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Evaluation of Organic Carbon from Anaerobic Sequencing Batch Reactor Effluent as a Carbon Source for Denitrification

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Abstract

The discharge of nitrate-rich effluent has adverse effect on the receiving environment and the public health of the polluted water users. The nitrates are eliminated in a denitrification step that requires reducing power in form of organic carbon. The objective of this study was to evaluate the potential of utilizing organic carbon in effluent from the anaerobic SBR as a carbon source for denitrification. Reactors were operated for one year using meat processing wastewater. Anaerobically treated abattoir wastewater equivalent to 5, 10 and 15% of aerobic SBR hydraulic volume were added to three separate reactors. A 12 h operating cycle consisted of the following periods: (a) filling, 0.30 h; (b) settling, 11 h and (d) decanting, 0.30 h for the anoxic reactor. A comparison between different carbon loads was performed based on biological carbon, nitrogen and phosphorus removal. Sufficient denitrification was achieved with 10% (aerobic SBR hydraulic volume) of anaerobically-treated abattoir wastewater. TCOD, BOD_5, TKN, NO_2^--N, NO_3^--N, PO_4^{3-}, TS, EC and temperature and turbidity were reduced by 78, 70, 91, 100, 98, 62, 39, 65, 71, 5 and 39% respectively, with effluent mean concentrations of 80 ± 5 mg/L, 54 ± 12 mg/L, 35 ± 4, 00 ± 0, 2 ± 1, 18 ± 1, 254 ± 12, 1.64 ± 0.01, 22.04 ± 0.02 and 738 ± 9 FAU. Organic carbon in effluent from the anaerobic SBR can be used as a carbon source for anoxic denitrification. However, the denitrification rate is affected by the organic carbon load used. Except TKN and $o-PO4^{3-}$ mg/L, all other parameters in the denitrified effluent met discharge standards.

Keywords: Biological treatment; Denitrification; Nitrate; Organic carbon; Sequencing batch reactors (SBR); Wastewater

Introduction

The discharge of nitrate-rich effluent has adverse effect on the receiving environment and the public health of the polluted water users [1,2]. Such effects manifest as toxicity to aquatic biota, eutrophication [3,4] and public health complications such as thyroid hypertrophy, methemoglobinemia, hypertension and cancer [5]. The World Health Organization has set a limit of 10 and 100 mg/L NO_3^- for human and animal consumption, respectively. Wastewater above these limits requires treatment [6-8].

Nitrates are biologically removed via denitrification, anoxic reduction of $NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$ by heterotrophic bacteria such as *Pseudonomas stutzeri, Alcaligenes faecalis* and *Ochrobactrum anthropi* [9,10]. Activated sludge based sequencing batch reactor (SBR) allows the removal of carbon, nitrogen and phosphorus using a single reactor [11-13]. The key to efficient biological pollutant removal is transitioning between anaerobic, aerobic, and anoxic phases. Doing so promotes transformation of the carbon, nitrogen and phosphorus by triggering utilization of different electron acceptors and donors [14].

However, simultaneous phosphorus and nitrogen removal is not always successful [13,15]. Polyphosphate-accumulating organisms (PAO) and denitrifying bacteria are both heterotrophic [9,10], and thus able to take up organic carbon under anaerobic conditions and store it for growth once a suitable electron acceptor is available [14,16-18]. In combined denitrification and EBPR systems, organic carbon availability is usually the limiting parameter [19,20], denitrifers and PAOs directly compete for the available organic carbon [11,21]. Both processes are inhibited by this competition [13,22].

Insufficient denitrification is improved by addition of an external organic carbon such as such as glucose, ethanol, acetate, methanol, aspartate, formic acid [18,23], molasses, sulfite waste liquor, whey and distillery stillage [24]. However, these solutions are costly, require an adaptation period, lead to excessive sludge production and are not always efficient [13]. Hence efficient and cost-effective solutions are needed.

The use of wastewater as an internal carbon source is a promising alternative [10]. However, denitrification is affected by the type and dose of the organic carbon source used, duration of each phase and time cycle. The choice of hydraulic retention times and sludge retention time is dependent on this optimization [25,26]. The objective of this study was to evaluate the potential of utilizing anaerobically treated meat processing wastewater as a carbon source for denitrification. A comparison between different carbon loads was performed based on biological carbon, nitrogen and phosphorus removal.

Materials and Methods

Model reactors

Three glass reactors (Figure 1), each with a total volume of 25 L and a working liquid volume of 20 L were set up at Makerere University Biochemistry Research Lab. These reactors were fed with nitrified effluent. To prepare the nitrified liquor, meat processing wastewater from City Abattoir, Kampala-Uganda was treated sequentially in anaerobic and aerobic SBR using a standard procedure as described by Mutua et al. [19].
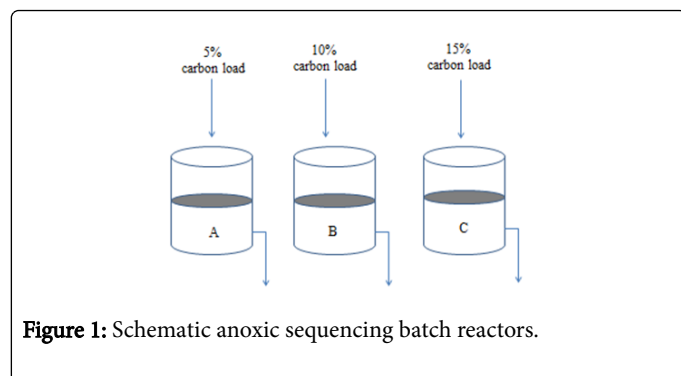

Figure 1: Schematic anoxic sequencing batch reactors.

Then, anaerobically treated abattoir wastewater equivalent to 5, 10 and 15% of aerobic SBR hydraulic volume were added to three separate reactors (the properties of the anaerobically treated wastewater are shown in Table 1.

Parameter	Outflow conc.
TCOD	3554 ± 58
SCOD	762 ± 3
BOD_5	1869 ± 27
TKN	400 ± 30
NH_4–N	288 ± 7
NO_2–N	NIL
NO_3–N	NIL
TP	129 ± 1
$\circ$-PO_4^3—P	82 ± 1
Turbidity	2800 ± 9
TS	2307 ± 21
pH	6.56 ± 0.03
EC	3.02 ± 0.01
Temperature	25.7 ± 0.2

Table 1: Mean ± standard error values of the physiochemical parameters determined for the anaerobic SBR effluent, (n=6).

Concentrations of TCOD, SCOD, BOD_5, TKN, NO_2^-N NO_3^-N, TP, o-PO_4^{3-}, Turbidity and TS are expressed in mg/L; Turbidity, EC, and temperature are expressed in (FAU), (ms/cm) and (∘C), respectively.

An 12 hour operating cycle consisted of the following periods: (a) filling, 0.30 h; (b) settling, 11 h and (d) decanting, 0.30 h for the anoxic reactors. At the end of each cycle, 10 litres of the supernatant was decanted, followed by feeding of an equal amount of wastewater. The system operated at a nominal Sludge Retention Time (SRT) of 5 days. The organic loading was 12.8 kg COD/m_3/day, during the study period. Steady-state conditions were obtained after 3 months.

Analytical procedure

Physical water quality variables (temperature, pH and electrical conductivity) were measured in situ per hour using portable WTW (Wissenchaftlich Technishe Werkstatten) microprocessor probes and meters. Chemical parameters such as total chemical oxygen demand (TCOD), biochemical oxygen demand (BOD_5), soluble chemical oxygen demand (SCOD), ammonia (NH_3), total kjeldahl nitrogen (TKN), nitrite (NO_2^-), nitrate-nitrogen (NO_3^-), total phosphorus (TP), ortho-phosphate (o-PO_4^{3-}), solids content (TSS) and turbidity were analyzed according to standard method.

Statistical analysis

One-way analysis of variance (ANOVA) was used to compare treatment means. The results were expressed as mean ± SEM. The differences were considered significant, when P<0.05.

Results

Figures 2 to 7 and Table 2 show the denitrification efficiencies obtained when 5, 10 and 15% organic carbon load were used.
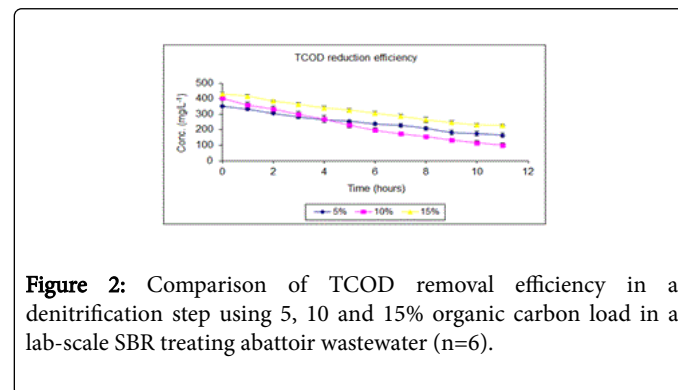

Figure 2: Comparison of TCOD removal efficiency in a denitrification step using 5, 10 and 15% organic carbon load in a lab-scale SBR treating abattoir wastewater (n=6).

Figure 3: Comparison of BOD_5 removal efficiency in a denitrification step using 5, 10 and 15% organic carbon load in a lab-scale SBR treating abattoir wastewater (n=6).
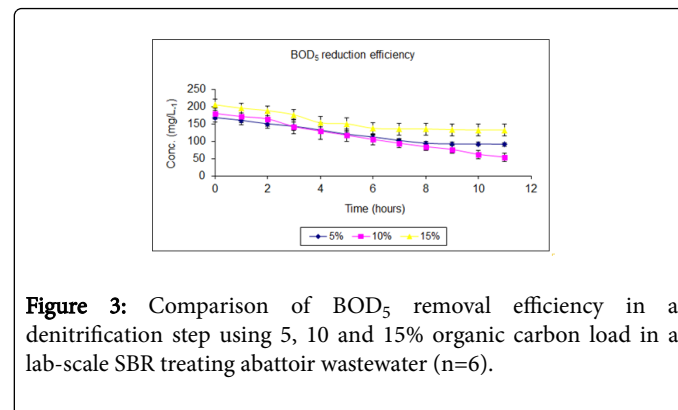
The TCOD removal efficiency significantly differed between 5% and each of 10% (p=0.000) and 15% (p=0.000) carbon load and the TCOD

removal efficiency significantly differed between 10% and 15% carbon load (p=0.000) (Figure 2, Table 2).

The BOD removal efficiency did not significantly differ between 5% and each of 10% and 15% carbon load. However, there was significant difference in BOD removal efficiency between 10 and 15% carbon load (p=0.007) (Figure 3, Table 2).

The TKN removal efficiency significantly differed between 5% and each of 10% (p=0.000) and 15% (p=0.000) organic carbon load and the TKN removal efficiency significantly differed between 10% and 15% organic carbon load (p=0.000) (Table 2).

Parameter	Aerobic SBR effluent	Carbon load (%)	Inflow conc.	Outflow Conc.	% Change
TCOD	332 ± 16	5	351 ± 8	165 ± 12[a]	-53
		10	359 ± 9	80 ± 5	-78
		15	430 ± 12	229 ± 7[bc]	-47
BOD5	164 ± 20	5	168 ± 13	91 ± 5	-46
		10	180 ± 16	54 ± 12	-70
		15	205 ± 16	132 ± 17[a]	-36
TKN	186 ± 12	5	209 ± 14	165 ± 18[a]	-21
		10	225 ± 2	35 ± 4	-91
		15	269 ± 11	229 ± 10b[c]	-15
NO_2N	115 ± 9	5	105 ± 4	43 ± 6[a]	-59
		10	93 ± 17	.00 ± 0	-100
		15	82 ± 9	29 ± 7[a]	-65
NO_3N	184 ± 15	5	172 ± 13	124 ± 15[a]	-28
		10	122 ± 1	2 ± 1	-98
		15	146 ± 9	115 ± 7[a]	-7
TP	22 ± 1	5	26 ± 4	27 ± 3	+4
		10	34 ± 1	18 ± 1	-47
		15	48 ± 9	32 ± 9	-33
PO_4^{3-}	18 ± 1	5	16 ± 1	14 ± 1	-1
		10	21 ± 1	8 ± 1	-62
		15	33 ± 6	21 ± 7	-36
Turbidity	1015 ± 6	5	1156 ± 28	683 ± 34	-41
		10	1210 ± 16	738 ± 9	-39
		15	1325 ± 54	912 ± 29[a]	-31
TS	655 ± 12	5	678 ± 13	217 ± 10	-68
		10	729 ± 7	254 ± 12	-65
		15	798 ± 15	365 ± 19[a]	-54
pH	7.05 ± 0.05	5	6.93 ± 0.03	6.96 ± 0.13[a]	0
		10	6.81 ± 0.04	7.00 ± 0.0	-27
		15	6.45 ± 0.13	6.75 ± 0.07[a]	-4
EC	4.18 ± 0.02	5	5.27 ± 0.02	3.34 ± 0.1[a]	-37

		10	5.69 ± 0.1	1.64 ± 0.01	-71
		15	5.86 ± 0.14	3.64 ± 0.09[a]	-38
Temperature	22.06 ± 0.1	5	23.02 ± 0.1	22.72 ± 0.02	-1
		10	23.23 ± 0.8	22.04 ± 0.02[a]	-5
		15	23.49 ± 0.11	22.09 ± 0.02[a]	-6

Table 2: Mean ± standard error values of physiochemical parameter determined in anoxic denitrification using 5, 10 and 15% (of the aerobic SBR hydraulic volume) carbon load from anaerobic SBR (n=6).

Concentrations of TCOD, BOD_5, TKN, NO_2^-N, NO_3^-N, TP, PO_4^{3-}, and TS are expressed in mg/L; Turbidity, EC, and temperature are expressed in (FAU), (ms/cm^3) and ($^\circ$C), respectively. - signifies reduction, + signifies increment.

The $NH4^-H$ removal efficiency did not significantly differ between 5% and each of 10%. However, there was significant difference in $NH4^-H$ removal efficiency between 15% each of 5 and 10% carbon load (p=0.007) (Table 2).

The NO_2^-N removal efficiency significantly differed between 10% and each of 5% (p=0.004) and 15% (p=0.004) carbon load. However, there was no significant difference in NO_2^-N removal efficiency between 5% and 15% carbon load (p>0.05) (Figure 4, Table 2).
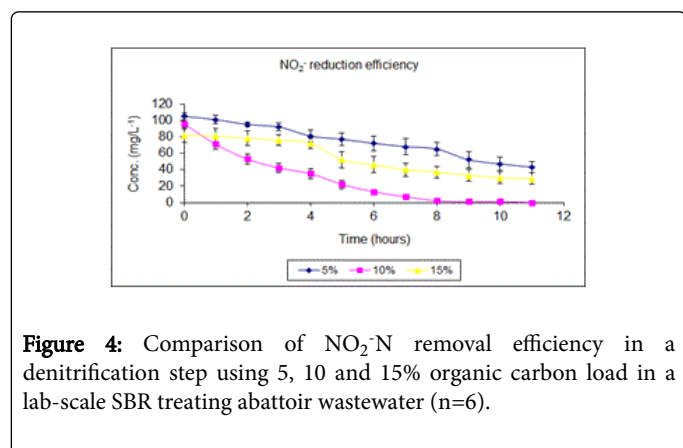

Figure 4: Comparison of NO_2^-N removal efficiency in a denitrification step using 5, 10 and 15% organic carbon load in a lab-scale SBR treating abattoir wastewater (n=6).
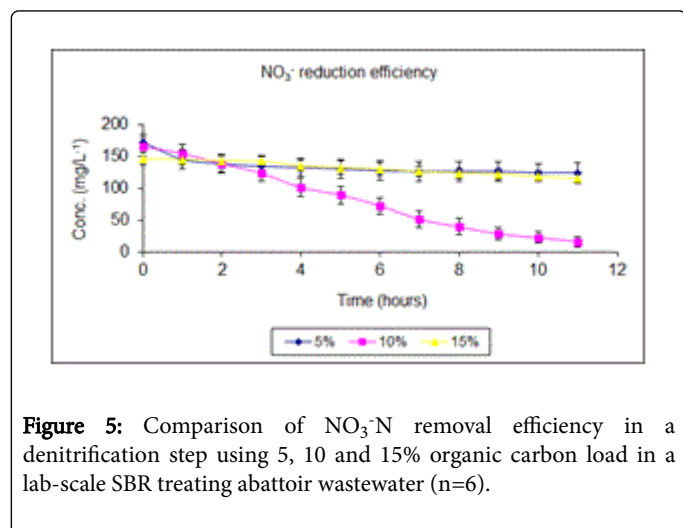

Figure 5: Comparison of NO_3^-N removal efficiency in a denitrification step using 5, 10 and 15% organic carbon load in a lab-scale SBR treating abattoir wastewater (n=6).

The NO_3^-N removal efficiency significantly differed between 10% and each of 5% (p=0.000) and 15% (p=0.000) carbon load. However, there was no significant difference in NO_3^-N removal efficiency between 5% and 15% carbon load (p>0.05) (Figure 5, Table 2). The TP and PO_4^{3-} removal efficiency did not significantly differ between and among 5%, 10% and 15% organic carbon load used (Figures 6 and 7, Table 2).
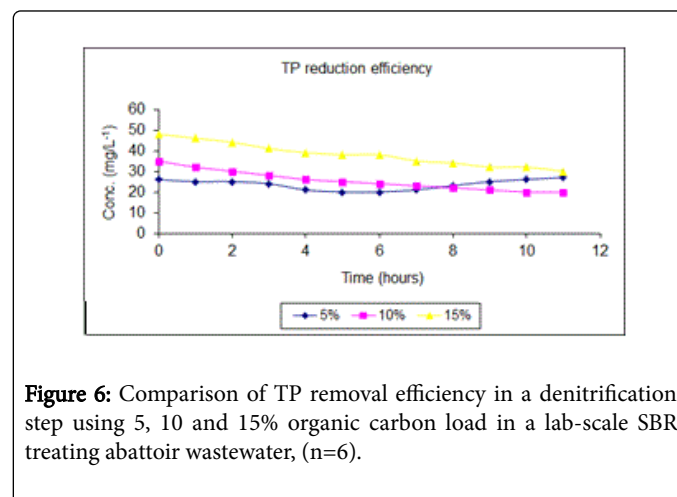

Figure 6: Comparison of TP removal efficiency in a denitrification step using 5, 10 and 15% organic carbon load in a lab-scale SBR treating abattoir wastewater, (n=6).

The turbidity removal efficiency did not significantly differ between 5% and 10% (p>0.05) carbon load. However, there was significant difference in turbidity removal efficiency between 15% and each of 5% and 10% organic carbon load (p<0.05) (Table 2).

The TS removal efficiency did not significantly differ between 5% and 10% (p>0.05) carbon load. However, there was significant difference in TS removal efficiency between 15% and each of 5% and 10% organic carbon load (p<0.05) (Table 2). The pH did not significantly differ between 5% and 15% (p>0.05) organic carbon load. However, there was significant difference in pH between 10% and each of 5% and 15% organic carbon load (p<0.05) (Table 2).

The EC did not significantly differ between 5% and 15% (p>0.05) organic carbon load. However, there was significant difference in EC between 10% and each of 5% and 15% organic carbon load (p<0.05) (Table 2). Temperature did not significantly differ between 10% and 15% (p>0.05) organic carbon load. However, there was significant difference in temperature between 5% and each of 10% and 15% organic carbon load (p<0.05) (Table 2).

Discussion

The National Environmental Management Authority (NEMA) has set wastewater discharge standards of: COD, 100 mg/L; NH_4^-N, 10 mg/L, TKN, 10 mg/L; TSS, 100 mg/L; ortho-P, 5 mg/L and total-P, 10 mg/L; and Turbidity, 300 NTU/FAU; [19]. Effluent above these limits needs further processing to reduce pollutant concentrations.

Denitrification is anoxic reduction of the $NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$ by heterotrophic bacteria such as *Pseudonomas stutzeri*, *Alcaligenes faecalis* and *Ochrobactrum anthropi* [9,10]. Most denitrifiers are facultative heterotrophic bacteria that use organic carbon as energy source and nitrite-nitrates as electron acceptors [15]. While most waters contain a reducing power in the form of organic substrate, it is difficult to preserve the reducing power required for denitrification, due to the necessary preceding aerobic oxidation step [24,26]. Consequently, sufficient organic carbon source must be provided for proper denitrification [18].

Limited carbon is known to cause repression of denitrifying enzymes [11] as shown when 5% organic carbon (C:N ratio 1.68) is added (Figures 2 and 3). The current interpretation of this phenomenon is that nitrate entering the anoxic phase is used as an electron acceptor in the growth of non-poly heterotrophs [14]. This reduces the amount of the substrate available for sequestration by the poly organisms and hence reduces the amount of phosphorous removal that can be achieved [15]. Moreover, phosphate removal efficiency is affected by competition for the organic substrate between denitrifiers and PAOs [13,19] leading to high phosphorus concentration in the effluent (Figure 7, Table 2).

10% organic carbon load had a C:N ratio of 1.89 mg $COD.L^{-1}$ N-TKN which achieved complete nitrite removal (Figure 4). Negligible amounts of nitrates (2 ± 1 mg/L) remained within the system (Figure 5, Table 2). This is consistent with research findings by Obaja et al. [25] and Rahman et al. [24] that complete denitrification is obtained when the C/N ratio is $\geq$ 1.7. According to Fabregas [27], the high DO concentration (14.40 mg/L^{-1}) at the beginning of anoxic period could have lowered the level of biodegradability of the wastewater and hence complete nitrate removal was not achieved. Carbon decrease observed was as a result of both assimilative and dissimilative carbon utilization by denitrifying and other bacteria [20,24]. The COD concentration measured at the end of the cycle was from the fraction of slowly biodegradable substrate contained in the abattoir wastewater [15]. Denitrification resulted in a rise in alkalinity of the system [13,28,29], with corresponding increase in pH (Table 2).

The rate of phosphate removal increases (Figures 6 and 7) after most of the denitrification had taken place because denitrifers have high affinity for organic carbon than PAOs [11]. The phosphorus uptake under anoxic conditions is attributable to the activity of denitrifying phosphorus accumulating organisms (DNPAO), capable of accumulating high amounts of polyphosphates [20]. Towards the end of anoxic phase, orthophosphorus had a slight increase with no corresponding total phosphorus increase. This observation can be attributed to anoxic orthophosphorus release by PAOs and total phosphorus absorption by sediments [22].

The use of 15% organic carbon load decreases C:N ratio (1.59). This system overload inhibits both denitrifiers and PAOs activity [13,15], decreasing nitrates and ortho-phosphorus removal efficiencies. The

initial high NO_2^- and NO_3^- removal efficiencies were likely due to dilution factor.
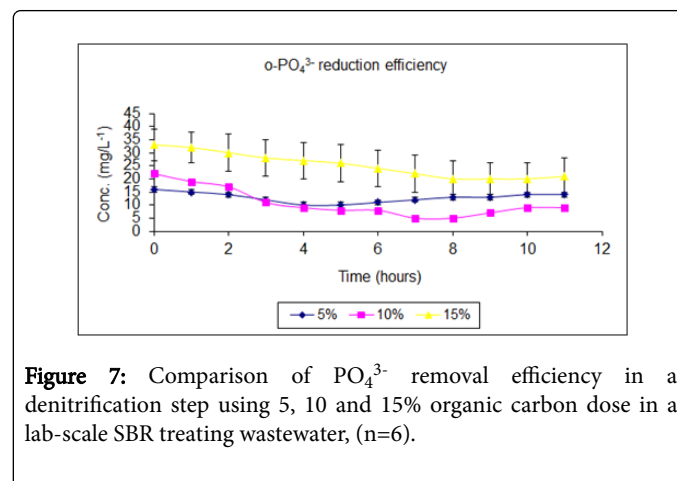

Figure 7: Comparison of PO_4^{3-} removal efficiency in a denitrification step using 5, 10 and 15% organic carbon dose in a lab-scale SBR treating wastewater, (n=6).

Conclusion

Organic carbon in effluent from the anaerobic SBR can be used as a carbon source for anoxic denitrification and phosphorus removal. However, the denitrification rate is affected by the organic carbon load used. The best denitrification and phosphorus removal efficiencies are achieved with 10% (of the anoxic SBR operational volume) of the anaerobic effluent. Except o-PO_4^{3-}–P (8 ± 1 mg/L) and TKN (35 ± 4 mg/L) all other parameters (BOD, TCOD, SCOD, TP, TSS, NH_{4+} -N and turbidity) in the denitrified effluent met permissible discharge standards when 10% (of anoxic bioreactor) organic load was used.

Acknowledgements

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