A Modified UCT Method for Enhanced Biological Phosphorus Removal

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Abstract

Biological Nutrient Removal Activated Sludge (BNRAS) systems remove carbon, nitrogen and phosphorus by biological means with low costs and less waste sludge production. One of the most commonly applied BNRAS methods for urban wastewater treatment relies on the University of Cape Town (UCT) concept. The pilot-scale prototype BNRAS system presented here combines both, the idea of UCT concept and the step denitrification cascade for integrated removal of carbon, nitrogen and phosphorus. The experimental set-up of 44 L operational volume consists of an anaerobic selector and stepwise feeding in subsequent anoxic and oxic tanks. Raw wastewater with influent flow rates ranging between 48 – 168 L/d was fed to the unit at Hydraulic Residence Times (HRT) of 5 – 18 h and specific BOD_5 loading rates of 0.08 – 0.82 kg BOD_5/ (kg MLVSS· d). Influent flow rate (Q_F) is distributed at percentages of 60/25/15, 40/30/30 and 25/40/35 % to the anaerobic selector (AN), 2nd (DN2) and 3rd (DN3) anoxic tank respectively. The overall Sludge Retention Time (SRT, θc) was kept constant at 10 d and temperature at 20˚C. The results of two year operation show high removal efficiencies of organic matter of 89% as total COD removal and 95% removal for BOD_5, complete nitrification (95% removal of ammonium-nitrogen), 90% removal of Total Kjeldahl Nitrogen (TKN) and total nitrogen removal through denitrification of 73%. Phosphorus removal attains a mean value of 67%. All removal efficiencies receive constantly the highest values at the feeding ratio of 60/25/15. Moreover, plant configuration provides operational conditions that suppress filamentous bacteria and favour growth of floc-formers, and thus leads to high sludge settleability characteristics (Sludge Volume Index ≈ 100 ml/g). The highest removal efficiency and the optimum operation are recorded at HRT of about 9 hrs and influent flow rate of 96 L/d, which is distributed by 60% to the anaerobic selector, by 25% to second anoxic tank and by 15% to the last anoxic tank. Removal efficiency of organic substrate is 94% and 98% for COD and BOD_5 respectively, 99% for ammonium nitrogen, 94% for TKN, 83% for total nitrogen and 93% for orthophosphates.

Keywords: UCT, wastewater, nutrients, phosphorus, step feeding, BNRAS

1. Introduction

Nitrogen and phosphorus are limiting nutrients in most freshwater systems and cause eutrophication. Recently, interest has been developed in the use of biological, rather than chemical processes for phosphorus and nitrogen removal from wastewater. Increasing demands for lower effluent concentrations of nutrients in combination with minimized cost requirements, has encouraged researchers and plant operators to focus on nutrients removal optimization.

Biological nitrogen removal is a two step process including ammonia oxidation by nitrification, followed by reduction of nitrogen oxides to nitrogen gaseous compounds by denitrification (Wagner et al., 2002). As a result, a sequence of oxic and anoxic conditions is required for nitrogen removal. Biological phosphorus removal from wastewaters exploits the potential of some microorganisms, known as Phosphate Accumulating Organisms (PAOs), to accumulate phosphate (as intracellular polyphosphate) in excess of their normal metabolic requirements under aerobic conditions (Seviour et al., 2003). In the anaerobic phase, sufficient readily biodegradable carbon sources, such as volatile fatty acids (VFAs), must be available to induce PAOs to take up the acids, store them as poly-β-hydroxyalkanoates (PHA) and release orthophosphates into the solution (Seviour et al., 2003). This means that under anaerobic conditions PAOs gain the selective advantage over other bacteria. The bottleneck of the process is that PAOs accumulate aerobically more phosphorus than the phosphorus released under anaerobic conditions. Sludge wasting after aeration leads to phosphorus removal. Key wastewater characteristics, such as VFA content, pH, cations etc, and operational parameters, such as temperature, oxygen concentration, sludge quality etc, affect the successful removing of phosphorus (Mullkerins et al., 2004).

Biological Nutrient Removal Activated Sludge (BNRAS) systems remove carbon, nitrogen and phosphorus by biological means with low costs and less waste sludge production. One of the most commonly applied BNRAS methods for urban wastewater treatment relies on the University of Cape Town (UCT) concept. The UCT process was designed to minimize the effect of nitrate to the anaerobic contact zone, which is crucial for maintaining truly anaerobic conditions and thus, allowing biological phosphorus release. In fact, the higher the phosphorus concentration released in the anaerobic tank, the higher is the phosphorus concentration taken up under aerobic conditions. An improvement of the UCT method could be obtained through recycling of sludge from the clarifier to an anoxic stage before entering the anaerobic tank. This would result in nitrate reduction by denitrification and thus, neither oxygen nor nitrate would enter the anaerobic reactor and damage the anaerobic conditions. From a biological aspect, the anaerobic selector not only contributes to phosphorus removal, but also forms heavy large flocs that enhance settleability (Metcalf & Eddy, 2003). Experiments and full scale applications have shown that this pre-denitrification – nitrification process, which includes an anaerobic selector and internal recycle mixed liquor, is efficient for removal of organic substrate, nitrogen and phosphorus from wastewater (Schlegel, 1992; Ekama and Wentzel, 1999; Metcalf & Eddy, 2003). The modified UCT method upgraded the process; however, an internal recycling of nitrified liquor to an anoxic tank is still needed (Metcalf & Eddy, 2003).

Modifications in such BNRAS systems have proved that step feeding is an attractive process to eliminate the need for internal recycling, sludge recirculation and to optimize organic carbon utilization for denitrification, resulting thus in energy savings (Metcalf & Eddy, 2003; Görgün et al., 1996; Pai et al., 2004; Schlegel, 1992; Vaiopoulou et al., 2007). On the other hand, multiple stage cascades optimize removal efficiency with minimum reactor volume. A cascade of three stages is expected to yield to optimum effluent quality, which is shown by model simulation results (Görgün et al., 1996). Experiments on nutrients removal in a two denitrification stage UCT system with step feeding and influent flow distribution by 50% to the anaerobic tank and the rest to the second anoxic tank showed high removal efficiencies (Schlegel, 1992). A similar attempt was undertaken by Pai et al. resulting to an economically efficient removal of organic substrate and nutrients with highly clarified effluent (2004). However, the effect of the step feeding process was minimized, whereas the plant was fed with a synthetic wastewater, which can not simulate successfully real wastewater. The regime and feed pattern in a wastewater treatment plant affects the microbial population synthesis and characteristics and thus, the removal efficiency of the unit (Wagner et al., 2002; Zeng et al., 2004). Indeed, personal previous experience using synthetic feed showed that artificial wastewater does not give a realistic view of the system performance (Vaiopoulou et al., 2007).

On this basis, the UCT approach could be enhanced by combining multiple stages of anoxic and oxic zones with the step feeding process. Herein a modified UCT method is proposed, which consists of an initial anaerobic selector followed by a cascade of three identical pairs of tanks. Each pair comprises of an anoxic and aerobic tank for denitrification and nitrification respectively. The initial anaerobic tank helps select phosphate accumulating organisms, according to the EBPR technique, as well as floc-formers over filamentous bacteria. The cascade is supposed to lead to high removal efficiency, optimum effluent quality and favorable residence time distribution with minimal reactor volume. The three-stage cascade is expected to yield a potential (theoretical) removal efficiency of total nitrogen around 83% (data not shown), when sludge recycle ratio is equal to the influent flow rate. Multiple stage cascades also offer the operational assurance of removing anything left untreated in a following stage. Step feeding strategy is adopted providing raw wastewater to the anaerobic selector, to the 2nd and 3rd anoxic tank transforming these tanks into selectors of floc-formers over filaments, whereas a cheap organic carbon source is available for denitrification. The feeding ratios of the influent flow rate to the anaerobic selector and anoxic tanks were calculated so that the carbon to nitrogen ratio is theoretically sufficient for denitrification. In particular, these influent percentages to the anaerobic selector, to the 2nd and 3rd anoxic tank are proposed as 60/25/15, 40/30/30 and 25/40/35 % of total influent flow rate respectively. Internal nitrified liquor recycle is not necessary, whereas sludge recycle is introduced to the anaerobic selector via the 1st anoxic tank. This results in 1) nitrate elimination before entering the anaerobic selector, 2) sludge recycle flow minimization and 3) supply of the anaerobic selector with biomass. Primary sedimentation is excluded and thus, organics and nutrients are maintained, which are essential to following procedures of phosphorus removal and denitrification.

Since such modifications would most probably result in a satisfactory performance with low costs, and since BNRAS systems are complex with many dependent interactions, experimental procedures are essential for evaluating their performance and design. Therefore, the objective of this work is to present a novel pilot-scale activated sludge system for biological removal of organic substrate, nitrogen and phosphorus, combining the idea of the UCT process with the step feeding approach, and also to confirm the theoretically expected design advantages. Performance, operation and removal efficiency of two alternative feeding schemes are also investigated aiming at process optimization.

2. Materials and Methods

2.1. Plant description

The experimental unit described here is an activated sludge system for enhanced biological phosphorus removal (Fig.1), which includes an anaerobic selector and stepwise feeding in subsequent anoxic and oxic vessels for simultaneous removal of BOD5/COD and suspended solids, as well as nitrogen and phosphorus. The pilot plant has total operational volume of 44 L and consists of an anaerobic selector (ANAER) of 3.5 L followed by a cascade of three identical pairs of anoxic/aerobic bioreactors. Denitrification takes place in the three anoxic bioreactors (DNi, i=1 to 3), which have an operational volume of 3.4 L each, whereas nitrification and carbon oxidation is accomplished in the three aerobic bioreactors (AEi), which have an operational volume of 7.3 L each. Switching between anaerobic and aerobic conditions favors the growth of PAOs, and thus, biological phosphorus removal (Wagner et al., 2002; Seviour et al., 2003). Finally, a secondary sedimentation tank (ST) of 8.3 L is used for sludge separation and recycling into the anaerobic selector via the first anoxic tank. Sludge wastage and recycle are controlled by a timer that switches on/off a magnetic three-way valve. Wastewater transfer from one tank to the following one is performed in part by free overflow and in part by use of level controllers and peristaltic pumps. In particular, the reactors are connected with a piping system so that the wastewater or mixed liquor suspended solids (MLSS) are introduced at the bottom and withdrawn from the top of each reactor. The piping system is so designed that unintentional oxygenation of the water or MLSS is kept at a minimum. Aeration is performed by the diffused aeration method, i.e. air is introduced at the bottom of the aerobic bioreactors through special porous material. Air flow meters are used for manual control of air flow rates in each aerobic tank. All tanks are gently stirred.

Figure 1. View of the Biological Nutrient Removal Activated Sludge (BNRAS) system.

The pilot-scale plant has been in operation for about two years. The plant was fed with raw municipal wastewater from the combined sewer system of Xanthi city in Greece. Particularly, charges of one cubic meter were delivered once or twice per week depending on influent volume needs. Influent average concentrations of BOD5 and COD were 320 mg/L and 510 mg/L respectively. Influent orthophosphate phosphorus and total phosphorus concentration spanned a range between 2-9 mg PO_4^{3-}-P/L and 2-15 mg TP/L. The percentage of orthophosphates content in total phosphorus was in average 63%. Total nitrogen influent concentration was typically around 55 mg/L. Wastewater characterization is presented in Table 1. No addition of chemicals took place to provide convenient values of wastewater pollutants. With the exception of some low phosphorus concentrations, organic substrate and nitrogen extent can be regarded as normal for a Greek municipal or domestic wastewater.

Table 1. Wastewater characterization of municipal origin.

Pollution parameter	Concentration (mg/L)		
	Min	Mean	Max
Total Suspended Solids (TSS)	60	198	784
Volatile Suspended Solids (VSS)	50	170	666
Non-Volatile SS (NVSS)	10	28	118
COD_p	233	518	782
BOD_5	170	311	540
Total Nitrogen (TN)	25	54	69
Total Kjedahl Nitrogen (TKN_p)	25	54	69
Ammonium nitrogen (NH_4^+-N)	21	39	54
Organic Nitrogen	2	15	23
Nitrate/ Nitrite (NO_x^--N)	0	0	0
Total Phosphorus (TP)	1,4	8	15
Orthophosphates (PO_4^{-3}-P)	0,3	5	9,2

Influent wastewater rate (Q_F) is distributed to the anaerobic selector with a flow rate of Q_{ANAER}, and to the second and third anoxic zone with flow rates of Q_{DN2} and Q_{DN3} respectively, as shown in Fig. 2 (influent flow distribution before the anaerobic selector). Incoming percentages are calculated according to the ratio of the tank flow rate to the total influent flow rate (e.g. Q_{DN2}/Q_F). During the experiments reported here, influent flow distribution to the anaerobic selector, and the second and third anoxic tank ($Q_{ANAER}/Q_{DN2}/Q_{DN3}$) was set to 60/25/15, 40/30/30 and 25/40/35 %. Influent flow rates (Q_F) into the activated sludge system were increased from 48 to 168 L/d in order to obtain a hydraulic residence time (HRT, θ) from 18 to 5 hours respectively. HRT is calculated excluding the clarifier at an operational working volume of 35.6 L. Sludge recycling rate (Q_R) was set equal to influent flow rate (Q_R= Q_F) for each run, whereas the overall sludge retention time (SRT, θc) was kept constant at 10 days by controlling sludge wastage flow rate.

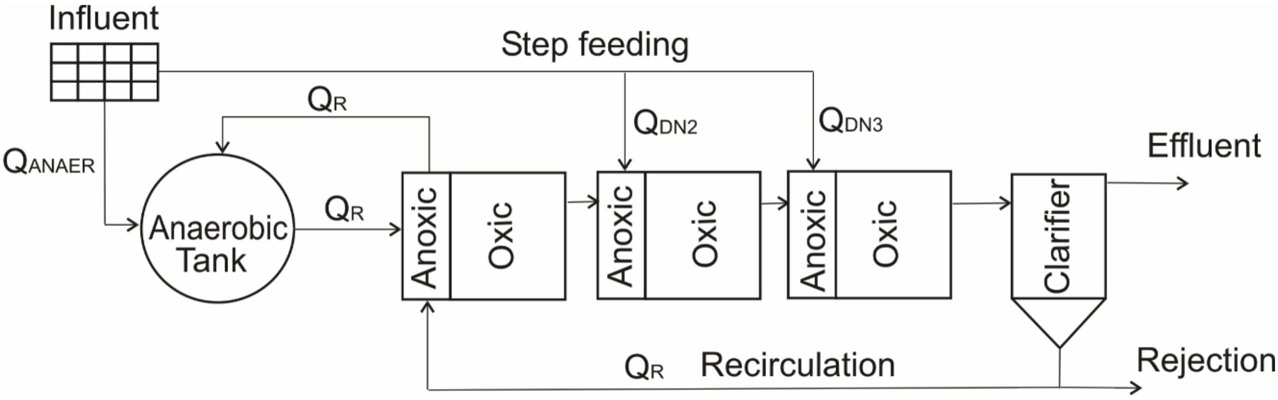
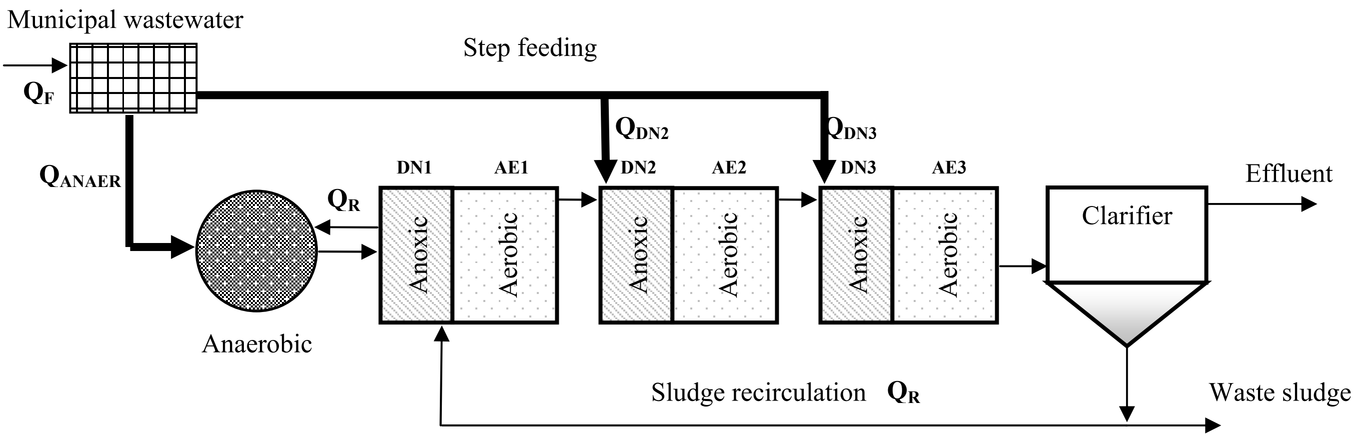


Figure 2. Experimental set-up of the BNRAS system.

2.2. Flow pattern alternatives

Two alternative feeding patterns of the described plant were investigated. The two influent flow pattern alternatives refer to the influent flow distribution before or after the anaerobic selector. This means that the influent wastewater is either distributed to the anaerobic tank, to the second and third anoxic tank (influent flow distribution before the anaerobic selector), or, in the alternative flow pattern design, the influent wastewater is first introduced to the anaerobic tank and afterwards distributed to the three anoxic tanks (influent flow distribution after the anaerobic selector) (Fig.3). Both flow schemes do not permit introduction of nitrate to the anaerobic selector, thus protecting the sensitive process of phosphorus release, whereas step feeding to anoxic tanks provides essential carbon compounds for the denitrification process.

(A)



(B)

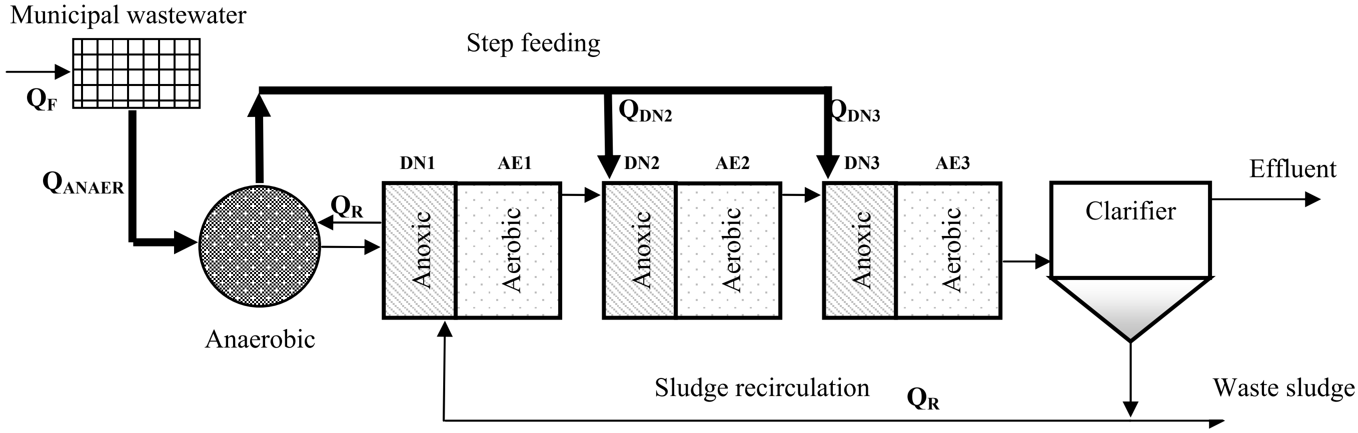


Figure 3. Schematic layout of the feed distribution pattern (A) before and (B) after the anaerobic selector in the pilot scale BNRAS plant.

The influent flow distribution pattern before the anaerobic selector was thoroughly investigated by introducing a wide range of influent flow rates (from 48 to 168 L/d) at different feed distribution percentages (60/25/15, 40/30/30 and 25/40/35 %), whereas the influent flow distribution pattern after the anaerobic selector was operated under optimum feed conditions with the aim to optimize the unit efficiency and performance. Therefore, HRTs in the influent flow distribution pattern before the anaerobic selector range between 5 and 18 hrs, whereas HRTs in the influent flow distribution pattern after the anaerobic selector range between 6 and 9 hrs. In Table 2 operational conditions, loadings and loading rates for organic, nitrogenous and phosphorus substrates are presented for each run. The main disadvantage of feeding with real wastewater is the fluctuations of substrate concentrations, which are evident in Table 2. However, loading rates of organics and nitrogen tend to increase when increasing the influent flow rate, as expected. On the other hand, intense fluctuations in influent orthophosphates concentration result to phosphorus loading rate fluctuations, which are hardly correlated to the influent flow rate.

Table 2. Operational parameters of the BNRAS plant.

St-st no.	Q_F [L/hr] (AN/DN2/DN3) [% Q_F]	Loading (g/d)				Sludge Loading Rate (L_{RX}) [kg_{BOD5}/($kg_{MLVSS}\cdot$d)]	HRT τ [d]
		COD	BOD_5	NH_4^+-N	PO_4^{3-}-P		
Influent flow distribution before the anaerobic selector							
1	2 (60/25/15)	22.8	9.6	1.5	0.42	0.09	17.80
2	2 (40/30/30)	11.2	9.6	1.9	0.44	0.12	17.80
3	2 (25/40/35)	32.6	11.5	1.4	0.19	0.15	17.80
4	3 (60/25/15)	37.4	21.6	3.4	0.54	0.16	11.87
5	3 (40/30/30)	34.2	27.4	3.0	0.50	0.33	11.87
6	3 (25/40/35)	29.3	17.3	2.5	0.32	0.12	11.87
7	4 (60/25/15)	65.7	36.5	4.6	0.35	0.33	8.90
8	4 (40/30/30)	44.6	17.3	4.1	0.22	0.15	8.90
9	4 (25/40/35)	39.3	37.4	5.2	0.26	0.39	8.90
10	5 (60/25/15)	79.8	62.4	4.5	0.72	0.52	7.12
11	5 (40/30/30)	85.5	60.0	4.5	0.68	0.42	7.12
12	5 (25/40/35)	91.0	31.2	4.2	0.50	0.27	7.12
13	6 (60/25/15)	69.5	77.8	5.3	0.58	0.82	5.93
14	7 (60/25/15)	91.9	53.8	5.8	0.74	0.56	5.09
St-st no.	**Q_F [L/hr] (DN1/DN2/DN3) [% Q_F]**	**Loading (g/d)**				**Sludge Loading Rate (L_{RX}) [kg_{BOD5}/($kg_{MLVSS}\cdot$d)]**	**HRT τ [d]**
Influent flow distribution after the anaerobic selector							
1	4 (60/25/15)	29.2	21.1	3.2	0.43	0.22	8.90
2	5 (60/25/15)	70.7	45.6	4.7	0.52	0.50	7.12
3	6 (60/25/15)	43.1	28.8	3.3	0.61	0.28	5.93

2.3. Water quality monitoring

Under steady-state conditions, samples were collected from the influent of the unit and the effluents of each tank, as well as from the separated sludge in the clarifier. The samples were analyzed following standard procedures for the determination of BOD_5, particulate and soluble COD, ammonia, particulate and soluble TKN (Total Kjedahl Nitrogen), nitrite, nitrate, total phosphorus, orthophosphate, VSS (Volatile

Suspended Solids) and SVI (Sludge Volume Index) (Standard Methods for the Examination of Water and Wastewater, 1998). A High Pressure Liquid Chromatography with a 550 conductivity detector and a 530 column heater of Alltech, USA was used for anion determination in isocratic mode after filtration of samples through 0.2 μm filters. A Marathon IV HPLC pump with flow rate of 2 ml/min was driving the filtered samples to pre-columns before entering the Hamilton PRP-X100 column. The system was running on EZ Chrom software. Wastewater temperature, pH and dissolved oxygen (DO) were monitored on a daily basis. Typical values for pH ranged between 7.4 and 7.6, whereas temperature was kept constant at 20 ºC. The DO in the aerated reactors was maintained above 2 mg/L during the experimental period. Sludge samples were also frequently taken and were observed within one hour under bright field and phase contrast microscopy.

A start-up period of about one month is required for biomass acclimation and stable unit performance. Once the operational conditions (i.e., influent flow rate and step feeding distribution) were set, the pilot plant was run until it reached steady state conditions. According to a rule of thumb, steady state conditions are reached after five times the HRT of the plant, since the unit consists of a Continuous Flow Stirred Tank Reactors (CFSTR) cascade. However, in order to assure steady state conditions in practice, samples were taken periodically to check the stability of the unit efficiency. When steady state conditions were confirmed, sampling, analyzing and finally set of operational conditions for the next run were taking place. Experiments were at least three times run to ensure results reproducibility.

3. Results

3.1. Unit performance

The results of two year operation running on influent flow distribution before the anaerobic selector show constantly high removal efficiencies of organic matter of 89% as total COD and 95% removal for BOD_5 averagely. Figure 4 depicts the influent and effluent concentrations of BOD_5 and COD for each steady state experiment including removal efficiency. Results for each pollution parameter, which are presented in charts, are divided by vertical lines in three sections corresponding to the three feeding ratios to the anaerobic selector, the second and third anoxic tank (60/25/15, 40/30/30 and 25/40/35). Total COD removal ranged between 80-98% and BOD_5 removal between 87-98%, receiving constantly the highest values at the feeding ratio of 60/25/15. A slight drop in BOD_5 and COD removal is observed at low HRTs (high influent flow rate), although the plant was fed with the ratio of 60/25/15, which is attributed both to the high hydraulic loading and loading rate of organic substrate. COD removal fluctuates around 88% in feeding schemes of 40/30/30 and 25/40/35, whereas it remains constantly above 90% during feeding at the ratio of 60/25/15. The influent total COD concentrations with a mean value of 540 mg/L spanned between 230-760 mg/L, whereas effluent concentrations with a mean value of 55 mg/L fluctuated between 15-95 mg/L. Accordingly, mean influent concentration of BOD5 was 330 mg/L with a width of 180-540 mg/L, whereas effluent concentrations ranged between 5-35 mg/L with an average value of 18 mg/L.

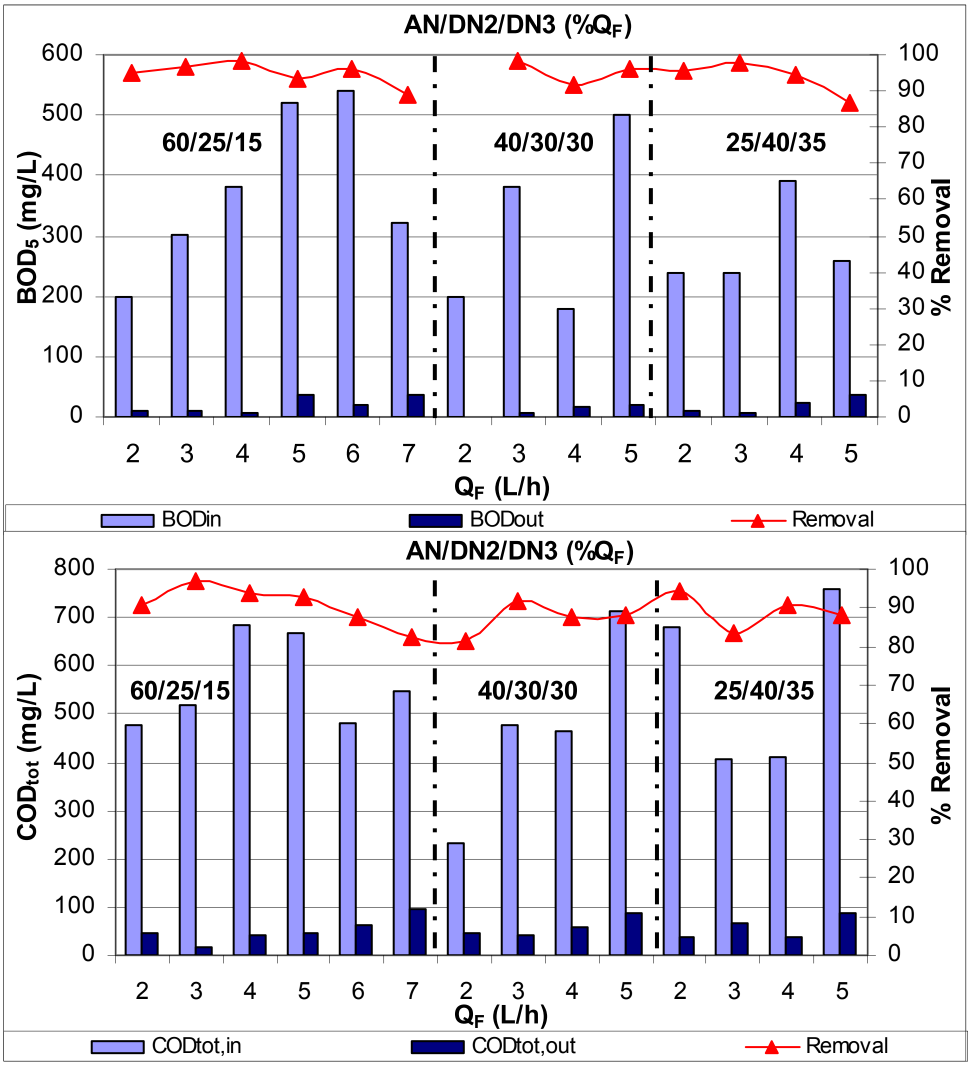


Figure 4. Removal efficiency of organic substrate under influent flow distribution before the anaerobic selector.

Complete nitrification is accomplished in all steady state experiments with the exception of low HRTs runs (Fig. 5). Mean removal of ammonium-nitrogen and TKN is 95% and 90% respectively. However, ammonium removal, and thus nitrification, is even higher since more ammonium nitrogen is produced from organic nitrogen in the plant after wastewater entrance. Removal efficiency is constantly higher at the feeding ratio of 60/25/15 with the exception of the steady state experiments at low HRTs (6 and 7 L/hr). The lowest TKN removal of 68% and the highest ammonium-nitrogen effluent concentration (7.8 mg/L) were recorded at HRT of about 5 hrs (QF = 7 L/hr) in the feed ratio of 60/25/15. Mean influent and effluent NH_4^+-N concentrations attain values of 40 and 2 mg/L respectively, whereas mean influent and effluent TKN concentrations receive values of 56 and 6 mg/L respectively.

On the other hand, denitrification efficiency fluctuated fairly at the feeding ratios of 40/30/30 and 25/40/35, whereas at the feeding ratio of 60/25/15 was constantly high attaining the highest extent at HRT of about 6 hrs (Q_F = 6 L/hr) (Fig. 5). Total nitrogen influent concentrations ranged between 40-60 mg/L with a mean value of 52 mg/L, whereas mean effluent concentration was 14 mg/L fluctuating between 8 and 19 mg/L. The low removal efficiency and the high effluent concentrations are attributed to the high nitrate effluent concentrations, which in some cases exceeded 10 mg/L in the feeding ratios of 40/30/30 and 25/40/35. This result is most probably attributed to the unit configuration, which ends to an aerobic tank, where nitrification is processed and nitrate is produced. However, aerobic conditions as the final step are essential for phosphate accumulation and maintenance of phosphorus effluent concentrations at low levels. Another explanation could be the accumulation of nitrate from previous aerobic stages on its way to the effluent.

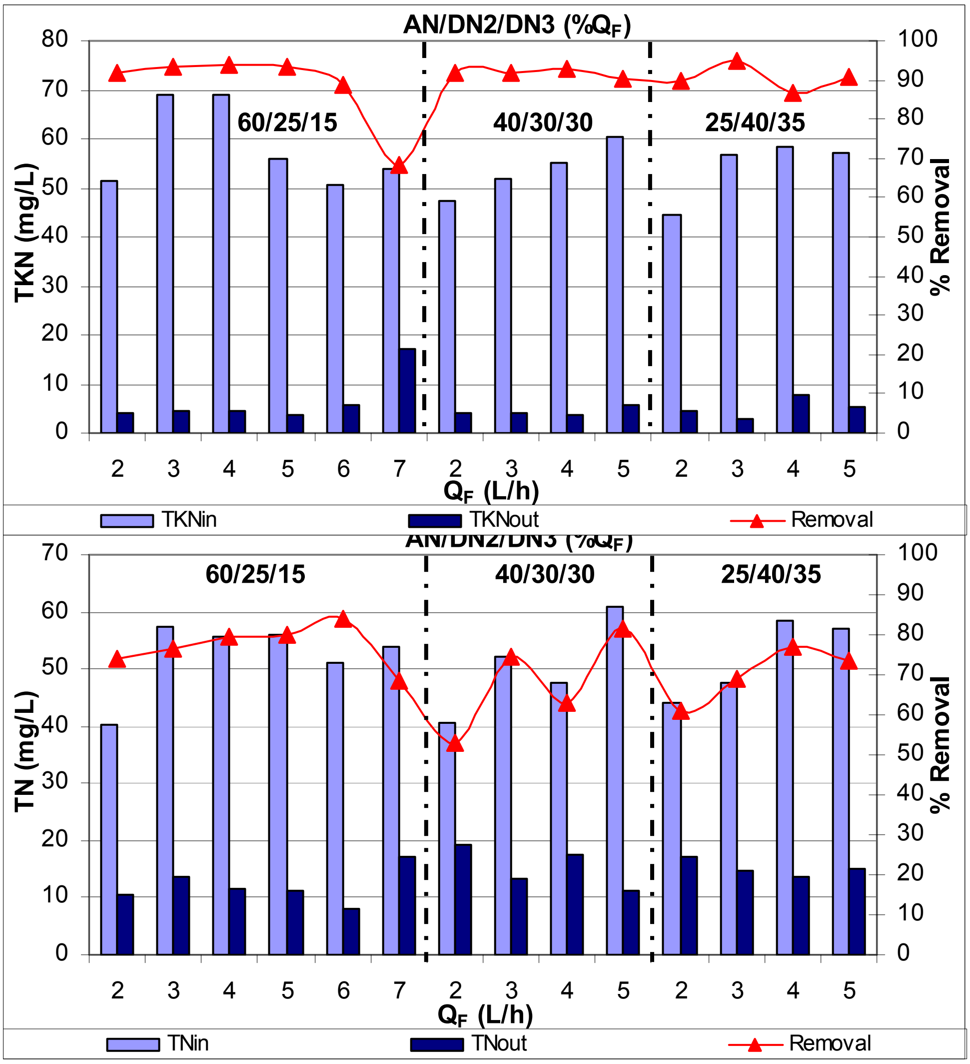


Figure 5. Removal efficiency of total Kjedahl nitrogen and total nitrogen under influent flow distribution before the anaerobic selector.

When compared to the results of a similar two-stage plant (Schlegel, 1992), the removal efficiencies of organic and nitrogenous pollutants are enhanced in the present BNR unit. This finding was expected since the cascade increase, besides being a safer mode of operation, may also result to higher plant removal efficiency. However, a further increase of the tank number could provide an even higher efficiency, but also a much higher construction and operation cost.

The phosphate removal efficiency ranges intensively between 27-96% with a mean value of 67% (Fig. 6), which is attributed to the influent concentration fluctuation. A revision paper underlines the significance of stable operating conditions and influent concentrations for a successful phosphorus removal (Mullkerins et al., 2004). Influent phosphate concentrations spanned between 2-9 mg/L with an average value of 5 mg/L, whereas mean effluent phosphate concentration was less than 2 mg/L with a width of 0.2-5.3 mg/L. The lowest removal efficiency was recorded at the highest HRT (Q_F = 2 L/hr). Constantly high removal efficiencies occurred at HRTs of 5 to 9 hrs at the feeding ratio of 60/25/15, during which the phosphate loading rate exceeded the 10 g/ (m^3 d). Total phosphorus removal efficiency followed the same profile with a mean value of 53% ranging between 30% and 79%. Mean influent total phosphorus concentration was about 8 with a width of 2-15 m/L, whereas mean effluent concentration was 3.4 mg/L fluctuating between 0.9-7.5 mg/L.

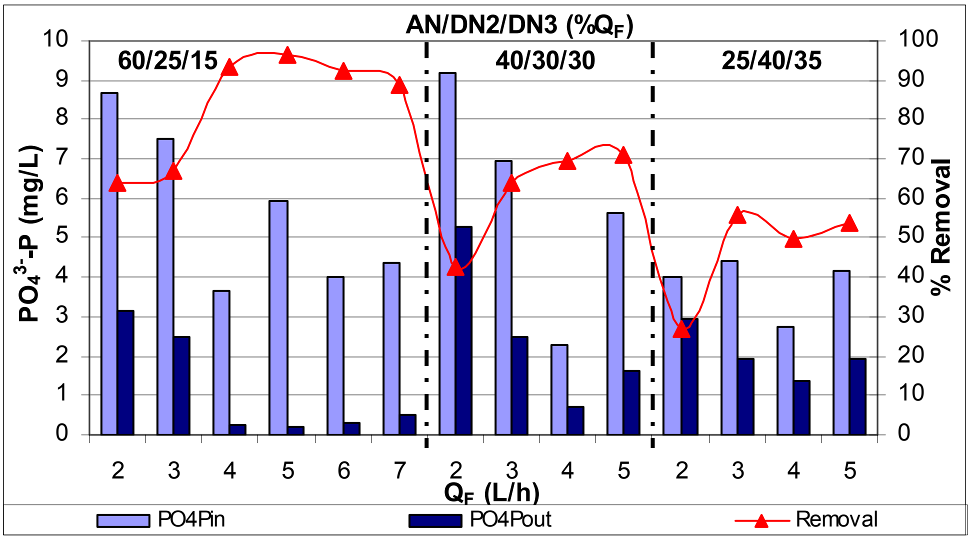


Figure 6. Removal efficiency of phosphate under influent flow distribution before the anaerobic selector.

Throughout the experimental period, long, robust and compact flocs comprised of a large species diversity could be observed under bright field and phase contrast microscopy. MLVSS concentration decreased averagely from 3.3 g/L in DN1 to 2.5 g/L in AE3 due to the sludge recycle entering DN1 tank, due to the dilution effect and to the higher HRTs in the initial tanks, which are attributed to the step feeding process. The same profile stands for Sludge Volume Index (SVI), which decreased averagely from 160 ml/g in AE1 and 130 ml/g in AE2 to 100 ml/g in AE3 and thus, in the clarifier. These SVI values are characterized as ideal (Jenkins et al., 1993) and show that the unit configuration actually results to enhanced sludge settleability. Accordingly, mean effluent MLVSS concentration was less than 25 mg/L which ensures a well-clarified effluent. However, SVI attains values higher than 150 ml/g at HRT of about 12 hrs at the feeding ratio of 25/40/35. Removal efficiency and sludge characteristics results are in agreement with previous experimental findings in similar units (Kayser et al., 1992; Pai et al., 2004; Schlegel, 1992).

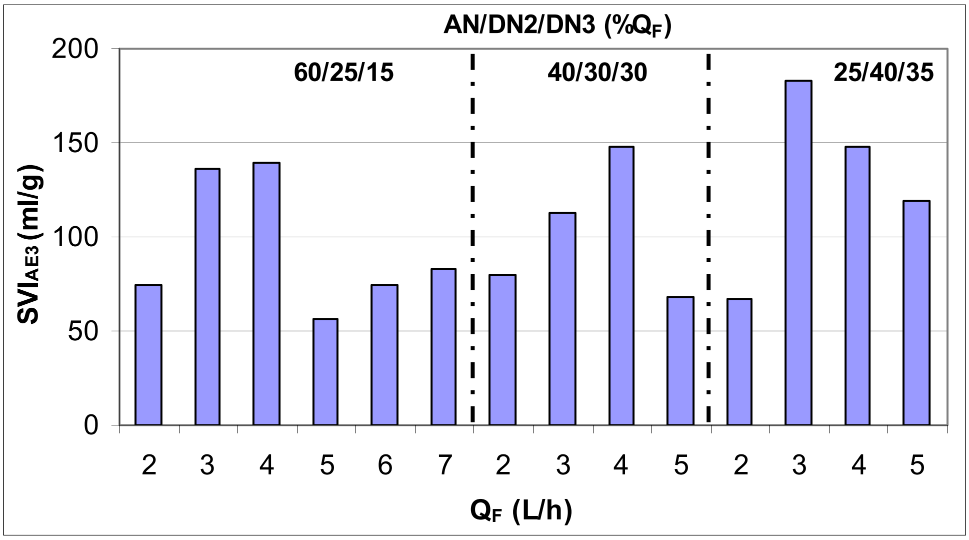


Figure 7. Sludge volume index (SVI) in the last aerated tank (AE3) under influent flow distribution before the anaerobic selector.

It is quite clear that the feeding ratio of 60/25/15 to the anaerobic selector, to the second and third anoxic tank results to the most efficient performance, since all removal efficiencies are constantly higher than in the other two feeding schemes. Additionally, the SVI remains lower than 150 ml/g, implying ideal sludge settleability. Figure 8 shows the removal efficiencies for the three feeding ratios under influent flow distribution before the anaerobic selector. In all cases, organic substrate removal and nitrification are almost complete. However, denitrification, expressed by total nitrogen removal, and phosphate removal fluctuated. Total nitrogen removal ranged between 60-75% in the feeding ratio of 25/40/35, between 60-80% in the feeding ratio of 40/30/30 and between 80-83% in the feeding ratio of 60/25/15. Accordingly, phosphate removal spanned 27-54% in the feeding ratio of 25/40/35, between 42-71% in the feeding ratio of 40/30/30 and between 64-93% in the feeding ratio of 60/25/15. That means that increasing flow rate of influent raw wastewater in the initial stages of the plant increases the removal efficiency.

Having detected the optimum feeding ratio (60/25/15), detection of the optimum hydraulic residence time (HRT) is the following step. Figure 8 shows that the highest removal efficiencies are recorded at HRTs of either 9 or 7 hrs, which correspond to an influent flow rate of 96 and 120 L/d. Since BOD removal represents the biodegradable part of organic pollution, NH_4^+-N removal stands for plant nitrification capability, PO_4^{3-}-P removal for desphosphatation capability and TN removal for plant denitrification capability, the mean removal efficiency calculated by using these data could be an indicator of the plant efficiency. SVI should also be taken into account as the settleability indicator. Comparing these data, the most efficient unit performance is recorded at HRT of 9 hrs and feeding ratio of 60/25/15 to the anaerobic selector, 2nd and 3rd anoxic tank respectively. This optimum steady state run will be stated as

4(60/25/15) for brevity reasons. This finding can be easily confirmed by data given in Table 3.

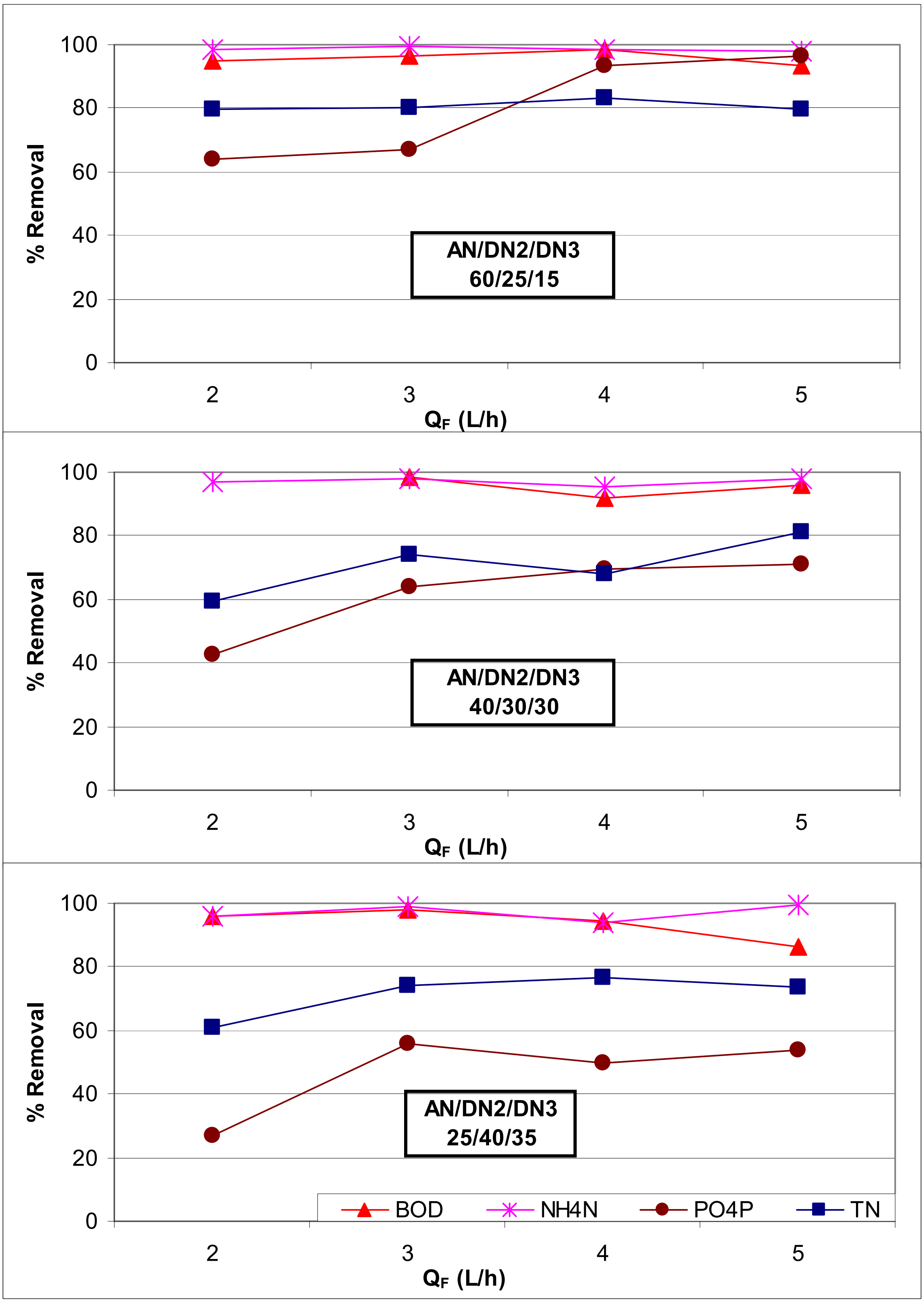


Figure 8. Removal efficiency for each feeding ratio under influent flow distribution before the anaerobic selector.

3.3. Flow pattern comparison

In both flow patterns, whose influent flow distribution was accomplished according to the quota of 60/25/15 either before or after the anaerobic selector, removal efficiency of organic, nitrogenous and phosphorous contaminants was satisfactory for influent flow rates of 96, 120 and 144 L/d. Figure 9 presents the mean removal efficiency for organic, nitrogenous and phosphorus contaminants for the two different feed patterns. It is obvious that the feed distribution before the anaerobic selector leads to higher removal efficiency in comparison to the scheme of feed distribution after the anaerobic selector. The one and only exception is the total phosphorus removal, which is enhanced from 71% to 78%, when the feed is distributed after the anaerobic selector. However, orthophosphates removal is slightly hindered, whereas nitrification remains on the same removal levels (95-96%) for both feed patterns.

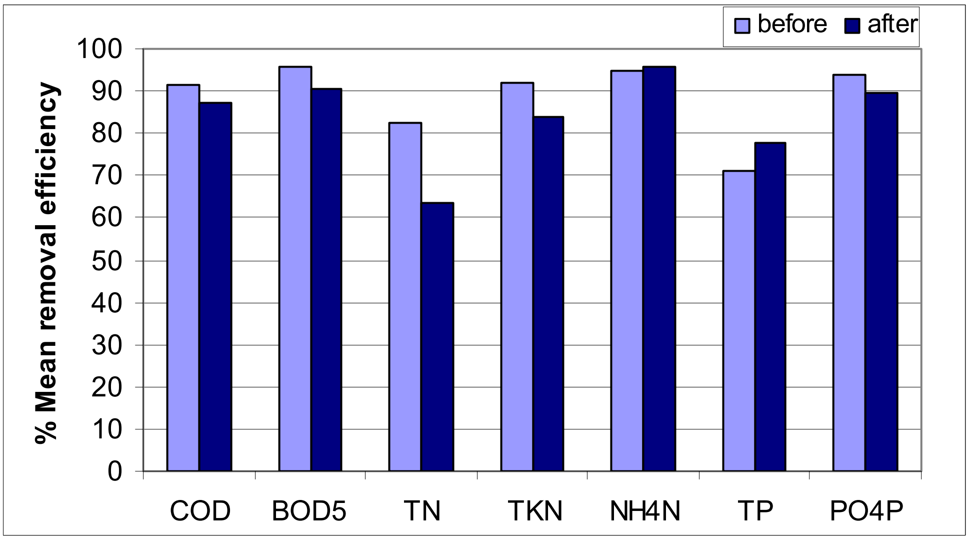


Figure 9. Comparison of removal efficiency for both feed patterns (influent flow distribution before and after the anaerobic selector).

The total phosphorous removal enhancement during the feed distribution pattern after the anaerobic selector is related to the phosphate release increase (Fig.10). Although the maximum phosphate release is performed in the first anoxic tank (DN1) in both feed patterns, total influent flow introduction to the anaerobic selector before step feeding results to a higher phosphate release, as shown in Figure 10. Higher phosphate release leads theoretically to greater phosphate uptake in the following aerobic conditions, and thus to higher removal efficiencies (Metcalf & Eddy, 2003). However, influent and effluent phosphate concentrations are equal in both feed patterns. Therefore, the total phosphorous removal enhancement in the feed distribution pattern after the anaerobic selector may be attributed to the higher phosphate release due to the higher readily biodegradable carbon substances concentration available under anaerobic conditions, when the total raw wastewater flow enters the anaerobic tank. Readily biodegradable carbon is stored by PAOs in form of PHA, which is then used aerobically for phosphate uptake. The limited organic components concentration in the distributed flows, that is in this case (influent flow distribution after anaerobic selector) available for denitrification in the anoxic zones, is actually insufficient and responsible for denitrification decrease (Fig.9).

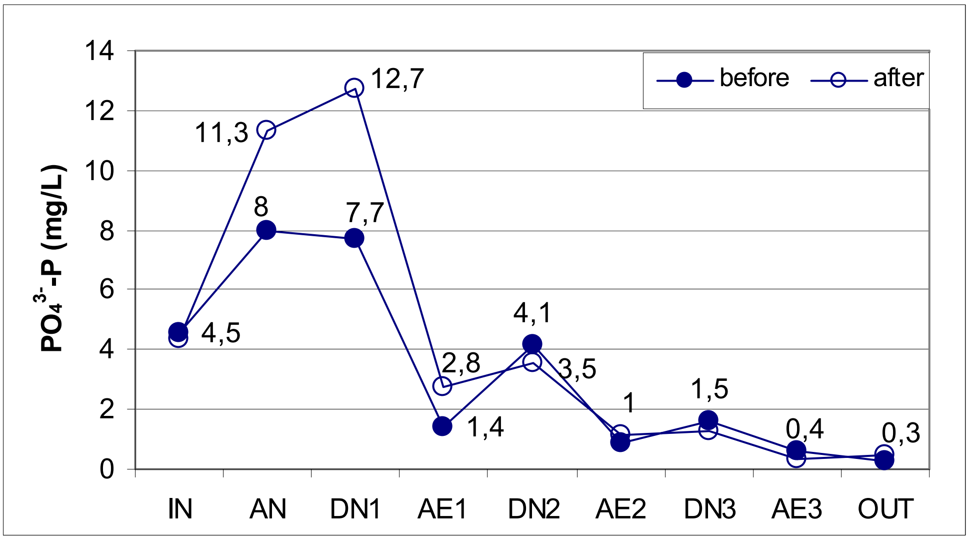


Figure 10. Orthophosphate concentration course along the unit for both flow patterns.

The highest efficiencies per parameter and the lowest nitrate nitrogen effluent concentration for both flow patterns are marked bold within Table 3 to facilitate tracking of the flow pattern with the most efficient performance. It is quite interesting to note that the influent flow rate of 4 L/h (HRT of 9 hrs) distributed by the ratio of 60/25/15 provides the highest removal efficiency in both feed distribution patterns. The effluent nitrate nitrogen concentration is 7 and 6 mg/L for the steady state run of 4(60/25/15) with influent flow distribution before and after the anaerobic selector respectively. Since the steady state run of 4(60/25/15) with influent flow distribution before the anaerobic selector gathers the highest removal efficiencies and lowest nitrate nitrogen effluent concentration (highest denitrification capacity), retains the operational conditions and feed distribution pattern according to which the herein BNRAS system can perform optimally.

Table 3. Removal efficiency and effluent NO_3^--N concentrations for both step feeding patterns.

	Influent flow distribution					
	BEFORE the anaerobic selector			**AFTER the anaerobic selector**		
Removal (%)	**Q_F (AN/DN2/DN3)= (60/25/15) [L/hr] (%Q_F)**			**Q_F (DN1/DN2/DN3)= (60/25/15) [L/hr] (%Q_F)**		
	4	**5**	**6**	**4**	**5**	**6**
COD	**94**	93	88	**92**	90	79
BOD_5	**98**	93	96	89	**92**	90
TN	83	80	**84**	**75**	73	43
TKN	**94**	93	89	**86**	**86**	79
NH_4^+-N	**99**	98	87	**96**	**96**	95
TP	-	63	**79**	80	70	**83**
PO_4^{3-}-P	93	**96**	92	88	84	**97**
Average	**94**	88	88	**87**	84	81

An unexpected effect noticed in the feed distribution pattern after the anaerobic selector was the extreme deterioration of the sludge settleability as indicated by the SVI measurements, which, however and most surprisingly, did not cause insufficient clarification. The mean SVI value in the last aerated tank under steady state condition was measured at 90 ml/g and 230 ml/g for feed distribution pattern before and after the anaerobic selector respectively (Fig.11). In the first case (SVI=90 ml/g), results indicate ideal sludge settleability characteristics, whereas in the latter case totally worrying (Jenkins et al., 1993).

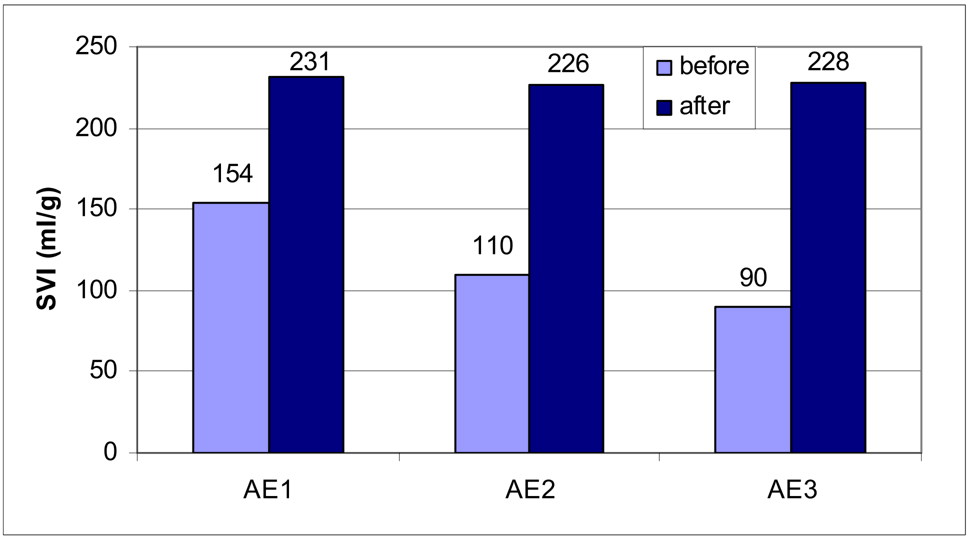


Figure 11. SVI values in aerobic tanks for feed distribution pattern before and after the anaerobic selector.

Microscopic observations showed a wide variety and high numbers of protozoa and metazoa, such as Aspidisca costata, rotifers, Vorticela, Epistylis, Opercularia etc., as well as medium compact flocs with irregular shape, revealing a healthy sludge. Actually, the microscopic image of the unit sludge characteristics was similar in both flow schemes with the exception of the floc size, which was larger in the influent flow distribution after the anaerobic selector. Larger flocs are expected to increase sludge settleability because of their weight. The increased SVI finding might be attributed to the reduction of the influent sulfate to sulfide by sulfate reducing bacteria (SRB) under anaerobic conditions as observed previously in the same BNRAS plant when fed with a synthetic medium (Vaiopoulou et al., 2007). It is suggested though, that the filamentous bacteria require a-two-month time to reach their cumulative length and thus, to cause bulking sludge (Jenkins et al., 1993; Vaiopoulou et al., 2007). Other researchers showed that when sulfate reduction rates are high, maximum phosphate release is most of the times also high, because PAOs may utilize the acetate produced by SRB (Yamamoto-Ikemoto et al., 1998). Although their observations seem to explain our findings, further investigation is required to reach a confident explanation. It is also noticeable that SVI in the influent flow distribution pattern after the anaerobic selector attains the same average values in all aerated zones (AE1, AE2, AE3), which is probably related to the fact that all of them are fed with the effluent of the anaerobic selector.

4. Discussion

Experiments on nutrients removal in a two denitrification stage UCT system with step feeding and influent flow distribution by 50% to the anaerobic tank and the rest to the second anoxic tank showed high removal efficiencies (Schlegel, 1992). According to these reported results, extensive reduction of carbon compounds was achieved, nitrogen removal attained values of 87%, phosphorus removal ranged between 70-80% and effluent nitrate nitrogen was low at 4.6 mg/L. A sludge thickener and a digester were used to provide a high sludge concentration in the recycle as well as VFAs to the anaerobic zone. As an initial treatment step, a primary sedimentation tank was included in the flow chart. A similar attempt was undertaken by Pai et al. resulting to an economically efficient removal of organic substrate and nutrients with highly clarified effluent (2004). In this research work, 70-100% of the influent flow rate was fed to the initial anaerobic stage and the rest to the 1st and 2nd anoxic stage of the cascade. However, high influent flow rate introduction to the initial stages of a unit minimizes the effect of the step feeding process. In addition, the researchers fed their plant with a synthetic wastewater, although it can not lead to a realistic view of the unit performance, since some real wastewater composition parameters, such as COD and VFAs content, phosphorus load, pH etc, are of crucial importance for the effective biological removal of phosphorus (Mullkerins et al., 2004) and most of the times can not be precisely simulated. Indeed, the authors have previously fed the herein presented experimental plant with synthetic wastewater, which led to filamentous bulking sludge and total system failure (Vaiopoulou et al., 2007).

The large number of BNRAS treatment methods, aiming at enhancing effluent characteristics and at optimizing the unit's efficiency, include external supply of organic substrate (usually acetic acid) (Satoh et al., 1992; Thomas et al., 2003), reduction of sludge age (Rodrigo et al., 1996), chemicals addition for precipitation, metals addition (Schoenborn et al., 2001), as well as minimization of nitrate and oxygen concentration entering the anaerobic tank (e.g. the UCT method) (de-Bashan and Bashan, 2004; Metcalf & Eddy, 2003; Mullkerins et al., 2004). However, these methods are not cost effective and require expertise when applied, e.g. waste sludge digestion for VFAs production in order to be added to the anaerobic tank for phosphate release enhancement. On the other hand, innovation in the feeding scheme may result to low cost and high efficiency removal. Having in mind that wastewater composition and feeding scheme determine the microbial synthesis, characteristics and thus, the unit performance and removal efficiency (Pai et al., 2004; Wagner et al., 2002; Zeng et al., 2004), detection of the optimum feeding pattern could enhance effluent quality without extra costs.

On this basis, the UCT method in combination with a three-stage denitrification cascade and the step-feeding strategy leads to no need for nitrified liquor recycle, reduces sludge recycle and volume requirements, eliminates nitrate/ oxygen intrusion to anaerobic conditions, optimizes organic carbon usage for denitrification and enhances effluent water characteristics. In particular, step-feeding configuration removes nitrogen by minimizing internal recycle and optimizing the carbon usage for denitrification (Görgün et al., 1996; Metcalf & Eddy, 2003; Kayser et al., 1992; Schlegel, 1992). The cascade enhances effluent water quality with the minimum reactor volume, whereas provides operational safety, since whatever is left untreated, may be treated in the next stage (Kayser et al., 1992; Schlegel, 1992). Furthermore, a three-stage cascade has been proved to lead to low effluent concentrations with low volume needs, whereas a two-stage system is insufficient (Görgün et al., 1996; Lesouef et al., 1992). The removal efficiency of such a full-scale system, operating at a temperature of 12˚C and without an anaerobic selector, depends on the sludge recycle rate and the flow-split percentages to the three anoxic tanks (Kayser et al., 1992). It has been proved that influent flow rate reduction to the last stages of the cascade is more efficient for nitrogen removal than recycle flow rate increase. Even though this system did not include an anaerobic selector, maximum phosphorus release was recorded in the first anoxic tank, which was afterwards taken up in the following aerobic tank. According to the same research work, the first anoxic tank of the cascade plays a crucial role in phosphorus removal. These conclusions are also confirmed by our results, since removal efficiency is maximized when the influent flow rate is distributed by 60% to the anaerobic selector, by 25% to the second and by 15% to the third anoxic tank, whereas under these operational conditions maximum phosphate release took place to the first anoxic tank.

Schlegel recommends multiple stage units for influent ratio of organics to nitrogen (BOD_5/TKN) above 5 (Schlegel, 1992), a value which attains the influent raw wastewater to our plant. In the same paper, a full-scale two-stage unit is presented, which includes primary sedimentation, an anaerobic selector and sludge digestion, for carbon, nitrogen and phosphorus removal. Phosphorus removal of 70-80% and effluent phosphorus concentration of 1.8 mg P/L is achieved at hydraulic residence time 2 hours in the anaerobic selector, when organic acids, produced by sludge digestion, into the influent. Influent flow rate was distributed by 50% to the anaerobic selector and 50% to the second anoxic tank. The herein presented plant consisting of three stages, operating at HRT of 33 min in the anaerobic selector, without the addition of any extra organic substrate and influent flow distribution of 60/25/15 succeeds 93% phosphorus removal and effluent concentration of 0.2 mg P/L. Operational results of a pilot-plant system with influent flow rate distribution by 70% to the anaerobic selector, by 20% to the first and by 10% to the second anoxic tank show 94% phosphorus removal, 75% total nitrogen removal και 87% organics removal (Pai et al., 2004), whereas our experimental results for influent flow distribution rates of 60%, 25% and 15% to the anaerobic selector, the second and third anoxic tank respectively show 93% phosphate removal, 83% total nitrogen removal και 94% COD removal.

Inclusion of an anaerobic selector to a plant configuration not only contributes to biological phosphorus removal, but also forms big and heavy flocs with excellent settleability (Metcalf & Eddy, 2003). A review paper referring to UCT and A_2N method comparison states that UCT systems reduce excess sludge production, whereas in warm climates, such as the Greek one, they are more efficient than the A_2N systems (Hao et al., 2001). Furthermore, recent experimental results confirmed the suggestion that nitrous oxide is produced by PHA-driven denitrification in activated sludge processes (e.g. A_2N) (Meyer et al., 2005). However, a comparative study of the herein presented system and a two-sludge system could actually give more efficient information. Therefore, such experiments are conducted currently in our laboratory.

Comparing the design parameters of the presented unit with other wide-spread methods, the sludge retention time (SRT=10d) and the hydraulic residence times in the anaerobic selector (0.55 h), in the anoxic (1.46 h) and aerobic zone (3.13 h) are quite short. No internal recycle is necessary, whereas recycle flow rate is relatively low at 100% of the influent wastewater flow rate. The required volume per population equivalent (PE) for an influent flow rate of 96 L/d is calculated at 68 L/PE, when assuming the daily wastewater production per population at about 185 L. This volumetric requirement per population equivalent is lower in comparison with other wastewater treatment systems and may be further reduced by increasing the treatment stages. Construction costs are limited respectively to volumetric needs reduction per population equivalent.

It must be clearly stated that the residence time distribution (RTD) of the presented pilot plant (data not shown here) plays a beneficial role regarding its enhanced response to the existing first order kinetics, due to the limited axial dispersion, and to the filaments suppression. RTD experiments revealed that the Bodenstein number (Bo) ranged from 5 to 6 (dispersion model), whereas the cascade number (N) has been determined between 2.5 and 3 (tanks in series model) in the pilot-scale plant. From a kinetic point of view, the plant behaves operationally as if a cascade of three continuous flow stirred tank reactors (CFSTR) and consequently, as if a plug flow reactor (PFR). The favorable plant characteristics, which are responsible for high removal efficiencies and good sludge settleability, are attributed to this PFR behavior.

In particular, the sludge volume index was less than 150 ml/g for influent wastewater distribution before the anaerobic selector, which means that the sludge settleability is excellent and may be added to the unit's advantages. Sludge formation with good settleability should not be only attributed to the anaerobic selector, but also to the previously mentioned operational behavior of the plant. Thus, the proposed configuration strongly supports the metabolic selection of floc-forming microorganisms resulting in excellent activated sludge settling properties.

The fact that the denitrification activity, and thus efficiency, was several times recorded below 83% is grounded on the experimental practice and mostly on the inconsistency of the influent carbon to nitrogen (C/N) ratio. Specifically, the piping system was so designed that unintentional oxygenation and anoxic conditions disruption is kept at a minimum. However, maintenance of a large quantity of wastewater, even under low temperature, inevitably leads to organics biodegradation, which may reach and exceed 30% under storage (4-5 days). Therefore, influent C/N ratio is sometimes so low that it is inefficient for denitrification. In case of full-scale application of the presented plant, where flow of influent wastewater will be constant and the C/N ratio constantly high, denitrification efficiency could reach higher values in the second and third anoxic tank.

5. Conclusions

The main characteristics of the proposed optimum plant configuration are:

1. The initial anaerobic tank helps select phosphate accumulating organisms, according to the EBPR technique, as well as floc-formers over filamentous bacteria.
2. A cascade of three identical bioreactor pairs, where each of them consists of two separate tanks with anoxic and oxic (aerobic) conditions. This cascade of three stages is expected to yield to a potential (theoretical) removal efficiency of total nitrogen around 83%, when sludge recycle ratio is equal to the influent flow rate. Multiple stage cascades also offer the operational safety that what leaves a stage untreated can be removed in the next stage.
3. The step-feeding process. Influent flow rate is distributed to anaerobic or anoxic tanks in order to provide cheap organic substrate for phosphate release or denitrification, as well as to transform these tanks into selectors of floc-formers over filaments.
4. The primary sedimentation tank is absent, which means that organic substrate is maintained for biological phosphate removal and denitrification.
5. The sludge recycle from the clarifier is introduced to the anaerobic selector via the first anoxic tank, resulting in nitrate elimination before entering the anaerobic selector, and thus, in real anaerobic conditions in the anaerobic selector, to sludge recycle flow minimization and to supply of the anaerobic selector with biomass.
6. The limited axial dispersion in the cascade (plug flow) due to the narrow spectrum of the Residence Time Distribution is ideal for first order kinetics, which in combination to the anaerobic selector may also suppress filamentous bacteria proliferation.

The highest removal efficiencies, the most favorable sludge characteristics and the lowest effluent concentrations were observed for influent flow distribution before the anaerobic selector at HRT of 8.9 hrs and ratios of 60% to the anaerobic selector, 25% to the second and 15% to the third anoxic tank. In particular, organics removal received values of 94% and 98% for COD and BOD5 respectively, whereas phosphate removal of 93%. Nitrogen removal was recorded at 83% as total nitrogen, 94% as total Kjedahl nitrogen and 99% as ammonium nitrogen. Effluent concentrations were measured at 43 mg COD/L, 6 mg BOD_5/L, 0.7 mg NH_4^+-N/L, 7 mg NO_3^--N/L, 4.4 mg TKN/L, 0.2 mg NO_2^--N/L and 0.2 mg PO_4^{3-}-P/L. SVI was satisfactory at 140 ml/g.

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