed et al., 2017). The process of sludge reduction during OSA are explained by three prominent approaches for secondary maintenance; as proposed by Chudoba (Chudoba et al., 1992a, 1992b; Wentzel et al., 2000); another by Chen (Chen et al. 2001a, 2003, 2001b) and third by Khursheed et al. (2015) on the basis of SOUR gradient between fasting and feasting conditions. The Chudoba reported about 38%–54% less sludge on the basis of uncoupling as described above (Chudoba et al., 1992a, 1992b) consists of an anaerobic reactor in the return sludge line of a CAS reactor. Chen et al. (2003) cited low ORP of −250 mV in the anaerobic reactor and consequently lower biomass respiratory activity as the reason of sludge reduction to 36% during the process of sludge fasting/feasting in comparison to 58% at +100 mV ORP in the CAS process. The lysis cryptic growth due to increase in soluble COD in the anaerobic reactor caused low sludge yield in the OSA process (Saby et al., 2003). Khursheed et al. (2015) further substantiated the phenomenon of reduced excess sludge production in OSA process from 3% to 51% (average = 14.6–39.8%), due to SOUR gradient created between fasting and feasting conditions due to increased energy uptake during prolonged fasting.

2. Material and methods

The new laboratory scale configuration consisted of three chambered sequencing batch reactor (SBR) namely; aeration tank of 8 L effective volume, 3.68 L (volume Vn) anaerobic digestion chamber at the inlet, 2 L pre-denitrification anoxic chamber divided equally by a baffle and a 2 L side stream anaerobic reactor. The overall size of the reactor was 410 × 245 × 262 mm excluding side stream anaerobic reactor. Four cycles of 6-h duration were maintained per day in order to attain 1 day HRT. The experimental set up, cycle time details and sludge recirculation arrangement are shown in Figs. 1 and 2. Fig. 1 (a) shows SBR reaction cycle and Fig. 1 (b) shows both MLSS recirculation (Qr) to anoxic chamber and settled sludge recirculation to side stream anaerobic reactor, Fig. 2 (a) and (b) comprised of schematic diagram of reactor and its labeled picture respectively.

In order to investigate concurrent enhanced biological phosphate removal and reduced sludge production by a novel reactor configuration, the settled SBR sludge was recycled (Qrs) to a side stream anaerobic reactor at the rate of 0.25, 0.5, 0.75 and 1.0 L/cycle for 0.5 h/cycle after end of decantation resulted in 1, 2, 3 and 4 L MLSS/d flow rate as given in Table 1 (Figs. 1 and 2). The synthetic wastewater was used as given by Khursheed et al. (2015) by maintaining C/N ratio from 6.9 to 8.4 (7.54 ± 0.23), rbCOD/sbCOD ratio from 0.209 to 0.259 (0.248 ± 0.008) and C/P from 38.8 to 58.2 (46.4 ± 4.23) including orthP/TP ratio of 0.22–0.3. The C/P ratio was maintained at higher value of 18.2–20.2 (19.12 ± 0.62) in the last stage of the study by enhancing the orthP/TP ratio of 0.88. The MLSS recirculation to anoxic chamber at the ratio of (Qr/Q) of 4 was also maintained during aeration in order to achieve optimum nitrogen removal through denitrification in pre-anoxic chamber as per the findings of Khursheed et al. (2018). The entry of Qr to anoxic chamber prevents the detrimental effect of nitrate/nitrite (as other electron acceptor) in anaerobic reactor (Reddy, 1998). The reactor was operated in 5-stages with respect to sludge recycle to side stream anaerobic reactor and the feed characteristics as mentioned in Table 1. The influent (Q) was received in the anaerobic chamber then to the main SBR via anoxic chamber. The recycled settled sludge (Qrs) from SBR to a 2 L side stream anaerobic reactor (Qrs) also re-joined SBR via anoxic chamber.

The sludge was wasted daily from the reactor at varying rate (Vwn). All chemical investigations were done according to Standard Methods (APHA, 2005). For the determination of readily biodegradable COD, the method modified by Wentzel et al. (2000) was used. The Stoichiometric TP cellular uptake was calculated as 0.0267 times the sludge (VSS) growth in the reactor. The resulting enhanced TP content was calculated as ratio of TP uptake/sludge growth while increase in TP content was sludge phosphorus enrichment against stoichiometric value.

Khursheed et al. (2015) described in detail the mechanism of excess sludge reduction by maintaining fasting/feasting condition to the exposed biomass through alternate oxic and anaerobic cycles for the OSA process in the side stream anaerobic reactor coupled SBR. The mass of settled sludge (Xs, settled sludge active VSS concentration) was recirculated to the side stream anaerobic reactor (Qr) was Qrs Xs mg VSS/d out of the mass of total sludge (VX) in mgVSS present in aeration tank. This resulted in recirculation rate ((Qrs Xs)/(V X)) of settled sludge from aeration tank to anaerobic side stream reactor in mgVSS/mgVSS.d. However, the recirculation ratio (Rs) of settled sludge to total sludge from aeration tank to side stream anaerobic rector was (Qrs Xs)/(V X) (HRTn) in mgVSS/mgVSS. Since, HRTn is (Vn)/Qrs, the resulting Rs was (Vn Xs)/(V X). The Rs value expresses the mass of the main SBR sludge subjected to anaerobic fasting, in order to fulfill the main requirement of OSA process consequently the new rector configuration. Where, Xs and Qrs were settled sludge VSS (active) conc. and recirculation rate to side stream anaerobic reactor in L/d. V and X were aeration tank volume and MLVSS (active) concentration. Vn and HRTn were volume and HRT of side stream anaerobic reactor.

3. Results and discussion

3.1. Enhanced biological phosphorus removal (EBPR)

The EBPR in SBR with OSA depends on mass of VSS fermented in inlet anaerobic reactor and the HRT or SRT of the VSS in the reactor, since the fermentation of active sludge depends on these two main factors apart from many others. The key to EBPR is growth of PAOs which are normally present in mixed culture. Since, phosphate can only be taken by microbes in ortho form therefore anaerobic treatment is essential for it; moreover, conversion of substrate to VFA is also essential for storage of PHA products. The VFA thus produced in the anaerobic reactor expectedly facilitated EBPR during this phase of study. The parameters under discussion were TP, Ortho-P and Poly-P in influent, effluent and during different conditions namely; aerobic, anoxic and anaerobic wherever required. Consequent upon the reactor operation under the influence of sludge recirculation (Qrs), the phosphate removal during the study period are shown in Table 2 and Fig. 3 (a), which shows temporal variations in influent effluent values during the study and Fig. 3 (b) shows TP uptake and sludge recycle at different C/P values during the study period.

It was observed that in each stage the reactor took time to reach a steady state with respect to TP removal. Particularly during stage 1 this

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[1] Only frequently used.

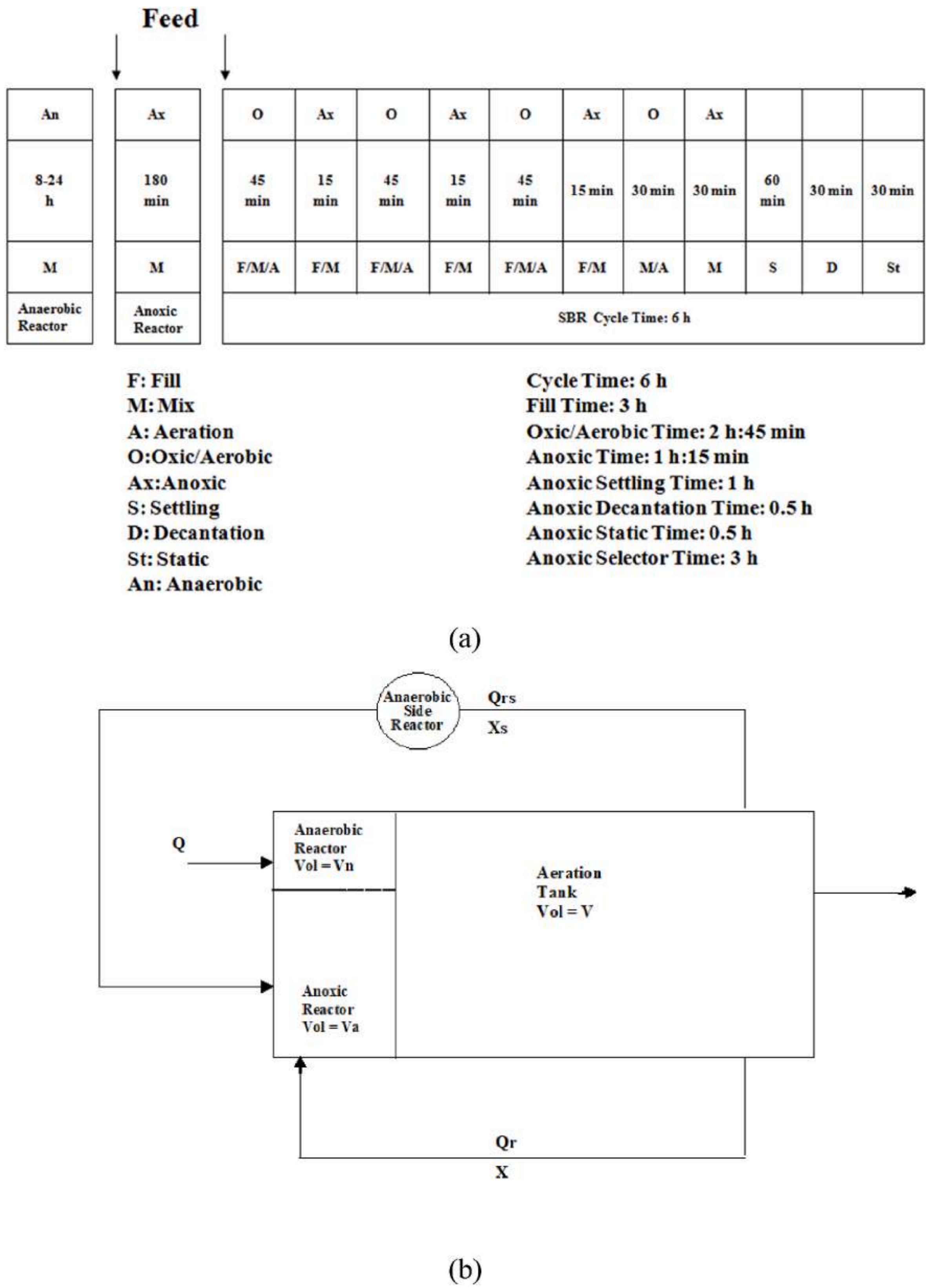


Fig. 1. (a) Reaction cycle showing durations of filling of SBR through inlet anaerobic reactor followed by pre-anoxic chamber, then alternate aerobic and anoxic operation of SBR and (b) Scheme of sludge recirculation (Qr) to pre-anoxic chamber during SBR operation and settled sludge recirculation through side stream anaerobic reactor to pre-anoxic chamber for OSA operation.

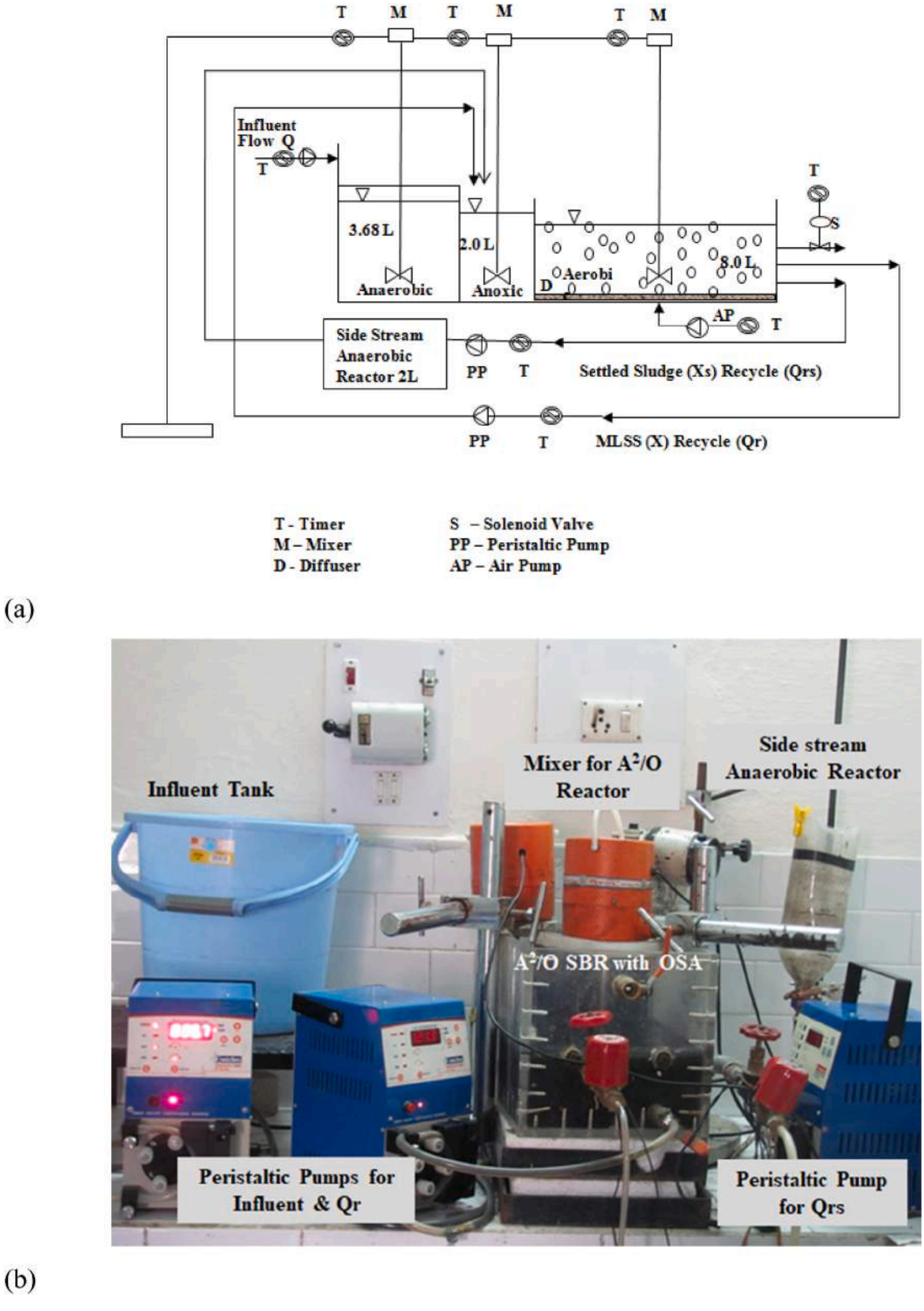


Fig. 2. (a) Schematic diagram of SBR with inlet anaerobic reactor, pre-anoxic chamber and side stream anaerobic reactors and (b) Picture of experimental set up.

Table 1
Reactor operation stages and feed characteristics.

Stage Qrs (L/d)	MLSS (Xs) recirculation to side stream anaerobic reactor	C/P Ratio	C/N Ratio	rbCOD/sbCOD Ratio	Inf TCOD (mg/L)	Inf bCOD (mg/L)	Inf BOD (mg/L)	Inf TN (mg/L as N)	Inf TP (mg/Las P)
1	0.25 L/cycle	48.86	7.51	0.248	456.7	304.5	190.3	40.5	6.23
2	0.5 L/cycle	42.97	7.56	0.248	460.1	306.7	191.7	40.5	7.14
3	0.75 L/cycle	46.98	7.54	0.250	460.3	306.8	191.8	40.7	6.53
4	1.0 L/cycle	42.94	7.54	0.248	462.8	308.5	192.8	40.9	7.19
5	0.5 L/cycle	19.12	7.66	0.244	463.6	309.0	193.2	40.4	16.17

***Settled MLSS (Xs) recirculation/cycle @ 4cycles/d and 0.5 h/cycle**.

Table 2
Effect of sludge recirculation ratio on TP removals, ortho-P removal, TP content of sludge and % change in the TP content.

Stage	Qrs, L/d (±σ)	C/P Ratio	Sludge VSSS Recy Ratio, g/g	Yobs/Yt	Inf TP, mg/L	Inf. Ortho P/TP Ratio	% TP Rem	Enhanced TP content, mgP/mgVSS	Increase in TP uptake	Ortho-P released in anaerobic, mgP/mgVSS
1	1.0	48.9	4.36	0.84	6.23	0.30	42.6	0.047	1.76	–
	± σ	1.03	1.08	0.09	0.11	0.09	12.1	0.016	0.61	–
2	2.0	42.8	6.41	0.75	7.14	0.23	76.1	0.092	3.46	0.019
	± σ	3.82	0.73	0.07	0.73	0.09	11.5	0.024	0.89	0.002
3	3.0	47.6	7.63	0.61	6.53	0.22	69.3	0.076	2.86	0.036
	± σ	5.88	1.34	0.08	0.78	0.13	6.4	0.010	0.39	0.003
	3.0	44.8	8.44	0.51	6.95	0.22	63.44	0.052	1.95	0.034
	± σ	6.07	0.27	0.08	0.80	0.13	8.87	0.01	0.38	0.001
4	4.0	43.1	8.24	0.59	7.19	0.28	59.4	0.078	2.93	0.029
	± σ	2.67	1.59	0.08	0.35	0.11	11.3	0.017	0.64	0.003
	4.0	44.2	10.06	0.50	7.04	0.28	64.53	0.036	1.36	0.053
	± σ	4.58	0.76	0.08	0.62	0.11	11.06	0.01	0.31	0.002
5	2.0	19.1	5.53	0.76	16.17	0.88	48.7	0.135	4.94	0.012
	± σ	0.62	1.24	0.08	0.16	0.24	2.3	0.017	0.63	0.002

time was more than 20 days. The phosphate removal is always independent of its initial concentration rather it depends on the growth of biomass in assimilative form and to a little extent it also removed through precipitation (Khursheed et al., 2022). Therefore, instead of its percent removal the actual TP uptake in to the sludge is considered more appropriate parameter of performance evaluation. Fig. 3 (b) shows TP uptake at each C/P ratio for the whole study period and Table 2 shows average variation in each stage. Fig. 3 and Table 2 show that the total phosphorus removal increased progressively from 42.6% to 76.1%. The highest rate was observed in stage 2 when the sludge recirculation to side stream anaerobic reactor was 6.41 gVSS/gVSS. The corresponding TP uptake into sludge was also highest at 3.46 times enrichment with respect to the stoichiometric value. The phosphorus enrichment in the form of increase in TP uptake receded in subsequent stages maintained at higher Rs except stage 5. Fig. 3 (b) also mentions average observed TP uptake when no anaerobic recirculation was observed, the average C/P ratio was 50.3 and the average TP uptake was 2.43 mg/L (σ ± 0.27). At the same time, TP uptake based on anticipated stoichiometric sludge growth calculated based on yield coefficient Yt mentioned by Khursheed et al. (2015) is also plotted (Avg. Obs TP uptake No recycle (C/P = 50.3) for the sake of wider choice of comparison.

3.2. *Effect of settled sludge recirculation to side stream anaerobic reactor*

The stoichiometric TP removal mentioned above was calculated based on 2.67% TP content in the sludge. The above Fig. 4 shows initial increase then decrease in phosphate uptake. Fig. 4 shows that the maximum uptake was obtained at 2 L/d Qrs sludge flow to the side stream anaerobic reactor resulting in 6.41 gVSS/gVSS recirculation ratio. The recycling of settled sludge to the side stream anaerobic reactor caused the release of phosphorus from the cell (VSS) in ortho form. This released ortho P rejoins the main SBR thereby reducing the actual removal of TP from the wastewater. The released orthoP varied from 0.019 to 0.053 mgOrthoP/mg VSS. The maximum average sludge recycling of 10.06 gVSS/gVSS was maintained in the last stage of reactor operation, however any further increase would have caused more leaching of TP than its uptake. The third and fourth stages of the operation at a recirculation rate of more than 8 and 9 were analyzed respectively as shown in Fig. 4 and Table 2. Fig. 3 (b) and Fig. 4 reveals that out of the two conflicting factors of EBPR and onsite sludge reduction any further anaerobic decimation of sludge from 2 L/d would reduce TP uptake despite continued enhancement in TP content of the sludge. Table 2 and Fig. 4 provides data on the effects of sludge recirculation ratio on TP removal, TP content of sludge, sludge TP enrichment in terms of increase with respect to stoichiometric uptake, and release of ortho P to the solution due to recycling to the side stream anaerobic reactor. The phosphorus enrichment under different stages of operation was 0.047–0.135 mgTP/mgVSS (4.7–13.5% phosphorus in the sludge) as against stoichiometric value of 0.0267 mgTP/mgVSS resulting in 1.76–5.05-fold increase in phosphorous content.

The dry sludge samples (taken randomly) were analyzed and their contents further confirmed the assimilation of nitrogen and phosphorus, as shown in Table 3. The measured values of N and P content in dry sample (unenhanced nutrients) were 0.119 (±0.008) mgN/mgVSS in comparison to 0.124 mgN/mgVSS and 0.080 (±0.019) dry mgP/mgVSS in comparison to experimentally observed in SBR of 0.074 (±0.013) mgP/mgVSS respectively are generally in agreement with each other.

3.3. *Effect of C/P ratio*

Nutrient removal processes depend on quantity and quality of carbon sources to achieve desired efficiency (Che, 2011). The nitrogen removal efficiency not only depends on C/N ratio but also on rbCOD/sbCOD ratio (Khursheed et al., 2018). Since biological phosphorus removal only occurs in assimilative form, the presence of higher C/P determines the ability of the system to grow more biomass and consequently to assimilate more or to remove more phosphorus. Generally, it is considered that a C/P ratio measured as BOD:P ratio greater than 20 is required to

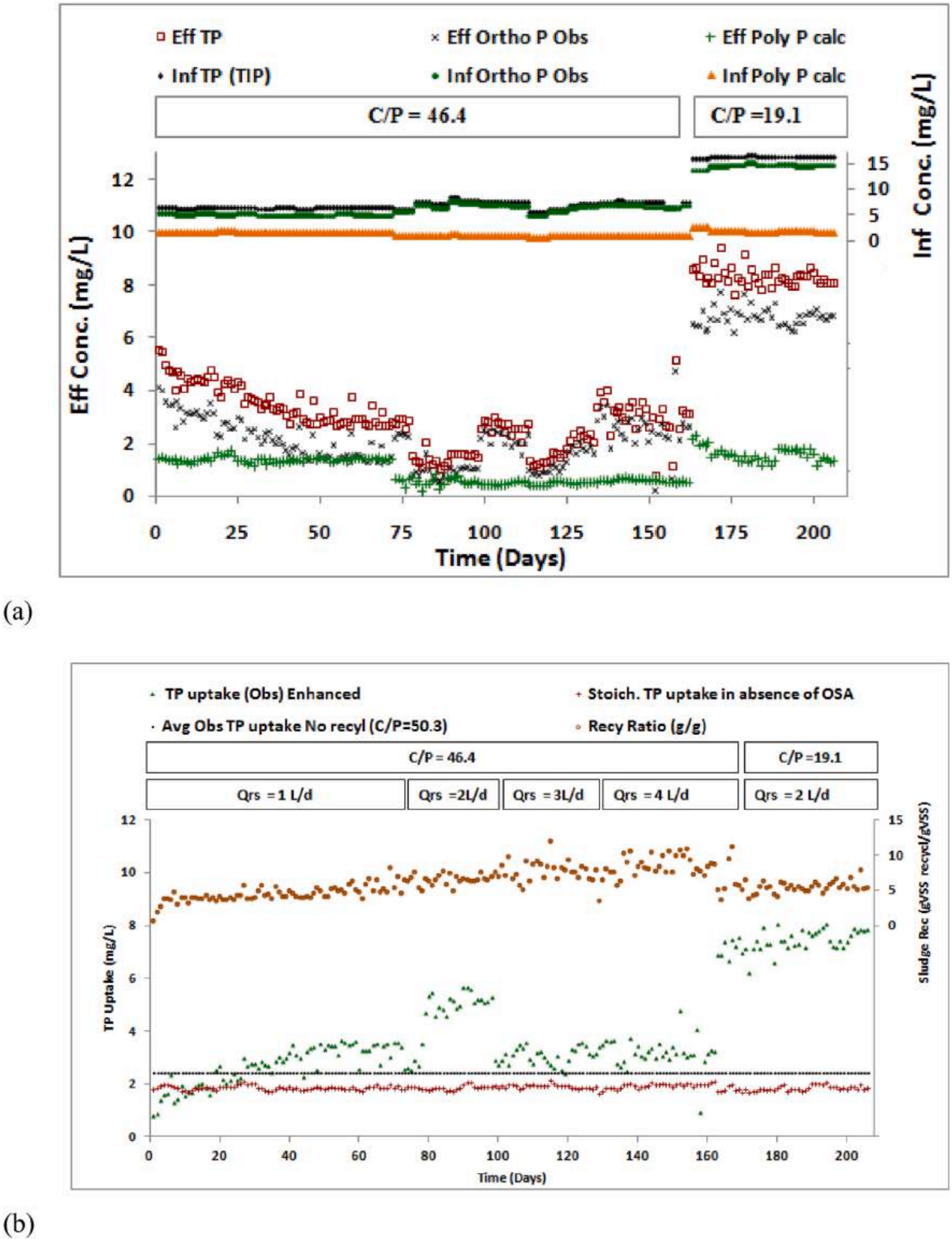


Fig. 3. Temporal variations in (a) influent and effluent values of phosphates including distribution of ortho and poly phosphates, (b) Observed and stoichiometric values of TP uptake with reference to C/P ratio and settled sludge recirculation through side stream anaerobic reactor (Qrs).

achieve lower phosphorus concentrations in the effluent (Metcalf and Eddy, 2003). The component bCOD was taken as C and, NH_4^+ as N, resulting in bCOD/NH_4^+ (as C/N) and bCOD consisting of rbCOD and sbCOD (in the ratio of rbCOD/sbCOD). Considering worst to average substrate constitution the C/P was kept at an average value of 50, and high value of 15. However, the actual carbon/phosphorus ratio (C/P) values maintained in the reactor were from 38.8 to 58.2 (46.4 ± 4.23). The C/P ratio was intended to be maintained at higher value of 15 in the last stage of the study also varied from 18.2 to 20.2 (19.12 ± 0.62). It was expected that the TP removal will be poor at lower C/P ratio, but the purpose of operation at lower C/P ratio (19.12) was to study the effect of higher TP content by virtue of higher Ortho P concentration in the reactor. This deliberate step was taken to see the extent to which EPBR could be achieved with higher ortho-P content which is similar to the

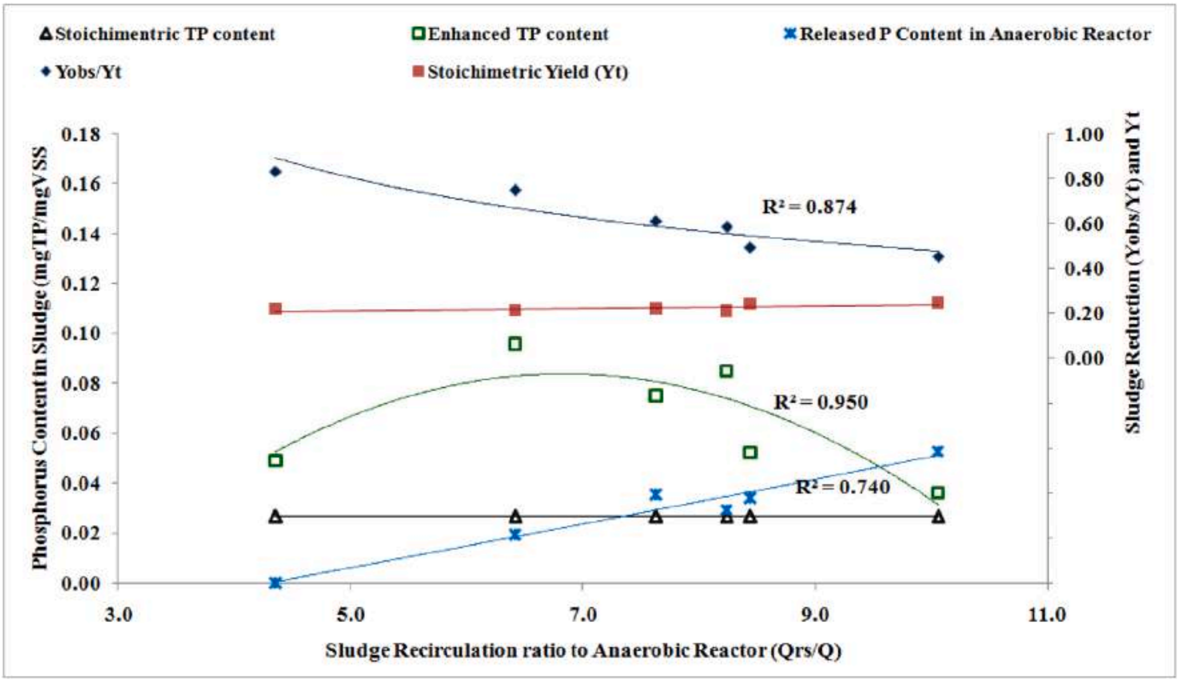


Fig. 4. Average TP uptake in every stage in relation to C/P ratio and settled sludge recirculation ratio (Rs) through side stream anaerobic reactor.

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

Day	Sludge N content		Sludge P content			Increase in TP uptake	
	gN/gVSS		gP/gVSS			gP(Obs)/gP(S)	
	Stoich.	Obs (Dry)	Obs	Stoich.	Obs (Dry)	Obs	Obs (Dry)
83	0.124	0.124	0.093	0.0267	0.077	3.48	2.88
88	0.124	0.132	0.083	0.0267	0.072	3.11	2.70
92	0.124	0.12	0.087	0.0267	0.086	3.26	3.22
98	0.124	0.13	0.089	0.0267	0.092	3.33	3.45
102	0.124	0.114	0.065	0.0267	0.056	2.43	2.10
110	0.124	0.103	0.054	0.0267	0.058	2.02	2.17
122	0.124	0.124	0.078	0.0267	0.086	2.92	3.22
128	0.124	0.11	0.069	0.0267	0.068	2.58	2.55
131	0.124	0.116	0.084	0.0267	0.082	3.15	3.07
141	0.124	0.108	0.061	0.0267	0.06	2.28	2.25
151	0.124	0.122	0.068	0.0267	0.068	2.55	2.55
162	0.124	0.115	0.057	0.0267	0.055	2.13	2.06
180	0.124	0.122	0.110	0.0267	0.104	4.11	3.90
188	0.124	0.128	0.116	0.0267	0.106	4.34	3.97
194	0.124	0.118	0.112	0.0267	0.115	4.21	4.31
199	0.124	0.117	0.108	0.0267	0.1	4.04	3.75
Avg.		0.119	0.083		0.080	3.12	3.01
± σ		0.008	0.020		0.019	0.762	0.720

release of ortho P from poly P during substrate fermentation in anaerobic process.

Corsino et al. (2020) observed simultaneous sludge reduction (64%), the removal of nitrogen (90%) and phosphorous (97%) at the phosphorus uptake rate 48.6 mgPO_4^{3-}-P/gVSS/h in an integrated anaerobic reactor in the mainstream of a pre-denitrification-MBR. The simultaneous high phosphorous removal at low sludge growth was cited due to the oxidation of low molecular weight compounds produced during bacterial lysis as secondary substrate by PAO. However, when Ortho-P was more in the influent 14.39 mg/L in stage 5 out of 16.17 mgTP/L as against 1.64 mg/L in stage 2 out of 7.14 mgTP/L, the corresponding increase in P uptake shoot up to 0.135 mgTP/mgVSS in stage 5 (5.05 times enrichment)as against 0.0092 mgTP/mgVSS in Stage2 (3.46 times enrichment). Table 2 clarifies this phenomenon further as to how TP uptake depends on Ortho-P concentration and the same was achieved by virtue of anaerobic treatment given to influent. Khursheed et al. (2015) found similar trends on those occasions when SOUR study was performed as shown in Fig. 4. It can be concluded that sludge recycle ratio shall be more if Ortho-P is less for high TP uptake.

3.4. *VFA production and ortho-P release in inlet anaerobic reactor*

The process of VFA sequestration is energy intensive, which is being released by the production of ATPs out of release of ortho-P from poly-P (Lanham et al., 2013; Welles et al., 2016). The rbCOD mainly consisted of VFAs present in digested anaerobic liquors (Vongvichiankul et al., 2017). Therefore, sludge samples taken from time to time were subjected to ortho-P and rbCOD determination as given in Table 4. The concentration of VFA as rbCOD given above reflects when converted to equivalent concentration in the SBR varied from 3.4 to 21.3 mg/L (9.7 ± 4.9). For the corresponding enhanced TP content in the present study, it amounted to 0.8–7.84 mg rbCOD VFA/mg of enhanced phosphorus uptake (3.4 ± 1.98), which was much less than the reported values of about 6–9 mg of rbCOD preferably in the form of VFA/mg of phosphorus (Mulkerrins et al., 2004). The concept of inability of PAOs to directly utilize carbon sources other than VFAs is no more valid and experimental evidences suggest that PAOs appear to have mechanisms to directly use unfermented non-VFA substrates by heterotrophic bacteria, nevertheless the extent of overall phosphorus removal capacity is yet to establish (Lanham et al., 2013; Welles et al., 2016). Similarly, the released Ortho-phosphate to acetate-uptake ratio are conflictingly reported by researchers from 0.2 to 0.73 mg PO_4–P/mg COD (Welles et al., 2016). Table 4 shows mgOrthoP released/mgrbCOD as VFA in the range of 0.33–0.76 (0.58 ± 0.11), which also shows existence in lower range than the required value.

3.5. *PHB production*

Han et al., (2012) and many more have already established that heterotrophs store polymers like PHA/PHB in the cell. This storage is mediated by a coenzyme HSCoA. Formation of HSCoA requires energy, which comes through hydrolysis of Poly-P from ATP during anaerobic

Table 4
Ortho-P released and VFA as rbCOD produced in inlet anaerobic reactor.

Stage	Day	TP uptake (Obs)	Enhanced TP content	Increase in TP uptake	Released Ortho-P (mg/L)	Ortho-P released Mass/cycle	Ortho-P released[a] (mg/L)	rbCOD (mg/L)	Ortho-P released/ rbCOD
	95	5.17	0.084	3.14	73.56	36.78	4.60	102.02	0.72
	96	5.06	0.089	3.32	80.58	40.29	5.04	112.79	0.71
	97	5.12	0.087	3.27	72.18	36.09	4.51	100.91	0.72
	98	5.28	0.089	3.35	67.83	33.91	4.24	98.30	0.69
3	105	3.31	0.063	2.37	53.01	39.76	4.97	95.05	0.56
	112	3.23	0.061	2.28	60.82	45.61	5.70	143.04	0.43
	122	3.25	0.078	2.93	55.83	41.87	5.23	170.13	0.33
	128	3.14	0.069	2.58	60.02	45.02	5.63	128.62	0.47
4	130	3.45	0.080	3.00	41.04	41.04	5.13	86.86	0.47
	139	3.14	0.059	2.21	34.03	34.03	4.25	56.10	0.61
	147	3.05	0.066	2.46	38.27	38.27	4.78	79.41	0.48
	148	3.22	0.050	1.87	21.64	21.64	2.71	28.44	0.76
	155	3.11	0.044	1.66	21.92	21.92	2.74	33.90	0.65
	160	3.14	0.058	2.15	32.87	32.87	4.11	64.03	0.51
	162	3.22	0.057	2.14	34.86	34.86	4.36	56.66	0.62
5	168	7.18	0.132	4.95	28.57	14.28	1.79	56.71	0.50
	175	7.11	0.115	4.32	38.19	19.09	2.39	68.57	0.56
	182	7.42	0.134	5.01	46.53	23.27	2.91	82.43	0.56
	195	7.41	0.115	4.31	22.68	11.34	1.42	35.26	0.64
	200	7.35	0.124	4.64	17.70	8.85	1.11	32.13	0.55
	201	7.63	0.127	4.76	25.12	12.56	1.57	40.83	0.62
	206	7.84	0.120	4.49	19.39	9.70	1.21	27.53	0.70

[a] Corresponding Concentration in the main SBR.

decimation of substrate. The PHB was therefore determined in the sludge samples as given in Table 5. Fig. 5 (a) shows TP uptake, increase in TP uptake and its enhanced content in sludge with progressive increase in PHB found in the sludge. Fig. 5 (b) shows all above parameters in relation to sludge recycle ratio (Rs). Figs. 4 and 5 (b) are very close to each other, in other words phosphorus enhancement and PHB increase were observed only up to 6.41 mgVSSrecycled/mgVSS. Rodgers and Wu (2010) quantified three PHB production rates of 70, 156 and 200 mg/gVSS.h under aerobic conditions, followed by anaerobic conditions, and under aerobic conditions on return with the supply of energy from polyphosphate conversion to orthophosphate respectively.

It can be summarized that EBPR can be successfully achieved by anaerobic treatment of aerobic sludge and sludge can be enhanced to 9.2% (13.5% provided high ortho form of P made available at nearly the same recirculation rate) of the cell as VSS as compared to normal content of 2.67%. However, it is the TP mass uptake which ultimately matters and the maximum observed uptake was 4.57 mgP/L at 6.41 gVSS/gVSS recycle ratio corresponding to the Qrs of 2 L/d. Further increase in recycle ratio deteriorated TP uptake despite enhancement in TP content of the sludge.

3.6. *Excess sludge reduction in the reactor*

In present reactor configuration excess sludge reduction by biological uncoupling of metabolism through the OSA process was observed. The overall observed Yield Coefficient (Yobs) during the OSA process conditions was calculated on the basis of daily sludge growth per unit daily bCOD removed and compared with stoichiometric yield coefficient (Yt) subject to SRT (θx) in order to evaluate sludge reduction potential. The Yobs is the yield based on active VSS growth while Yobs(Gross) is the observed yield inclusive of growth based on non-biological VSS also. In this study all growths are excluding non-biological VSS. Similarly, the observed yield coefficients (Yobs) was also compared to stoichiometric yield (Yt). The C/N ratio and rbCOD/sbCOD ratio of 7.5 and 0.25 respectively were maintained and the MLSS was also recirculated to preanoxic chamber at the rate of Qr/Q of 4 as per the findings of Khursheed et al. (2018). As mentioned above the recirculation flow rates of settled sludge and corresponding sludge recirculation ratio to the side stream anaerobic reactor and observed gross (overall), active observed and stoichiometric biomass growth rates during entire study period are plotted in Fig. 6 (a) and (b). Fig. 5 (a) gives complete picture of all forms of yield coefficients at different sludge recycle ratios at C/P values during the study period. Fig. 6 (b) shows trend of sludge reduction at increasing sludge recycle values.

Both gross and active observed growth rates experienced steady decline due to sludge recirculation. The average growth yields Yobs (gross), Yobs and Yt at Qrs of 1 L/d, 2 L/d, 3 L/d, 4 L/d at C/P ratio of 46.4 and Qrs of 2 L/d only at C/P ratio of 19.1 were Yobs(gross) 0.26 (±0.024), 0.23 (±0.010), 0.19 (±0.027), 0.20 (±0.028), 0.24 (±0.021), Yobs 0.18 (±0.020), 0.16 (±0.016), 0.14 (±0.017), 0.13 (±0.016), 0.16

Table 5
PHB values in relation to phosphorus uptake and enhanced P-content.

Stage	Day	Recycle Ratio	PHB	mgPHB/mgVSS	TP uptake (Obs)	Enhanced TP content[a]	Increase in TP uptake[b]
		(gVSSrecyl/gVSS)	(mg/L)		(mgP)	(mgP/mgVSS)	(mgP/mgVSS)
1	6	3.57	138.17	1.94	2.34	0.033	1.24
	7	4.74	73.53	1.08	1.28	0.019	0.70
	53	5.26	304.35	5.32	3.42	0.060	2.24
2	77	5.36	315.51	4.11	2.65	0.051	1.89
	78	4.78	136.89	7.43	3.49	0.059	2.22
4	147	9.86	228.39	7.09	3.05	0.066	2.46
	148	7.58	244.63	3.78	3.22	0.050	1.87
5	200	5.13	343.84	7.49	7.35	0.124	4.64
	201	6.72	289.47	10.49	7.63	0.127	4.76

[a] TP uptake/sludge growth.

[b] Enhanced TP content/0.0267.

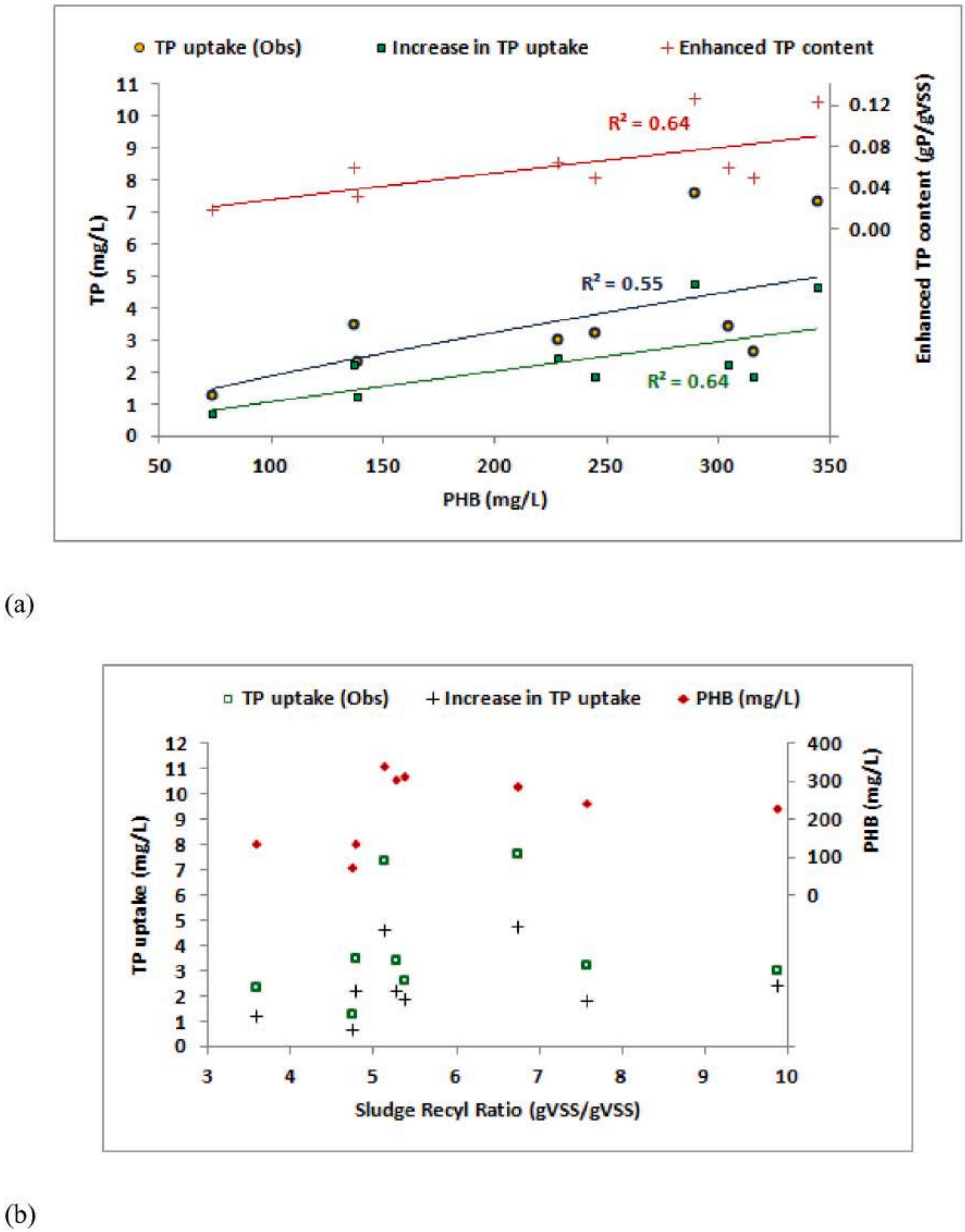


Fig. 5. (a) EBPR with progressive increase in PHB, (b) EBPR in relation to sludge recycle ratio to the side stream anaerobic reactor.

(±0.017) and Yt 0.22 (±0.008), 0.21 (±0.009), 0.22 (±0.008), 0.21 (±0.005), 0.21 (±0.005) respectively. As mentioned earlier Khursheed et al. (2015) observed the phenomenon of reduced excess sludge production in OSA process from 3% to 51% (average = 14.6–39.8%), at sludge recirculation from the SBR to a side stream anaerobic reactor of 3.78–9.78 gVSSrecycled/gVSS. In addition to the earlier identified reasons of sludge reduction namely; uncoupling of anabolism and catabolism (Chudoba et al., 1992a, 1992b) and ORP (Chen et al. 2001a, 2001b), the specific oxygen uptake rate (SOUR) gradient between sludge recycles and no recycle condition is reported as other possible reason. The SOUR gradient is the ratio of $[(\mathrm{SOUR})_{\mathrm{aeration}}\text{-}(\mathrm{SOUR})_{\mathrm{settling}}]/[(\mathrm{SOUR})_{\mathrm{aeration}}\text{-}(\mathrm{SOUR})_{\mathrm{anaerobic}}]$ difference between fasting when sludge came out of inlet anaerobic reactor and feasting conditions during aeration, and the SOUR difference between aeration and termination of settling in the absence of any sludge recycle. During prolonged fasting (in anaerobic) a high demand of energy does exist, therefore, when sludge enters aeration phase and experience feasting conditions (in aeration) increased energy uptake takes place. The increased cell

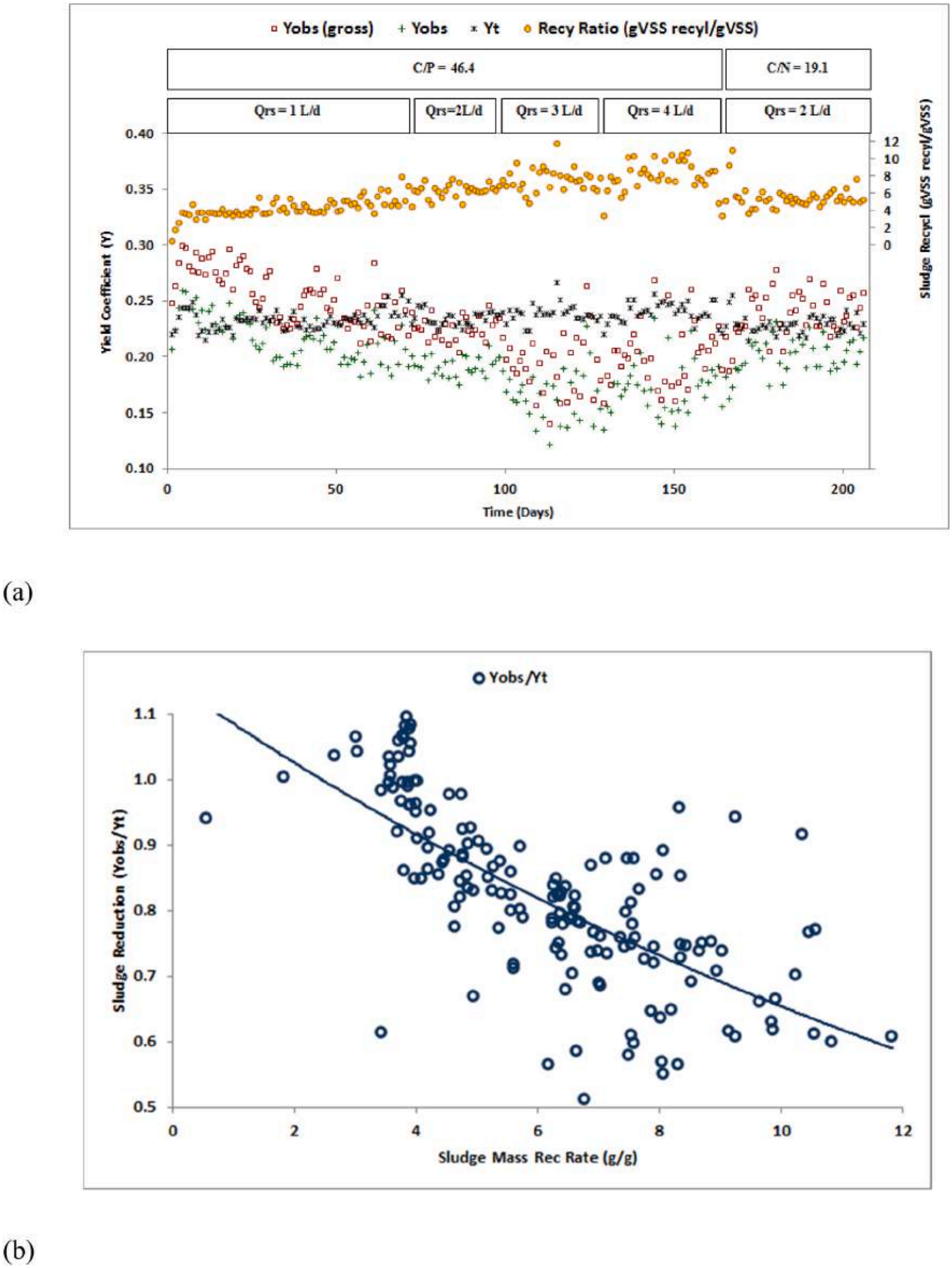


Fig. 6. (a) Temporal variations in observed yield coefficient in comparison to maximum and stoichiometric growth at different C/P ratios, (b) Sludge reductions for varying sludge recycle rate and ratio to side stream anaerobic reactor.

maintenance also validated the effect of SOUR gradient in direct proportion (Khursheed et al., 2015, 2017).

The DPAOs use nitrate and/or nitrite as an electron acceptor for P removal instead of oxygen and PHAs in the anoxic chamber existing between anaerobic and aerobic regimes resulting in simultaneous denitrification and P removal with 20–30% lower cell yield (Kim et al., 2013). The fasting microorganisms consumed stored energy during uncoupling replenish their consumed energy on their return to aerobic feasting environment. This uncoupling leave either less or no energy for growth resulting in significantly lowered biomass growth by about

38%–54%. (Chudoba et al., 1992a, 1992b). The controlled ORP of −250 mV in the anaerobic reactor resulted in 36% less sludge growth (Chen et al., 2001a, 2003, 2001b).

On the analogy of proportional relationship between the specific biomass growth rate (μ) and the specific substrate utilization rate (q), Lawrence and McCarty (1970) described that sludge yield (Y_s) decreased with increased SRT (θ_x) with reference to maximum theoretical or true yield (Y_m). The observed yield (Y_s) and maximum or true yield (Y_m or Y_G) in relation to Sp. SUR for maintenance requirement (q_m or mp) was also reported by Pirt (1975). The preference of energy utilization by the microorganisms for cell maintenance was established by Low and Chase (1999) while quantifying the biomass production per unit volume, which is the sum of the substrate utilized by anabolism and the biomass for their maintenance requirements. Khursheed et al. (2015, 2017) substantiated sludge reduction to the increased maintenance due to anaerobic stress and gave relationship between observed reduced sludge growth (Y_{obs}) due to sludge recirculation Rs (settled sludge recirculation ratio to side stream anaerobic reactor) during the OSA and stoichiometric value (Y_t) without OSA in terms of Yobs/Yt. However, the process of increased sludge reduction with higher recirculation to side stream anaerobic reactor may not be continuing as further sludge reduction would cause overloading food to microorganism ratios and ultimately bCOD reduction would expectedly be hampered. But the peak anaerobic stress causing breakdown of the process was not obtained during the study as it was not within the stated objectives.

3.7. Combined effect of A^2/O and OSA coupling in a single rector

The present reactor configuration under study is coupling of A^2/O and OSA; and therefore, novel in its present form as it delivers the conflicting achievement of EBPR and reduced sludge growth as mentioned in the introduction.

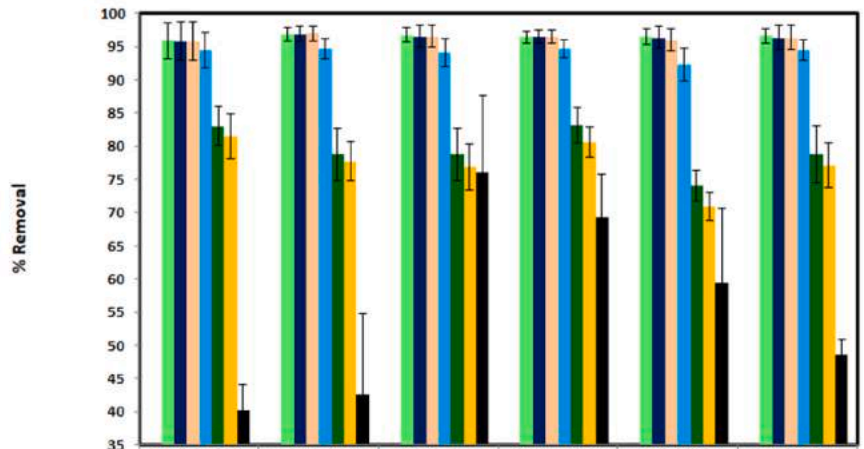
The EBPR in SBR with OSA depends on mass of VSS fermented in inlet anaerobic reactor and the HRT or SRT of the VSS in the reactor, since the fermentation of active sludge depends on these two main factors apart from many others. The results in Table 4 show 0.33–0.76 (0.58 ± 0.11) mgOrthoP released/mgrbCOD as VFA in rector. The sequestering of VFA into PHA/PHB is essentially required for EBPR, the progressive increase in PHB from 1.08 to 10.49 mgPHB/mgVSS found during different stages of study as shown in Fig. 5 and Table 5. This concluded that EBPR was successfully achieved in this novel reactor arrangement.

In present reactor configuration excess sludge reduction by biological uncoupling of metabolism through the OSA process was observed. During prolonged fasting (in anaerobic) a high demand of energy does exist, therefore, when sludge enters aeration phase and experience feasting conditions (in aeration) increased energy uptake takes place. The increased cell maintenance also validated the effect of SOUR gradient (degree of fasting to feasting shift) from 1.043 to 0.465 (average 0.68 ± 0.15) at VSS recycle ratio of 3.8–9.8 gVSS recycled/gVSS resulted in 3–51% sludge reduction (Khursheed et al., 2015, 2017) and low ORP of −59.4 to −478.6 mV on reduced sludge growth.

The novel reactor arrangement thus resulted in sludge reduction by 25% and phosphorus uptake enhancement by 3.46 times up to 6.41gVSSrecycled/gVSS and any further increase in sludge recirculation deteriorates phosphorus removal or uptake as released phosphorus from side stream reactor also recycled back to main SBR causing looping and at more than 6.41gVSSrecycled/gVSS it nullified the enhanced effect.

3.8. Other parameters during treatment process

Fig. 7 shows the average removal efficiency of all the parameters studied during different stages of the reactor operation. Definite trend was not observed except reduction in influent TP of 6 mg/L to effluent TP from 3.6 to 1.7 and then increase to 2.9 mg/L on increasing the sludge recirculation ratio from 0 to 6.4 and then to 8.2 gVSS recycled/gVSS at average C/P ratio of around 50. The optimum C/N and rbCOD/sbCOD ratios of 7.5 C/N and 0.25 ratio respectively was used at the MLSS recirculation to influent flow ratio from intermittent aeration SBR to pre-anoxic chamber of 4 as per the findings of Khursheed et al. (2018). At average wastewater quality in conjunction with the values given in Table 1; the TN removal was not more than 83% (76.9% in stage 2 at R value of 6.41). All the parameters were found independent of sludge recirculation ratio except TP removal as described above. Peak TP



	0 (C/P = 50.3)	1 (C/P = 48.9)	2 (C/P =42.8)	3 (C/P =47.6)	4 (C/P =43.1)	2 (C/P =19.1)
🟥 Sludge Recl (g/g)	0	4.4	6.4	7.6	8.2	5.5
🟩 % TCOD Rem	95.9	96.9	96.7	96.4	96.4	96.6
🟦 % TBOD Rem	95.8	96.9	96.6	96.5	96.4	96.4
🟧 % bCOD Rem	95.8	97.0	96.6	96.5	96.0	96.4
🟦 % NH4 Rem	94.5	94.6	94.1	94.6	92.3	94.4
🟩 %NO3 Rem	83.0	78.8	78.7	83.2	74.0	78.8
🟨 % TN Rem	81.5	77.8	76.9	80.6	70.9	77.2
⬛ % TP Rem	40.1	42.6	76.1	69.3	59.4	48.7

Fig. 7. Average removal efficiency of all the reactor parameters namely; total COD, BOD, bCOD, NH_4+, NO_3^-, TN and TP in all the stages under study.

removal of 76.1% was observed at Rs value of 6.41 gmVSSrecycled/gVSS. All the parameters excluding TP were maintained a very high level of removal in all the stages.

3.9. Statistical analysis

The influence of C/N ratio on phosphorus uptake was examined statistically using analysis of variance (ANOVA). The p-values smaller than 0.05 indicate statistically significant difference at 95% confidence level, [Gao et al., 2019; Sokkanathan et al., 2018]. The total phosphorus removal by virtue of cellular uptake at different settled sludge recirculation to side stream anaerobic reactor from 3.5 ± 0.5 to 10.5 ± 0.5 at an equal interval of 1.0 was subjected to one factor ANOVA to find the statistical significance of the difference between their mean values. The results from the statistical analysis have been compiled in tabular form as shown in Table 6(a) and (b). Table 6 (a) shows the mean and variance of the individual groups. Table 6 (b) shows the ANOVA results, the F-Value was obtained as 13.3789 which is far greater that the F-Critical value of 2.12. The corresponding p-Value was obtained as 2.62×10^{-11} ($\ll 0.05$), hence null hypothesis can be rejected as the difference between the means of these groups is statistically significant (Singh et al., 2020). These results suggest that the samples for each group i.e., we can conclude that total P uptake depends on recirculation ratio.

Table 6a
One-way ANOVA test: mean and variance of the individual groups of C/N ratios.

	SUMMARY				
	Groups	Count	Sum	Average	Variance
Sludge Recycle Ratio to Side stream Reactor	$3.5 \pm .5$	11	386.5094	35.13722	132.3333
	$4.5 \pm .5$	11	498.6935	45.33577	89.75042
	$5.5 \pm .5$	11	596.852	54.25927	138.2641
	$6.5 \pm .5$	11	737.2494	67.02268	201.8919
	$7.5 \pm .5$	11	763.7934	69.43576	111.1425
	$8.5 \pm .5$	11	723.4192	65.76538	94.30169
	$9.5 \pm .5$	11	704.5494	64.04994	44.89865
	$10.5 \pm .5$	11	705.4975	64.13613	168.088

Table 6b
one-way ANOVA results.

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	11480.26	7	1640.037	13.3789	2.62E-11	2.126324283
Within Groups	9806.705	80	122.5838			
Total	21286.96	87				

4. Conclusions

The sludge recirculation to side stream anaerobic reaction from 3.78 to 9.78 gVSS recycled/gVSS present resulted in successful sludge reduction from 3% to 51%. Low ORP values from −50 to −500mV in the inlet anaerobic reactor and around −50 to +100 mV in the pre-anoxic chamber may be a cause of lower sludge production. Also, worth consideration, the lower OUR in the anaerobic sludge created a SOUR gradient which has been cited as an additional reason of lower sludge yield. The successful reduced sludge yield under the influence of anaerobic stress proved to be effective. The further increase in sludge recirculation ratio may eventually cause highly unsustainable f/m ratio. EBPR is linked to recirculation ratio of settled sludge to a side stream anaerobic reactor from the aeration tank. The phosphorus enrichment under different stages of operation was 0.047–0.135 mgTP/mgVSS (4.7–13.5% phosphorus in the sludge) as against stoichiometric value of 0.0267 mgTP/mgVSS resulting in 1.76–5.05-fold increase in phosphorous enrichment. Total TP uptake, and its enhanced content in sludge was consistent with PHB found in the sludge despite lesser presence of 3.4 ± 1.98 mgrbCODVFA/(mg of enhanced phosphorus uptake). The TP uptake also depends on availability of PO_4^{3-} in ortho form, thus when Ortho-P was more in the influent the corresponding increase in P enrichment shoot up to 5.05 times in stage 5 as against 3.46 times in Stage 2 of reactor operation.

In summary, sludge reduction by 25% and phosphorus uptake enhancement by 3.46 times took place under sludge recirculation to side stream anaerobic reactor. Any further increase in settled sludge recirculation beyond 6.41gVSSrecycled/gVSS would increase the sludge reduction but at the cost of phosphorus removal or uptake. It precisely, appeared to be the "**point of reconciliation"** of these two conflicting parameters.

Contribution of the authors

Anwar Khursheed, Conceptualization, Formal analysis, Writing - Original Draft, Review & editing. Faris Mohammad A. Munshi, ~, Project administration, Funding acquisition, manuscript editing. Abdullah A. Almajid, ~, Project administration, Funding acquisition, manuscript editing. Sunita Varjani, ~, Writing – Review & editing. Ibrahim Almohana, ~, Investigation, Methodology. Shamshad Alam, ~, Writing – Review & editing. Omar Alrehaili, ~, Project administration, manuscript editing for resubmission. Dar Tafazul Islam, ~, Laboratory analysis, statistical analysis. Abdulrhman Fahmi Alali, ~, Laboratory analysis, manuscript editing. Vinay K. Tyagi, ~, Writing – Review & editing. Mohab Amin Kamal, ~, Writing – Review & editing. Manish Kumar, ~, Writing – Review & editing. A. A. Kazmi, ~, 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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References

APHA, 2005. Standard Methods for the Examination of Water and Wastewater, twentieth ed. American Public Health Association, Washington, DC, USA.
Che, H.M., 2011. Insight Enhanced Biological Phosphorus Removal (EBPR) Systems: EBPR Process, Mechanism and Microbiology. Lambert Academic Publishing.
Chen, G.H., An, K.J., Saby, S., Brois, E., Djafer, M., 2003. Possible cause of excess sludge reduction in an oxic-settling anaerobic activated sludge process (OSA process). Water Res. 37 (16), 3855–3866.
Chen, G.H., Saby, S., Djafer, M., Mo, H.K., 2001a. New approaches to minimize excess sludge in activated sludge systems. Water Sci. Technol. 44 (10), 203–208.
Chen, G.H., Yip, W.K., Mo, H.K., Liu, Y., 2001b. Effect of sludge fasting/feasting on growth of activated sludge cultures. Water Res. 35 (4), 1029–1037.
Chudoba, B., Morel, A., Capdeville, B., 1992a. The case of both energetic uncoupling and metabolic selection of microorganisms in the OSA activated sludge system. Environ. Technol. 13, 761–770.
Chudoba, P., Chudoba, J., Capdeville, B., 1992b. The aspect of energetic uncoupling of microbial growth in the activated sludge process: OSA system. Water Sci. Technol. 26 (9–11), 2477–2480.
Corsino, S.F., de Oliveira, T.S., Trapani, D.D., Torregrossa, M., Viviani, G., 2020. Simultaneous sludge minimization, biological phosphorous removal and membrane fouling mitigation in a novel plant layout for MBR. J. Environ. Manag. 259, 109826.
Gao, Y., Peng, Y., Zhang, J., Wang, S., Guo, J., Ye, L., 2011. Biological sludge reduction and enhanced nutrient removal in a pilot-scale system with 2-step sludge alkaline fermentation and A2O process. Bioresour. Technol. 102, 4091–4097.

Gao, M., Zhang, L., Florentino, A.P., Liu, Y., 2019. Performance of anaerobic treatment of blackwater collected from different toilet flushing systems: can we achieve both energy recovery and water conservation? J. Hazard Mater. 365, 44–52.

Ge, H., Batstone, D.J., Keller, J., 2015. Biological phosphorus removal from abattoir wastewater at very short sludge ages mediated by novel PAO *clade comamonadaceae*. Water Res. 69, 173–182.

Guo, J.-S., Fang, F., Yan, P., Chen, Y.-P., 2020. Sludge reduction based on microbial metabolism for sustainable wastewater treatment. Bioresour. Technol. 297, 122506.

Han, W., Yuan, L., Chai, L., Zhao, K., Li, G., 2012. The effect of PHA on enhanced biological phosphorus removal. Advanced Materials Research Advanced Materials Research 610–613. https://dx.doi.org/10.4028/www.scientific.net/AMR.610-613.1343.

Henze, M., 2008. Wastewater characterization. In: Henze, M., Loosdrecht, M.C.M.V., Ekama, G.A., Brdjanovic, D. (Eds.), Biological Wastewater Treatment: Principles, Modelling and Design. IWA Publishing, London, pp. 34–52.

Kim, J.M., Lee, H.J., Lee, D.S., Jeon, C.O., 2013. Characterization of the denitrification-associated phosphorus uptake properties of “candidatus accumulibacter phosphatis” clades in sludge subjected to enhanced biological phosphorus removal. Appl. Environ. Microbiol. 79 (6), 1969–1979. https://doi.org/10.1128/AEM.03464-12.

Khursheed, A., Ali, M., Munshi, F., M, A., Alali, A.F., Kamal, M.A., Almohana, A.I., Alrehaili, O., Gaur, R.Z., Tyagi, V.K., Khan, A., , A., Goel, G., 2022. Enhanced Combined Assimilative and Bound Phosphorus Uptake in Concurrence with Nitrate Removal in Pre-anoxic Cyclic Sequencing Batch Reactor. Environmental Technology & Innovation. https://doi.org/10.1016/j.eti.2022.102909.

Khursheed, A., Gaur, R.Z., Sharma, M.K., Tyagi, V.K., Khan, A.A., Kazmi, A.A., 2018. Dependence of enhanced biological nitrogen removal on carbon to nitrogen and rbCOD to sbCOD ratios during sewage treatment in sequencing batch reactor. J. Clean. Prod. 171, 1244–1254.

Khursheed, A., Kazmi, A.A., 2011. Retrospective of ecological approaches to excess sludge reduction. Water Res. 45, 4287–4310.

Khursheed, A., Sharma, M.K., Tyagi, V.K., Khan, A.A., Kazmi, A.A., 2015. Specific oxygen uptake rate gradient–another possible cause of excess sludge reduction in oxic-settling-anaerobic (OSA) process. Chem. Eng. J. 281 (1), 613–622.

Khursheed, A., Tyagi, V.K., Gaur, R.Z., Sharma, M.K., Khan, A.A., Kazmi, A.A., 2017. Reduced sludge growth at high bulk liquor dissolved oxygen induced by increased secondary cell maintenance. Chemosphere 184, 636–641.

Lawrence, A.W., McCarty, P.L., 1970. Unified basis for biological treatment design and operation. ASCE J. Sanitary Eng. Div 96 (SA3), 757.

Li, D., Lv, Y., Zeng, H., Zhang, J., 2016. Effect of sludge retention time on continuous-flow system with enhanced biological phosphorus removal granules at different COD loading. Bioresour. Technol. 219, 14–20.

Low, E.W., Chase, H.A., 1999. The effect of maintenance energy requirements on biomass production during wastewater treatment. Water Res. 33 (3), 847–853.

Metcalf, Eddy, Inc, 2003. Wastewater Engineering: Treatment, Disposal and Reuse, 4th rev. Tata McGraw-Hill, New Delhi.

Mulkerrins, D., Dobson, A.D.W., Colleran, E., 2004. Parameters affecting biological phosphate removal from wastewaters. Environ. Int. 30 (2), 249–259.

Oehmen, A., Lemos, P.C., Carvalho, G., Yuan, Z., Keller, J., Blackall, L.L., Reis, M.A.M., 2007. Advances in enhanced biological phosphorus removal: from micro to macro scale. Water Res. 41 (11), 2271–2300.

Pirt, S.J., 1975. Principles of Microbe and Cell Cultivation, first ed. Blackwell Scientific Publications, Oxford.

Rodgers, M., Wu, G., 2010. Production of polyhydroxy butyrate by activated sludge performing enhanced biological phosphorus removal. Bioresour. Technol. 101, 1049–1053.

Saby, S., Djafer, M., Chen, G.H., 2003. Effect of low ORP in anoxic sludge zone on excess sludge production in oxic-settling anoxic activated sludge process. Water Res. 37 (1), 11–20.

Semblante, G.U., Hai, F.I., Ngo, H.H., Guo, W., You, S.J., Price, W.E., Nghiem, L.D., 2014. Sludge cycling between aerobic, anoxic and anaerobic regimes to reduce sludge production during wastewater treatment: performance, mechanisms, and implications. Bioresour. Technol. 155, 395–409.

Semblante, G.U., Hai, F.I., Bustamante, H., Guevara, N., Price, W.E., Nghiem, L.D., 2016. Biosolids Reduction by the Oxic-Settling-Anoxic Process: Impact of Sludge Interchange Rate, vol. 210. Bioresource Technology, pp. 167–173.

Seviour, R.J., Mino, T., Onuki, M., 2003. The microbiology of biological phosphorus removal in activated sludge systems. FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Rev. 27 (1), 99–127.

Singh, B., Szamosi, Z., Siménfalvi, Z., 2020. Impact of mixing intensity and duration on biogas production in an anaerobic digester: a review. Crit. Rev. Biotechnol. 40 (4), 508–521. https://doi.org/10.1080/07388551.2020.1731413.

Sokkanathan, G., Sharmila, V.G., Kaliappan, S., Banu, J.R., Yeom, I.T., Rani, R.U., 2018. Combinative treatment of phenol-rich retting-pond wastewater by a hybrid upflow anaerobic sludge blanket reactor and solar photo Fenton process. J. Environ. Manag. 206, 999–1006.

Vongvichiankul, C., Deebao, J., Khongnakorn, W., 2017. Relationship between pH, oxidation reduction potential (ORP) and biogas production in mesophilic screw anaerobic digester. Energy Proc. 138, 877–882. https://doi.org/10.1016/j.egypro.2017.10.113.

Wang, X., Zhen, J., Zhao, J., Yu, D., Du, Y., Du, S., Yuan, M., Zhang, F., 2019. Effect of different sludge retention time (SRT) operations on the nutrient removal characteristics of a SNEDPR system. Europe PMC, Language: chi 40 (1), 352–359. https://doi.org/10.13227/j.hjkx.201807016.

Welles, L., Abbas, B., Sorokin, D.Y., Lopez-Vazquez, C.M., Hooijmans, C.M., van Loosdrecht, M.C.M., Brdjanovic, D., 2016. Metabolic response of “candidatus accumulibacter phosphatis” clade II C to changes in influent P/C ratio. Front. Microbiol. 7, 2121. https://doi.org/10.3389/fmicb.2016.02121.

Wentzel, M.C., Mbewe, A., Lakay, M.T., Ekama, G.A., 2000. Evaluation of a Modified Flocculation Filtration Method to Determine Wastewater Readily Biodegradable COD. WISA Biennial Conference, Sun City, South Africa.

Zhang, L., Zhang, S., Wang, S., Wu, C., Chen, Y., Wang, Y., Peng, Y., 2013. Enhanced biological nutrient removal in a simultaneous fermentation, denitrification and phosphate removal reactor using primary sludge as internal carbon source. Chemosphere 91 (5), 635–640. https://doi.org/10.1016/j.chemosphere.2012.12.071.