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RESEARCH ARTICLE

SIMULTANEOUS NITROGEN AND PHOSPHORUS REMOVALIN A SUBMERGED MOVING MEDIA REACTOR (SMMR)

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Manuscript Info *Abstract*

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Asubmerged moving media reactor(SMMR) was operated tosimultaneously remove COD, N and Punder anoxic and anaerobic conditions.The removal efficiencies of COD, N and P in SMMR with different COD/NH_4^+-N/NO_3^--N/P ratiosof 49.3/6.25/2.78/1 for case 1, 19.7/5.62/2.69/1 for case 2, and 10.3/4.61/3.21/1 for case 3, respectively, at various COD loading rate from 877 to 3,830 mg COD/d were investigated. Over 90% of COD was removed in SMMR for all COD loading rates butP removal and denitrification (nitrate removal) efficiencies in the range of 51.1 to 63.4 % and 54.5 to 72.2 %, respectively were depended on COD loading rate.Kinetic experiments were also additionally conducted to estimate the effect of COD and nitrate concentrations on P release rate (PRR), P uptake rate (PUR) and denitrification rate (DNR).The kinetic tests showed that the organisms in SMMR could remove P with nitrateand PUR was more affected by DNR than by PRR. Conclusively, SMMR can remove N and Psimultaneouslybymixed sludge containing DPAOcapable of P release, P uptake and denitrification.

Introduction:-

As global warming has been increasing, the average temperature in air and water onearth's surface has been also increasing.To date, algal blooming is one of the most serious problems in river and reservoir environments and thus the effluent standards of nitrogen and phosphate have been getting tightened in Korea. Over the last few decades, numerous studies on the removal of excess nitrates and phosphate, two major causes of eutrophication, have been conducted.

Enhanced biological phosphorus removal (EBPR) considering an effective and low-cost process enhances the proliferation of phosphate accumulating organisms (PAOs) (Kapagiannidis et al., 2013). In EBPR process, P release and organic removal take place in anaerobic reactor followed by P uptake in aerobic reactor. The biological nutrient removal (BNR) process, including the EBPR and biological nitrogen removal processes, requires considerable amount of COD in influent for both N and P removal, which isfrequently limited in the wastewater (Zhang et al., 2009). Randall et al. (1992) recommended over 6 and 40 of COD/TN and COD/TP ratios for effective BNR efficiency.

The enhanced P uptake with denitrifying phosphate accumulating organisms (DPAOs) in the anoxic condition has been considered a useful process in treating the deficient carbon source of influent (Johwan et al. 2001; Kapagiannidis et al., 2013; Kuba et al., 1993, 1996; Zhang et al., 2009). DPAOs can utilize NO_3^-in anoxic condition as an available electron acceptor (Kuba et al., 1993) and alsocan accumulate polyhydroxylakanoate (PHA) with P release under anaerobic condition.Denitrification and P uptake by DPAOs can occur simultaneously with PHA decomposition in the anoxic reactor, which was beneficial to reduce COD consumption (Ahn et al., 2001; Hu et al., 2002, 2003; Jiang et al., 2005; Kuba et al., 1996; Lee et al., 2001; Seojima et al., 2998; Wang et al., 2007; Zeng et

al., 2003), aeration costs (Choi et al., 2008) and sludge production (Kapagiannidis et al., 2013; Murnleitner et al., 1997). In addition, Zeng et al. (2003) insisted that PAOs and DPAOscould be the same microorganisms whichcan change theirmetabolism. In this study, a submerged moving media reactor (hereafter SMMR) used was designed to attach various kind of microorganisms including PAO and DPAO on submerged rotating disks (Kim et al., 1999).

The aim of this study was to perform the applicability of simultaneous N and P removal in SMMR including DPAOs. After the anaerobicandanoxic processes were combined using submerged media, we examined the removal efficiencies of organic matter, nitrogen, and phosphorus to deduce the most suitable operation conditions by altering the carbon source concentration of the influent.

Materials and Methods:-

Set-up of a submerged moving media reactor (SMMR):-

Fig. 1 shows the schematic diagram of the SMMR process made of acrylic resin. The SMMR with effective volume of 10 L was cylindricality with an internal diameter of 15.0 cm and a total height of 60.0 cm.52 acryl disks with 10 cm in diameter with a 0.8168 m^2 of total surface area wereinstalled with 1-2 mm of intervals and connected via a stainless steel shaft to motor and rotated at 60 rpm to attach biofilms with constant thickness and to mix mixed liquor suspended solids (MLSS) in the reactor. The SMMR was designed to co-exist anoxic and anaerobic zones outside and inside the rotating disks in the reactor. Due to the high amount of the condensed biofilm attached on the surface of rotating disks, anaerobic zone inside and around the biofilm was formed. Phosphate release took place in the anaerobic zone within the disks whereas denitrification and phosphate uptake occurred by MLSS in anoxic condition outside the anaerobic zone. The reactor was airtight and filled with the mixed liquor to prevent a direct exposure to O_2 in the air. A secondary sedimentation reactor of 2 L was used for sludge separation. Sludge recycle was performed to keep MLSS concentration constantly in the reactor using peristaltic pump.

Reactor inoculation:-

Inoculation sludge wasobtained from an anoxic tank of a municipal biological nutrient treatment plant(modified A2O process) located in Daegu, Korea. To obtain DPAO enriched biomass and to attach anaerobic bacteria onto the disks, the inoculated sludge was acclimatized in the SMMR under an anaerobic/anoxic condition for about 1 month as an adaptation stage before starting experimental phases.

Synthetic wastewater:-

Two synthetic wastewaters (influents 1 and 2) were separately fed into the SMMR in order to prevent an oxidation of organics and ammonium ioncoupled to nitrate reductionin the feed tank. The influent 1 contained (per liter): 76.9 to 384.4 mg of CH_3COONa (60 to 300 mg/Las COD basis), 76.4 mg of NH_4Cl (20 mg/Las N), 26.4 mg of KH_2PO_4 (6 mg/Las P), 150 mg of $MgSO_4 \cdot 7H_2O$, 200 mg of $CaCl_2 \cdot 2H_2O$, 50 mg of NaCl, 420 mg of $NaHCO_3$ and 0.3 mL of trace nutrient solution (TES). The TES consisted of the following compounds (per liter): 1.5 g of $FeCl_3 \cdot 6H_2O$, 0.03 g of $CuSO_4 \cdot 5H_2O$, 0.12 g of $MnSO_4 \cdot H_2O$ and 0.12 g of $ZnSO_4 \cdot 7H_2O$. The influent 2 was separately used to provide only nitrate (NO_3^-) as an electron acceptor: 142.9 mg of KNO_3 per liter (20 mg/L as N). The pH of the synthetic wastewater was adjusted at around 7.5 with 1.0 M HCl and 1.0 M NaOH.

SMMR operating conditions:-

As mentioned in previous section, the flow rates of the influents 1 and 2 were both 12 L/day and therefore those of the total influent into SMMR was 24 L/day. Sludge recycling (12 L/day) was also conducted from the bottom of the separation tank to the SMMR (Fig. 1). Thetotal flow-rate into the SMMR was 36 L/day. The MLSS and MLVSS concentrationsin the SMMR wereabout 3,510 and 2,230mg/Lfor phase 1, 1,740 and 1,060 mg/kg for phase 2,and 1,180 and 680 mg/L for phase 3, respectively, by controlling the appropriate amount of the disposed sludge. The MLVSS/MLSS ratio was in the range of 0.58 to 0.64. The hydraulic retention times (HRTs) of the SMMR and the separation tank were 10 h and 2 h, respectively. The SMMR has operated at low DO level (less than 0.5 mg/L) without aeration during operation period.

The operation of the SMMR was divided into three phasesaccording to SCOD concentrations in influent 1: 319 mg/L (Phase 1), 133 mg/L (Phase 2), and 73 mg/L (Phase 3). NH_4^+-N, NO_3^--N and PO_4-P concentrations are 24.3, 18.4 and 6.22 mg/L for phase 1, 21.9, 18.4 and 5.27 mg/L for phase 2, and 21.4, 19.9 and 6.34 mg/L for phase 3, respectively. The amounts of SCOD, N and P in the system were calculated by multiplying their concentrations with flow rates in order to estimate the COD, N and P removal efficiencies in SMMR. All tests performed when a steady-

state condition was achieved at different operational phases. The operating conditions of the SMMR system are presented in Table 1.

Kineticexperiments:-

Kinetic experiments were carried out to investigate P release, P uptake and denitrification rates of the activated sludgein SMMR under anaerobic and anoxic conditions. For P release, each 50 mL of the activated sludge taken from SMMRafter phase 1 finishedwastransferred into a dozen ofamber bottles (100 mL) purged withnitrogen gas and fed with 20 mL of sodium acetate of 1,000 mg/L as COD,20 mL of 100 mg N/L of NH_4Cl and 10 mL of 20 mg P/L of $Na_2HPO_4$and then capped tightly with a rubber cap. The bottles were shaken with an orbital shaker at200 rpmat 30°C for sampling time intervals. Two bottles were taken out from the shaker at each sampling time with 0, 0.25, 0.5, 1, 1.5 and 2 h and then settled for 0.5 h. The supernatant was quickly sampledfrom the bottle with a 20 mL syringe.

For estimation of P uptake and denitrification rates, 3 dozens of amber bottles (100 mL) were prepared. After P release steps for 2 h as described above, 20 mL of NO_3^--N solutions with 100 to 200 mg N/L were injected into the bottle. The bottles were shaken again at 200 rpm at 30°C for 0.25, 0.5, 1.0, 1.5, and 2 h, separately and settled again for 0.5 h.

SCOD, NH_4^+-N, NO_3^--N and PO_4-P were analyzed for each sample and phosphate release rate (PRR),phosphate uptake rate (PUR) and denitrification rate (DNR) were calculated from the analytical results.

Analytic methods:-

Under steady state conditions, samples from influent 1 and 2 and effluent were collected and filtered through 0.45 µm filter paper (GF/C-Whatman) before analysis. Soluble chemical oxygen demand (SCOD), ammonia nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), total phosphorus (TP) were analyzed according to the standard methods for the examination of water and wastewater (APHA, 2005).

Results and Discussion:-

The effect of COD/P and F/Mv ratios on COD removal in SMMR:-

The COD/P ratio is one of the key factors for the operation of EBPR (Zhang et al., 2011). The mass-based COD removal efficiencies in the SMMR were calculated as shown in Table 2.

Phase 1 was set with 49 of COD/P ratio in influent and 0.178 d^{-1} of F/Mv ratio, the amounts of COD in influent and effluent were 3,960 and 431 mg/d. The removed amount of COD in the SMMR was 3,526 mg/d. Carrera et al. (2001) reported the optimum COD/P ratio of 41 to 48 for EBPR. Phase 1 was conducted to provide the optimum condition for EBPR. The removed amount of COD was 3,526 mg/d and the COD removal efficiency in the SMMR was 89.1%. Considering addition removal of COD in the clarifier, the total removal efficiency of COD in the system was raised to 93.4%. In phase 2, COD/P ratio in influent was 24 and F/Mv ratio was 0.156 d^{-1}. The removed amount of SCOD in the SMMR was 1,395 mg/d with 84.1% of COD removal efficiency and the total removal efficiency in the system was 91.7%. In phase 3 with 10 of COD/P ratio and 0.146 d^{-1} of F/Mv ratio, the COD removal efficiencies in the SMMR and in the whole system were 76.5% and 91.5%, respectively. It may be associated with the consumption of organic matter related to P release during PHA and glycogen storage by DPAO, PAO and Glycogen Accumulating Organisms(GAOs), and with the biodegradation by other heterotrophic microorganisms. Therefore, it was not possible to estimate any difference in COD removal efficiency from them.

Fig. 2 shows that COD removal efficiency slightly increased with COD/P and F/Mv ratios. It is probably related to low nitrate and phosphate removal efficiencies as shown in Fig. 3 because COD is additionally removed at denitrification and P release.

Phosphate and nitrogen removals in SMMR:-

As shown in Table 2, NH_4^+-N removal in SMMR did not work at all phases but most of NO_3^--N were removed at relatively high organic/NO_3-N ratio at 17.73 and 7.50 g/g of COD/NO_3^--N in phases 1 and 2, respectively not at 3.22 COD/NO_3^--N in phase 3. SCOD/NH_4^+-N and SCOD/NO_3^--N ratios in influent were 7.89, 3.51 and 2.24 for cases 1, 2, and 3, and 17.73, 7.50 and 3.22, respectively. Lack of NH_4^+-N removal was probably attributed to the release of NH_4^+ from biofilm attached upon the submerged disks.

The amount of P, NO_3^--N and NH_4^+-N removed in the SMMR were 58.0 mg/d as P, 210.2 mg/d as N, and -132.0 mg/d as N, respectively. The P and NO_3^--N were removed by 72.2% and 94.2%in the SMMR, whereas NH_4^+-N was not removed. It indicates that there was no oxidation of ammonium in the SMMR because nitrate fed from influent 2 should be a sole electron acceptor. On the other hand, ammonium concentration in the reactor slightly increased because of ammonification of nitrate or release from the dead bacteria.

Fig. 4 presents the relationship between nitrate and phosphate removal rates (Fig. 4a) and the effect of COD/P on phosphate/nitrate removal ratio (Fig. 4b). As shown in Fig. 4a, nitrate removal efficiency increased with phosphate removal efficiency. It indicates that P uptake occurred coupled to denitrification under anoxic condition.

The observed ratio of P to COD removal was 0.0164 mgP/mgCOD which was higher than theoretically metabolic P/COD ratio (0 to 0.01 mg P/mg COD). The high ratio indicates that less organic carbon was required for P removal by PAOs in this system. Unfortunately, P release and excessive uptake could not be measured because both reactions occurred in the moving media on which the anoxic and anaerobic microbes were attached. The P concentration measured in the SMMR was lower than that in influent. The detailed operation results of the SMMR for each phase were summarized in Table 2.

Kinetic experiments:-

Because all of the reactions of organic removal, phosphate release and uptake, and denitrification were executed in the single reactor, we could not measure the efficiency of each reaction. Alternatively, we tested further the sequentially anaerobic and anoxic kinetic experimentsusing the bacteria taken from the SMMR to estimate the P release and COD removal rates under anaerobic condition and the P uptake and denitrification rates under anoxic condition.

Kinetic experiments consisted of anaerobic reaction time for 2h and anoxic reaction time for 2h by means of nitrate addition. Fig. 5 illustrates the profile of COD removal as well as nitrogen and phosphate removal via operating times in the batch reactor. It could be seen that almost all of the COD was removed within 2 h under anaerobic condition (> 94%) and different initial COD concentrations had no effect on the COD removal (Fig. 5a). Therefore, we concluded that the remained COD concentration after anaerobic reaction did not differentiate denitrification and P uptake from each phases.

Fig. 5b shows one-cycle profiles of N and P on the addition of nitrate at the 2h of operating time. P concentrations increased to 2.5 to 3.0 times within 2h (anaerobic) and then decreased due to enhanced uptake less than initial P concentrations. At the beginning, the initial P concentrations for cases 1 to 3 reached to 0.22, 0.21 and 0.21 mg in 100 mL. After anaerobic period (2 h), P concentrations increased to 0.55, 0.59 and 0.62 mg in 100 mL for case 1, 2 and 3, respectively.

Fig. 6a shows the ratio of COD removal and P release via time. The ratio reached the highest value of 200, 80 and 100 at 0.25, 0.5 and 0.5 hfor case 1, 2 and 3, respectively.As time went, the COD/P ratio also decreased to 54.9, 44.7 and 42.3 mgCOD/mgP for cases 1, 2 and 3, respectively, but more than 40 of COD/P ratio was required for P release.

Denitrification and P uptake were simultaneously achieved under anoxic conditions. Fig. 6b shows the P uptake/denitrification ratio via time. It exhibited low variable value such as 0.16~0.19 for case 1, 0.12~0.15 for case 2 and 0.12~0.13 for case 3 during whole time except for 0.25 min. It was in the order of case 1 > case 2 > case 3 after 2hrs of anoxic condition.

Fig. 7 describes the relationship of P release rate (PRR) vs. P uptake rate (PUR) (Fig. 7a) and PUR vs. denitrification rate (DNR) (Fig. 7b). PRR, PUR and DNR were 3.38, 3.68 and 19.0 for case 1, 3.37, 4.32 and 28.3 for case 2, and 3.39, 4.80 and 35.9 mg/g/h for case 3, respectively. PUR was affected by DNR more than by PRR. It indicates that P uptake was dependent on the supply of electron acceptor (ie, nitrate) and insufficient nitrate supply can cause the limitation of P uptake.

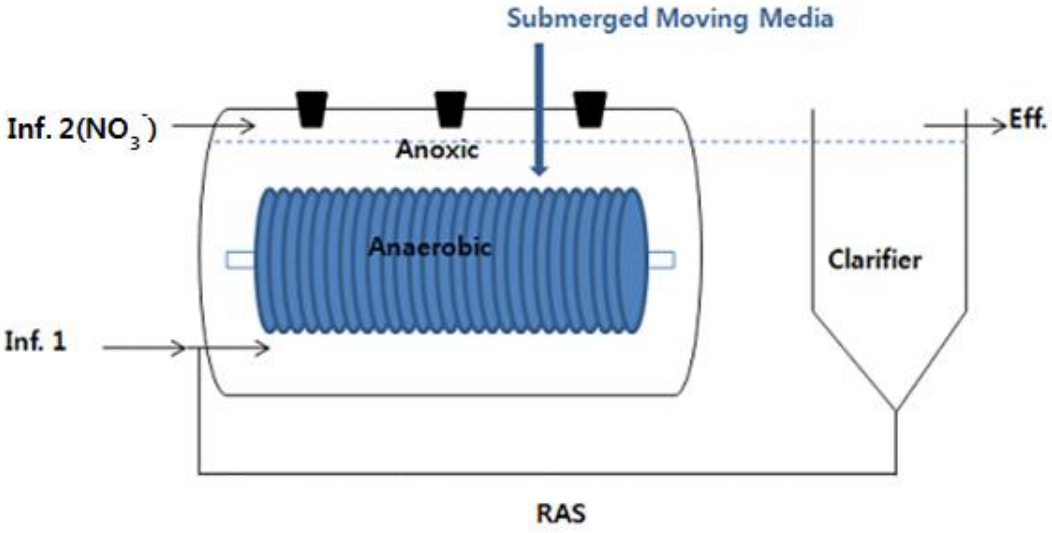

Fig.1:Schematic diagram of the experimental SMMR.

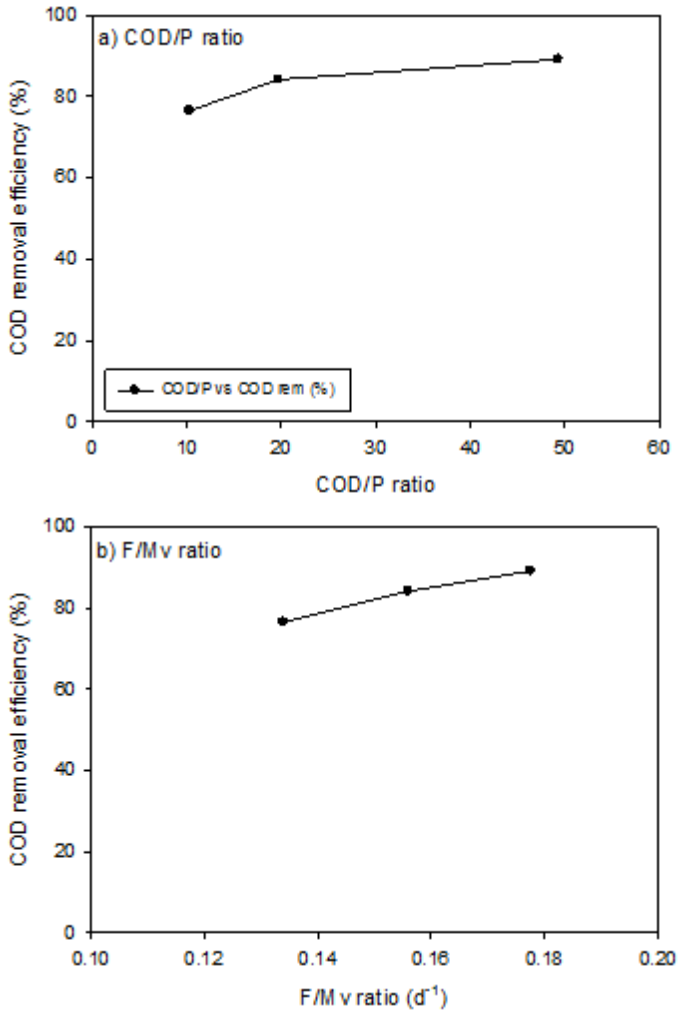

Fig. 2: Effects of (a) COD/P and (b) F/Mv ratios on COD removal efficiency (%)

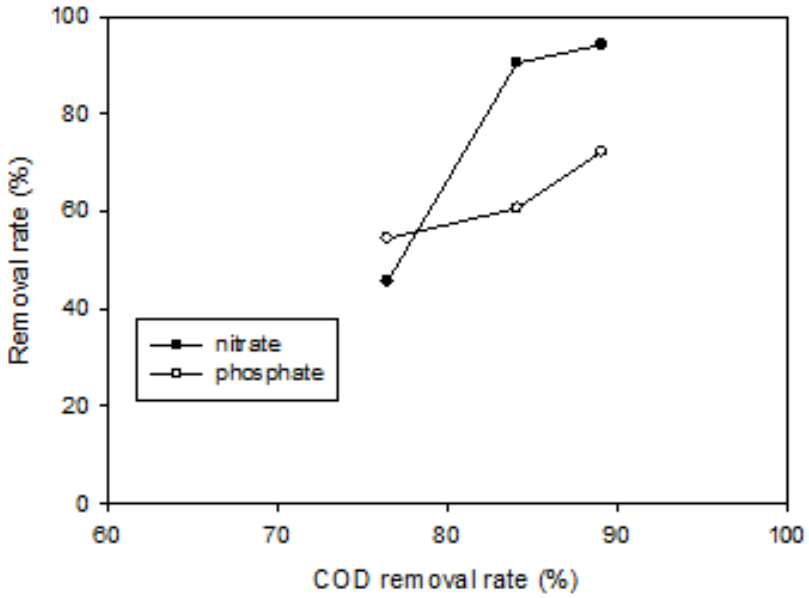

Fig. 3: Relationship between nitrate, phosphate and COD removal rates.

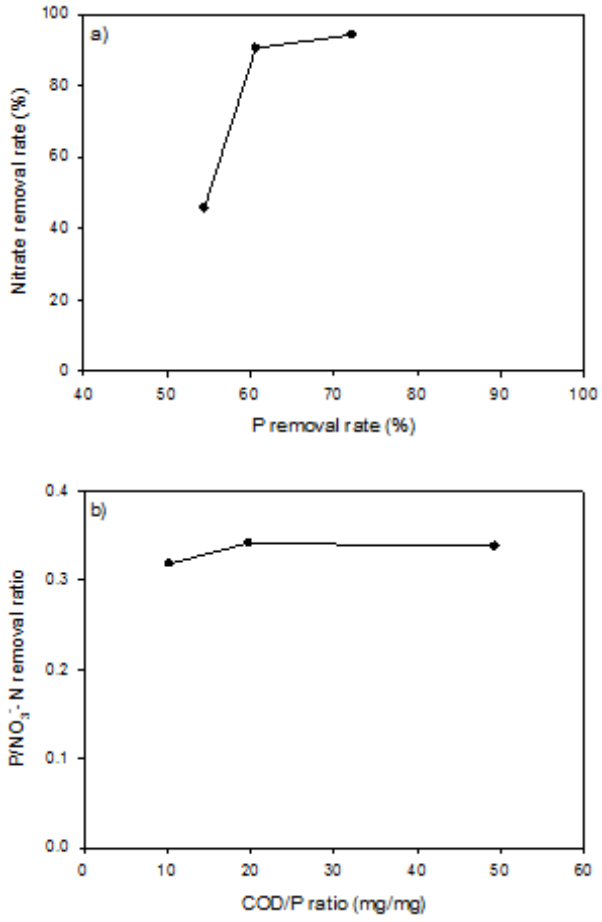

Fig. 4:(a)the relationship between nitrate and phosphate removal rates and (b) the effect of COD/P on phosphate/nitrate removal ratio.

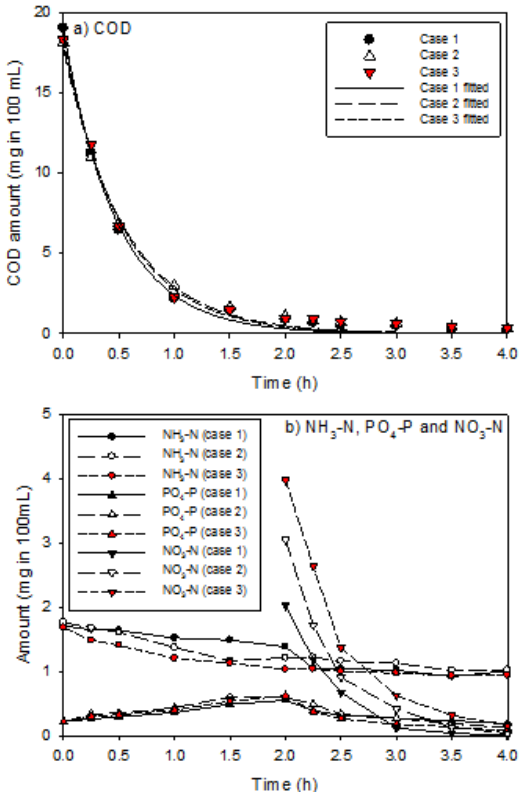

Fig. 5: Kinetic results on (a) COD, (b) NH_3-N, PO_4-P and NO_3-N.

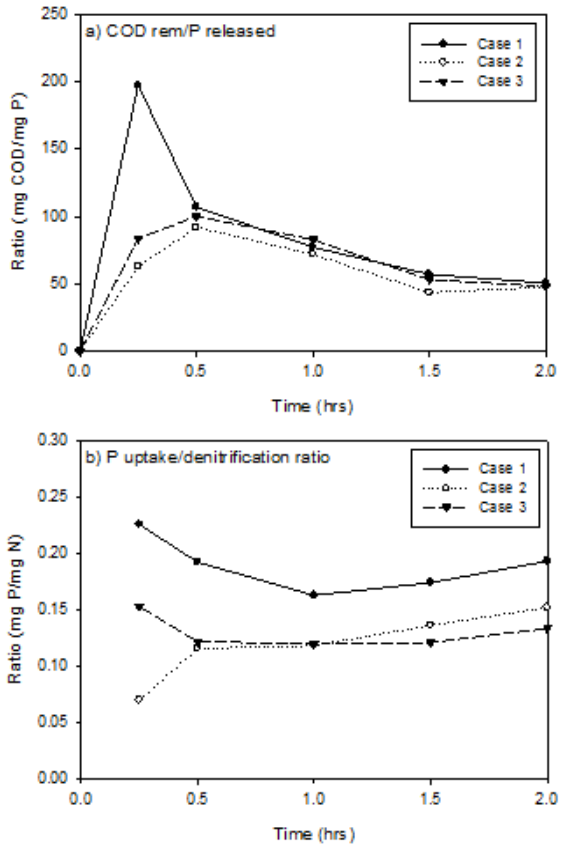

Fig. 6: Kinetic results on (a) COD removal/P release under anaerobic condition and (b) P uptake/denitrification under anoxic condition

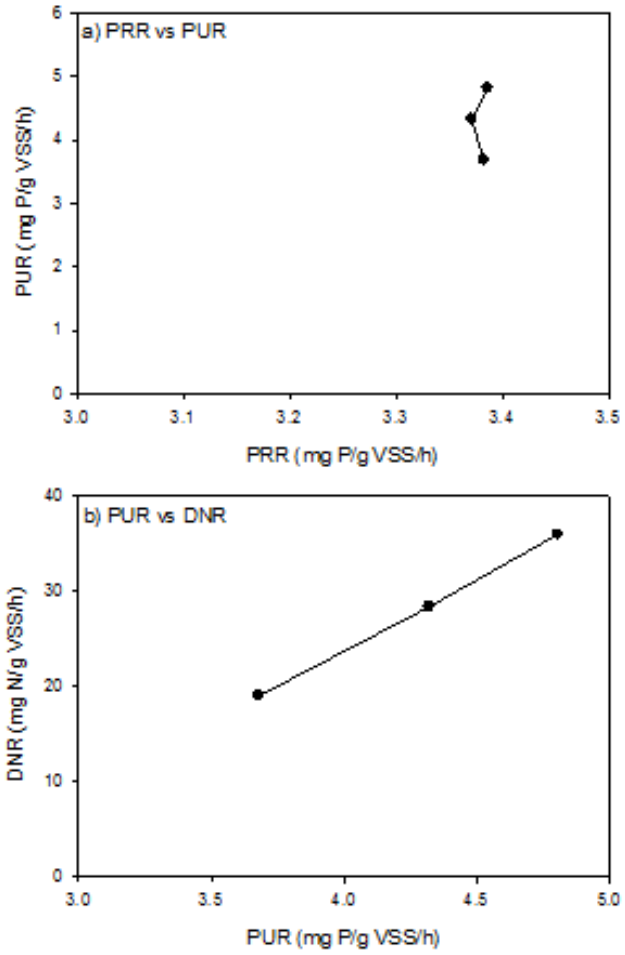

Fig. 7: Kinetic results on (a) COD removal/P release (PRR), (b) P uptake/denitrification (PUR)

Table 1: Operating conditions of the SMMR system.

Item		SMMR		
		Phase 1	Phase 2	Phase 3
From Influent 1	SCOD (mg/d)	3,957	1,658	914.0
	SP (mg/d)	80.3	84.1	88.6
	NH_3-N (mg/d)	501.6	472.8	408.0
	COD/P	49.3	19.7	10.3
	COD/NH_3-N	7.89	3.51	2.24
From Influent 2	NO_3-N (mg/d)	223.2	226.4	284.2
	COD/NO_3-N	17.73	7.32	3.22
MLVSS (mg/L) / MLSS (mg/L)		2,228/3,509	1,063/1,743	683/1,177
F/Mv ratio (mgCOD/mgMLVSS/d)		0.178	0.156	0.134
Surface loading rate for disc ($g/m^2/d$)	SCOD	4.84	2.03	1.12
	SP	0.098	0.088	0.108
	NH_3-N	0.614	0.579	0.500
	NO_3-N	0.273	0.271	0.348

Table 2: Operational results of the SMMR system in each phase.

Phase	Item	Inf. (mg/d)	SMMR				Clarifier Effluent (mg/d)	Total Removal (md/d)	Removal Effi. (%)
			In (mg/d)	Out (mg/d)	Removed (mg/d)	Removal Effi. (%)			
Phase 1	SCOD	3,830.2	3,956.5	430.6	3,526.0	89.1	252.7	3,577.4	93.4
	S-P	74.6	80.3	22.3	58.0	72.2	11.3	63.4	84.9
	NO_3-N	220.9	223.2	13.0	210.2	94.2	4.6	216.4	97.9
	NH_3-N	291.6	501.6	633.6	-132.0	-	420.0	-128.4	-
Phase 2	SCOD	1,592.2	1,658.3	263.2	1,395.1	84.1	132.2	1,459.9	91.7
	S-P	63.2	72.1	33.12	39.0	54.1	17.8	45.5	71.9
	NO_3-N	220.4	221.0	2.16	218.9	99.0	1.2	219.2	99.5
	NH_3-N	262.8	472.8	604.8	-132.0	-	420.0	-157.2	-
Phase 3	SCOD	877.3	914.4	214.9	699.5	76.5	74.2	803.2	91.5
	S-P	76.1	88.6	40.3	48.2	54.5	25.0	51.1	67.2
	NO_3-N	239.0	284.2	154.4	129.7	45.7	90.2	148.8	62.2
	NH_3-N	256.8	408.0	475.2	-67.2	-	302.4	-45.6	-

Conclusions:-

Weconducted to simultaneously remove NO_3^- and P in the SMMR withanaerobic and anoxic areas, which hasan advantage on both N and P removal in a single reactor and no-external carbon source. The removal efficiencies of organic matter, NO_3^-, and phosphorus were examined by changing the COD/N and COD/P ratios. Over 90% of COD was removed in SMMR for all COD loading rates. P removal and denitrification (nitrate removal) efficiencies were also dependent on COD loading rate. Kinetic experiments were conducted to support the evidence for the presence of mixed activated sludge in SMMR including DPAOs by analyzing the effect of COD and nitrate concentrations on P release rate (PRR), P uptake rate (PUR) and denitrification rate (DNR) as an electron acceptor.

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