INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 9, No. 4, p. 182-199, 2016

RESEARCH PAPER OPEN ACCESS

Sequential aerobic-anaerobic treatment of municipal wastewater: effect of different parameters

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Key words: Municipal wastewater, Sequential aerobic-anaerobic treatment, Dual treatment, Wastewater treatment, Biological treatment

http://dx.doi.org/10.12692/ijb/9.4.182-199 Article published on October 27, 2016

Abstract

Sequential application of aerobic and anaerobic system for domestic wastewater treatment experiences certain limitations under low temperature regime and further requires optimization of exogenous inoculum size and treatment time. In order to address the aforesaid issues, a sequential aerobic-anaerobic digester was locally designed and operated for 8-14 days for wastewater treatment. Overall, the treatment efficiency varied from 92-100 % in terms of biochemical oxygen demand (BOD), chemical oxygen demand (COD), turbidity and NO_2-N at 25°C (non-significantly differed at 45°C). Increase in aerobic retention time from 1-3 days helped improvement in treatment efficiency by 5-20 % in most of the parameters. However, a slight increase in COD (94 - 97%) and BOD (95- 97%) removal was observed when temperature raised from 25 to 45°C. Whereas, almost 60% decrease in treatment efficiency was observed in term of BOD and COD removal when temperature decreased from 45-5°C.The low temperature treatment efficiency of the whole system was recovered to maximum within 6-8 days when reactor was bioaugmented with activated sludge (amount/L). Nitrifying bacteria including *Nitrososmonas* sp. and *Nitrobacter* sp were enriched and identified from wastewater and activated sludge using specific activity analysis on different concentration of substrates. The specific activity verification was confirmed with their oxidizing capability of utilizing ammonium nitrogen and nitrite nitrogen. Thus, integrated aerobic and anaerobic system in sequence showed its feasibility to treat municipal wastewater under low to moderate temperature regimes.

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Introduction

Biological treatment of wastewater and sludge under aerobic and anaerobic conditions has been practiced globally mainly due to their low operational cost, easy handling and have less harmful effects on the corresponding environment compared to physical and chemical methods (Shalayby, *et al*.; 2008; Naz *et al*., 2014). Different aerobic and anaerobic processes include activated sludge systems, trickling filters, rotating biological filters, aerobic treatment, anaerobic treatment, septic tanks etc. Each system is limited to treatment of certain environmental and associated economical factors that ask for further improvement in process variables (USEPA, 2000; UN, 2003; Ali, *et al*. 2012; Naz *et al*., 2014). Activated sludge technology is most commonly used for domestic wastewater, but it still faces few problems when dealing with high concentration of effluent solids due to their poor settleability along with maintenance cost. Moreover, it aggravates environmental risk due to high bacterial contamination, thereby creating high risks to public health and water ecosystem (Martins *et al*., 2004; Kim *et al*., 2010).

Trickling filter system is used for the removal of organic matter from wastewater, however, requires additional treatment due to low BOD removal rates at high concentration of suspended solids in the effluent (20-30 mg/L). Besides, this system has a very little operational control (USEPA, 2000). Aerobic treatments remarkably reduce organic and inorganic pollutants, reduces 1-2 log unit of pathogens, require relatively little working space, and produce comparatively less undesirable odors but require additional mechanical aeration and produce high concentration of suspended solids. Anaerobic treatments comparatively reduce more solid content, produce renewable energy and comparably cost-effective. Anaerobic treatment comparatively reduces more solid content, and produces energy, however it is more complex and difficult to manage and control. Moreover, low pathogen removal rates and moderate acidogenic and methanogenic activity have been reported if only anaerobic treatment is utilized alone (Demirel and Yenigun, 2002; Keller *et al*., 2004; Pant and Mittal, 2007; Yenigun and Demirel, 2013).

So both processes have strong and weak features to deal with and to consider for performance optimization. In the light of these individualities, many researches proposed the combination of two processes in sequence, as a promising strategy. In the last decades, the dual anaerobic-aerobic digestion process has gained increased interest due to the validity of this approach to improve the performance of conventional digestion processing (Kumar *et al*., 2006; Zupancic and Ros, 2008; Kassab *et al*., 2010; John *et al*., 2011; Shi *et al*., 2014). However, very few studies have been investigated the sequential application of aerobic-anaerobic digestion for the treatment of wastewater specifically at low to moderate temperature (5-25°C). This is due to the assumption that limited activity of microorganisms occurs within this temperature range.

Despite some drawbacks, the obvious economic and environmental advantage of biological treatment over other processes has cemented its pivotal place in any integrated wastewater management system (Hosseini Koupaie *et al*., 2014; Crites *et al*., 2014). Comparatively, dual or sequential aerobic-anaerobic treatment has been considered as a viable alternative by harmonizing the advantages and disadvantages of both systems in a most cost effective setup. An additional positive effect of the aerobic pretreatment step is the ammonia nitrogen removal (about 85-90%) in the wastewater that can significantly reduce the nitrogen load recycled to the environment (Zupancic and Ros, 2008; Kim and Novak, 2011). Although anaerobic process has been increasingly used for organic matter removal, its effluent is rich in ammonia nitrogen, COD, suspended solids, nitrogen, phosphorus and sulfate (John *et al*., 2011). Additionally, denitrification takes place over a time and sometimes requires an additional anaerobic phase when the sequential anaerobic-aerobic configuration is applied to the waste treatment system (Kumar *et al*., 2006; John, *et al*., 2011). For this reason, the integration of an aerobic treatment step prior to anaerobic digestion promotes the simultaneous carbon oxidation, nitrification/denitrification, and phosphorus removal (Florante, 2009).

Thus, application of sequential aerobic-anaerobic treatment could provide dual opportunity at both technological and economical level in terms of resource recovery and compliance with current legislation on effluent discharge. Still these conventional dual treatments confront with issues of low treatment efficiency under low to moderate temperature ranges (Chan *et al*., 2009). The present study is designed considering the aforesaid issues mentioned in sequential anaerobic-aerobic treatment of wastewater and sludge. A simple and low cost laboratory scale dual/sequential aerobic-anaerobic digestion system was evaluated for the treatment of municipal wastewater and to produce pathogen free water. The treatment efficiency of the system was evaluated under varying aerobic treatment time (1-3 days), temperatures (5, 25 and 45°C) and inoculum sizes of activated sludge (2, 4 and 6g/L) in order to reduce the overall treatment time (retention time). The different effective bacteria were enriched and analyzed for their nitrification ability.

Materials and methods

Environmental samples collection and handling

Municipal wastewater collected from residential colony (population equivalent of about 1000 persons) of Quaid-i-Azam University, Islamabad, while the activated sludge inocula used in bioaugmentation strategy was taken from Wastewater Treatment Plant I-9, Islamabad, Pakistan using grab 1 manual method according to NPDES Compliance Inspection 14 (Operating Procedures Wastewater Sampling, 2013).

Sequential aerobic-anaerobic setup and operation

The experimental set-up was based upon integrated aerobic-anaerobic bioreactors was built from thermos table plastic material (height 12″, diameter 8″, radius 4″) as shown in the Fig.1. The first aerobic reactor was cylindrical in shape and has 8Liter of volumetric capacity. It consisted of single recipient aeration tank of slight conical bottom, used to process wastewater. It was made up of single outlet at the bottom for sampling and transferring the effluent. For aeration, it was operated with an overhead stirrer at 150 rpm (VELP Scientifica's electronic).

The second anaerobic reactor of 8 Liter volumetric capacity made upon of thermos table plastic material was run separately (Figure 1). It was similar to aerobic reactor except that the tank was permanently covered from the top to avoid contact with air, containing three ports for sample feeding (at the top left), decanting and N_2 purging (at bottom right and center) and biogas collection and measurement (at the top left). The working volume of wastewater was kept 6 Liter. The hydraulic retention time (HRT) of influent was kept 24hours and flow rate was calculated as 1.5 L/min (0.0015m^3/min). The hydraulic loading rate of the digester was 0.25 m/m^3.

Anaerobic culture was obtained from wastewater treatment plant, while the aerobic cultures were obtained from the aeration tanks of the activated sludge (sludge age = 12 days) units of the Islamabad Sewage Treatment Plant.

Experiments were conducted in three phases. In the first phase of the research the effect of aerobic operation time on the wastewater treatment was evaluated. The wastewater samples (feed) were aerobically digested for 1, 2 and 3 days and then subjected to 8-14 days anaerobic treatment at 25°C with 24 hours HRT. In the second phase of the study, the effect of incubation temperature was considered on the sequential aerobic and anaerobic treatment of wastewater.

The wastewater samples (three) were aerobically treated at different temperatures; i.e. 5°C, 25°C and 45°C for 2 day and then exposed to anaerobic treatment for 12 days. In the final phase, the effect of seed size on wastewater treatment efficiency by integrated aerobic and anaerobic system was evaluated. For this purpose three different concentrations of activated sludge such as 2, 4 and 6g/L were used as inocula (seed) in the reactors at 5 and 25°C. On the other side, in order to investigate autotrophic bacteria specifically nitrifiers within municipal wastewater and activated sludge, five batch experiments were conducted with different concentrations of NH_3-N and NO_2-N in modified specific media.

Analysis

Physico-chemical characterization of wastewater

In order to estimate the wastewater treatment efficiency of the integrated aerobic and anaerobic system, the influent and effluent samples were analyzed by considering parameters like; chemical oxygen demand (COD), biochemical oxygen demand (BOD), total dissolved suspended (TDS), Total suspended solid (TSS), pH, turbidity, phosphates (PO_4), sulfates (SO_4), nitrates nitrogen (NO_3-N) and nitrates nitrogen (NO_2-N) according to standard methods for water and wastewater analysis (APHA, 2005).

Microbiological characterization of wastewater and activated sludge

Verification of pathogenic bacteria

The pathogen removal efficiency of the sequential aerobic-anaerobic system was evaluated by considering Most Probable Number (MPN) technique to determine the presence and absence of coliforms and Viable cell counts of *Salmonella* sp., *Shigella* sp., *Klebsiella* sp., *Proteus sp.*, *E. coli*, *Pseudomonas* sp., *Streptococcus* sp., *Staphylococcus* sp. and *Bacillus* sp. were conducted in terms Colony Forming Unit (CFU) following Bergeys Manual of Bacteriology 9th Edition (Bergey *et al.*, 1994).

Verification of nitrifying bacteria

Ammonium oxidizing activity of *Nitrosomonas* sp. was carried out by using *Nitrosomons europaea* medium. Whereas, *Nitrobacter* medium B was used to check the nitrite oxidizing ability of *Nitrobcater* sp. according to the hand book of media for environmental microbiology, 2nd edition in 1000 ml flask (Atlas, 2005). The *Nitrososmonas* medium contained 0.15 g of K_2HPO_4, 0.2 g of $MgSO_4.7H_2O$, 0.02 g of $CaCl2.2H_2O$, 1.7 g of $(NH_4)_2SO_4$, and 1.0 g of Ferric EDTA per Liter and 1ml of trace element solution. *Nitrobacter* medium B contained 2.0 g of MnSO4, 0.5g of K2HPO4, 0.5g of MgSO4, 1.0 g of NaNO2, 0.3g of NaCl, 5.0 g of Fe2(SO4)3 per Liter and 1 ml of trace elements.

Trace elements including MnCl2.4H2O, 0.02; CuSO4.5H2O, 2.0g/L; CoCl2.6H2O, 0.2g/L; ZnSO4.7H2O, 0.01g/L; Na2MoO4.2H2O,0.01g/L, were added in both media. The medium was sterilized by autoclaving and then cooled. The pH was adjusted to 7.5 ± 2.

For the screening of ammonia oxidizers (*Nitrosomonas* sp), activity verification regarding the strength of nitrites (NO_2-N) in the liquid growth medium containing inoculum isolated and identified from waste water and activated sludge samples. Different concentrations (5, 10, 15, 20 and 25 mM) of Ammonium sulphate and sodium nitrite were used in *Nitrosomonas* medium and Nitrobacter B medium were taken in 250 mL Erlenmeyer flasks. After sterilization and cooling at room temperature, media were inoculated with 2 mL volume of enriched cultures isolated from waste water and sludge separately and then were incubated on shaking incubator at 30°C. For activity measurement, the strength of nitrites nitrogen and nitrate nitrogen formed from ammonium sulphate and sodium nitrite by pure isolated cultures was determined before incubation and periodically after incubation i.e. after 24, 48, and 72 hrs by standard method 4500 (NO2-N) for water and waste water (APHA, 2005).

Results and discussion

The efficiency of sequential aerobic-anaerobic digestion system for the treatment of municipal wastewater was evaluated under varying operational conditions. Overall, the treatment efficiency of the system improved from moderate to significant levels when operational conditions specifically treatment time and temperature were manipulated. Besides, supplementation of process with activated sludge (Bioaugumentation) proved to be considerably improving the efficiency of the process and making it feasible even at low temperature condition.

Physicochemical properties of untreated municipal wastewater

Municipal wastewater samples were physico-chemically analyzed by considering COD, BOD, TDS, TSS, Turbidity, etc.

These parameters vary strongly depending upon the loading rate of organic and inorganic materials. Levels of COD (249 ± 3.5 mg/L), BOD (175.2 ± 5.7 mg/L), TDS (784.6 ± 2.0 mg/L), TSS (1110 ± 9.8 mg/L), alkalinity (53 ± 5.0 mg/L) and turbidity (3175 ± 1.8 NTU) was significantly higher than Environmental Protection Agency (2004) and World Health Organization standards (2006) as shown in the Table 1.

Table 1. Characteristics of untreated wastewater and its comparison with USEPA and WHO guidelines.

Parameters	EPA Guidelines	WHO Guidelines	Real Municipal Wastewater (Ave ± SD)
COD (mg/L)	8-10	NGV	249.3 ± 3.5
BOD (mg/L)	5-8	NGV	175.2 ± 5.7
TDS (mg/L)	500-1000	<1000	784.6 ± 2.0
TSS (mg/L)	NGV	NGV	1110 ± 9.8
Phosphates(mg/L)	0.05	NGV	0.847 ± 1.3
Sulphates (mg/L)	NGV	250	0.721 ± 0.3
Nitrites (mg/L)	<0.5	3	2.870 ± 1.4
Nitrates (mg/L)	<0.05	50	0.980 ± 0.9
pH	6.0-9.5	6.5-8.5	6.671-7.00
Alkalinity (mg/L)	0.05	250	53.0 ± 5.00
Turbidity (NTU)	<0.5	NGV	3175 ± 1.8
EC (µS/cm)	NGV	400-1215	713.0 ± 1.3
Fecal Coliform (CFU/100mL)	10^3-10^4	10^3-10^4	2×10^9 ± 2.0

Key: NGV=not given value.

While, PO_4 (0.847 ± 1.3 mg/L) and SO_4 (0.721 ± 0.3) were lying within the permissible limits of EPA and WHO standards. Though, these parameters followed the same trends of a typical medium strength untreated domestic wastewater. However, the value of alkalinity (53 mg/L) was noticeably less than high strength domestic wastewater but was much higher than medium strength wastewater. On the other hand, concentration of nitrite (2.87 ± 1.4 mg/L) and nitrate (0.98 ± 0.9 mg/L) was significantly higher compared to high strength raw domestic wastewater. The bacterial count, MPN index of 1100 (150 – 4800) and 2 × 10^9 CFU/mL of raw wastewater exceeded the said standards. Likewise, Metcalf and Eddy (2004) reported that bacterial count ranged from 10^5 to 10^8 in real wastewater from domestic use (Table 1). Thus, the wastewater originated from local colony of QAU was certainly objectionable and required treatment before release into water ways.

Effect of the aerobic treatment time on wastewater treatment efficiency of the sequential aerobic-anaerobic digestion system

In the first phase of the study, the effect of different aerobic treatment time (1, 2 and 3 days) on waste water treatment efficiency was studied in terms of significant reduction in physicochemical and bacterial parameters. The treatment efficiency of the process increased (9-20%) when the aerobic treatment time increased from 1 to 3 days specifically in case of COD (61.8 ± 1.9% to 76.8 ± 1.9%), BOD (63.2 ± 1.2% to 81.5 ± 1.2%), TDS (24.2 ± 0.8% to 38.9 ± 1.7%) and NO_2-N (99.5% to 99.9 ± 0.2%) relative to the overall system efficiency (Table 2).

However, concentration of nitrate was increased by 10% (Table 2) suggesting process of nitrification within the system (Sakuma *et al.*, 2008). The bacterial count also followed the same pattern of reduction. Overall, total treatment efficiencies in dual treatment (aerobic = 1-3 days; anaerobic = 12-14 days) remained almost same in parameters like COD, BOD, nitrite, nitrates and turbidity. However, there was observed a significant variation in parameters like TDS (10%), PO_4 (8%) when aerobic retention time increased from 1-3 days (Table 2).

Table 2. Effect of aerobic retention time on the overall sequential aerobic and anaerobic treatment of domestic wastewater.

Parameters	*Influent Concentration (Ave ± SD)*	*Temperature*	*Aerobic Retention time (Days)*	*Treatment Efficiency (% ± SD)* Aerobic	Aerobic-Anaerobic (Total)
COD (mg/L)	249.3 ± 3.5		1	61.8 ± 1.9	90.8 ± 1.70
		25°C	2	73.4 ± 1.7	94.2 ± 1.71
			3	76.8 ± 1.9	94.6 ± 1.11
BOD (mg/L)	175.7 ± 5.7		1	63.2 ± 1.2	93.2 ± 1.40
		25°C	2	79.0 ± 1.3	95.6 ± 1.70
			3	81.5 ± 1.2	95.9 ± 1.80
	784.6 ± 2.0		1	24.2 ± 0.8	37.2 ± 0.60
TDS (mg/L)		25°C	2	38.7 ± 1.3	45.8 ± 1.42
			3	38.9 ± 1.7	47.3 ± 0.58
TSS (mg/L)	1110 ± 2.0	25°C	1	20.8 ± 2.1	25.5 ± 2.42
			2	28.3 ± 1.8	33.2 ± 1.02
			3	28.8 ± 2.5	37.9 ± 1.50
PO_4 (mg/L)	0.847 ± 1.3		1	32.6 ± 0.5	32.9 ± 0.50
		25°C	2	39.0 ± 0.7	40 5 ± 1.20
			3	40.2 ± 0.5	40.9 ± 1.60
SO_4 (mg/L)	0.721 ± 0.3		1	22.3 ± 0.5	59.8 ± 3.80
		25°C	2	25.1 ± 1.0	60.9 ± 0.10
			3	25.1 ± 0.7	60.3 ± 2.20
NO_2 (mg/L)	2.870 ± 1.4		1	99.5 ± 0.0	99.5 ± 0.00
		25°C	2	99.6 ± 0.2	99.6 ± 0.20
			3	99.9 ± 0.1	99.9 ± 0.06
NO_3 (mg/L)	0.980 ± 0.9		1	- 74.0	97.3 ± 0.50
		25°C	2	- 84.6	97.8 ± 1.00
			3	- 84.6	98.2 ± 0.40
Turbidity (NTU)	3175 ± 1.8		1	80.0 ± 2.1	99.6 ± 1.40
		25°C	2	99.4 ± 1.5	99.6 ± 1.50
			3	99.5 ± 2.0	99.9 ± 2.00
MPN/100mL	1100		1	240	<3
		25°C	2	210	<3
			3	240	<3
	2×10^9 ± 2.0		1	2.4×10^4	<30
CFU/mL		25°C	2	2.4×10^4	<30
			3	2.1×10^4	<30

Key: SE = Standard Error; SD = Standard Deviation; Ave = Average.

Biochemical changes in the wastewater have always been attributed to the metabolic capabilities of enriched indigenous microbial community and it has been well established in the said system. Overall, aerobic step contributed about 70-90% in the overall organic and inorganic matter removal efficiency of the whole system. Previously, in one case it was reported that the efficient solubilization and partial digestion (acidification) of particulate organic matter took about 12–24 hours, through the fermentative metabolism of thermophilic bacteria (Warakomski *et al*., 1967). Therefore, overall operation time i.e. 2 day aerobic first phase followed by 12-14 days of anaerobic treatment was considered to be optimum for producing high quality effluent having no hazardous effects on the corresponding environment at 25°C temperature.

Both COD and BOD are two key factors that indicate respective total and only biodegradable compounds in the wastewater. Comparatively, COD has been considered better than BOD for monitoring of total energy removal rates (Makaya *et al*., 2007). Time did not effect on the overall treatment of waste water in terms of COD and BOD reduction (90-95 %). However, under aerobic treatment COD and BOD removal rates varied by 20 and 16 % when treatment time increased from 1-3 days (Fig. 2 and Table 2).

Singh and Srivastava (2010) reported the sequencing batch reactor (SBR) treatment of 82% reduction in carbonaceous energy. An additional 20% COD destruction was achieved with an aerobic digestion after anaerobic digestion.

In an another report, Gonzalez-Gonzalez and Cuadros (2014) studied the influence of aerobic treatment time of 5 and 7 days on the anaerobic treatment of olive oil mill wastewater and they suggested 5 days aerobic pretreatment for the complete digestion and stabilization of wastewater with a total COD reduction of 65% along with 0.38 m3/kg COD methane production.

Table 3. Microscopic and biochemical characteristics of bacterial strains from sludge samples.

Strains Codes	Microscopy		Biochemical Characteristics													Identified Species
	Gram's reaction	Shape	Lactose Fermentation	Dextrose Fermentation	Sucrose Fermentation	Manitol Fermentation	H_2S Production	NO_3 test	Indole Production	MR Reaction	VP Reaction	Citrate Use	Urease Activity	Catalase activity	TSI reaction	
Secondary Sludge (SS)																
1	Gram negative	Rod	AG	AG	AG		--	+	--	±	±	+	+	+	--	*Klebsiella pneumonia*
2	Gram negative	Rod	--	AG	AG±		+	+	+	+	--	±	+	+	--	*Proteus vulgaris*
3	Gram negative	Rod	AG	AG	A±		--	+	+	+	--	--	--	+	A/NC	*Escherichia coli*
4	Gram negative	Rod	--	A+	--		--	+	--	+	--	+	--	+	K/NC	*Pseudomonas fluorescens*
5	Gram positive	Cocci	--	--	--	+	--	±	--	--	--	--	+	+	K/NC	*Streptococcus lactis*
6	Gram positive	Cocci bunches	A	A	A	+	--	--	--	+	--	--	--	--	--	*Bacillus subtilus*
7	Gram positive	Diplo cocci	--	A+	A+	+	--	+	--	--	--	--	--	+	--	*Enterobacter aerogenes*
8	Gram negative	Rod	AG	AG	AG		--	+	--	--	+	+	--	+	K/A	*Shigella dysenteriae*
9	Gram negative	Short Rods	--	A	A±		--	+	±	+	--	--	--	+	K/A, H_2S	*Alcaligenes faecalis*
10	Gram negative	Rod	--	--	--		--	--	--	--	--	±	--	+	--	*Pseudomonas aeruginosa*
11	Gram negative	Cocci	--	--	--	+	--	+	--	--	--	+	--	+	--	*Bacillus cereus*
12	Gram positive	Rod	--	A	A	+	--	+	--	--	±	--	--	+	A/NC	*Staphylococcus aureus*
13	Gram positive	Cocci	A	A	A	+	--	+	--	+	±	--	--	+	A/A	*Salmonella typhimurium*
14	Gram negative	Cocco-bacillus	--	AG±	A±		+	+	--	+	--	+	--	+	K/A, H_2S	*Klebsiella pneumonia*

Key: AG = Acid and gas; + = Positive; -- = negative; ± = Variable reaction; A = Acid production; K = alkaline reaction; NC = No change; H2S = Sulfur reduction; K/A = Red/yellow; K/NC = Red/no color change; K/A, H2S = Red/yellow with bubble and black precipitate; K/A, H2S = Red/yellow with black precipitate; A/NC = Acid/no color change; A/A = Yellow/yellow.

An about 10-15% increase in the removal of TDS (24.2-38.9%), TSS (20.8-28.8%) and PO4 (32.6-40.2%) was recorded from 1-3 day of aerobic treatment (Table 2). Reduction in the concentration of the PO4 (32.9-40.9%) might be due the presence of the phosphate accumulating microorganisms (PAOs). Strom (2006) also reported PO_4 removal by intercellular accumulation of PAOs. Further, more reduction was observed during aerobic conditions, as PAO's require aerobic and anaerobic conditions for phosphorus uptake. Kerrn-Jespersen and Henze (1993) also observed rapid phosphorus uptake in aerobic conditions. Helness and Odegaard (1999) investigated simultaneous nitrification and phosphorus removal under aerobic conditions than under anoxic conditions. Peng *et al.*, (2011) reported denitrifying phosphorous reduction in sequencing batch reactor (SBR). Considering an overall low removal rates i.e., 45-55% TDS, 40.5-57% TSS and 32.9-40.9% PO_4, further finishing of treated effluent is recommended.

Still high contents of these two said factors purely reflected that digestion of organic fraction of effluent is almost completed. This observation is positively correlated with the high removal of BOD and COD in effluent.

During the experiments, the pH remained 6.4-7.9. Generally, acidic conditions are created due to accumulation of different acidic products during anaerobic digestion (Lahav and Morgan, 2004).

While, buffering capacity of the system tends to change the pH (neutral) by neutralizing the acidic conditions. The optimal pH for nitrifying and denitrifying bacteria has been reported between 7.5 to 8.5 for complete nitrification and denitrification (Metcalf and Eddy, 2004).

Table 4. Pathogen removal efficiency of the sequential aerobic-anaerobic treatment of wastewater at different operational conditions.

Treatments of Untreated Water	Aerobic Treatment Time (days)			Incubation temperature (°C)			Activated Sludge Inoculum (g/L)		
	1	2	3	5	25	45	2	4	6
MPN/100mL	1100	1100	1100	1100	1100	1100	1100	1100	1100
CFU/mL	TNC	TNC	TNC	TNC	TNC	TNC	TNC	TNC	TNC
MPN After Aerobic	240	210	240	120	210	240	64	43	39
MPN After Anaerobic	<3	<3	<3	<3	<3	<3	<3	<3	<3
CFU After Aerobic	2.4×10^4	2.4×10^4	2.1×10^4	$2.5x\ 10^3$	$1.5x\ 10^3$	$3.5x\ 10^2$	1.9×10^3	1.7×10^3	2.0×10^3
CFU After Anaerobic	<30	<30	<30	<30	<30	<30	<30	<30	<30

In an aerobic system, nitrification and denitrification tends to change the pH of the system. Nitrification consumes alkalinity and decreases pH; it converts ammonium to nitrite and nitrate under high DO level (2 ± 0.2 mg/L). Denitrification reduces nitrate to nitrogen gas under reducing or low DO conditions (0.1–0.2 mg/L) and increases the pH. Nitrate serves as the terminal electron acceptor under anaerobic condition.

When both nitrification and denitrification are occurring together, all the ammonium is converted to nitrogen gas, as given by the equation $4C_5H_7O_2N + 23O_2 \rightarrow 20CO_2 + 2N_2 + 14H_2O$ (Grady, *et al.*, 1999). Therefore, nitrification and denitrification are the processes occurring at different environmental conditions that can be achieved in a system composed of aerobic and anaerobic reactors sequentially arranged.

Table 5. Morphological, microscopic and biochemical characterization of enriched cultures of wastewater and sludge.

Growth media	Isolated strains	Morphology				Microscopy		Biochemical Characteristics								Identified strain
		Size	Pigment	Form	Margins	Gram's reaction	Shape	H_2S Production	Indole Production	MR & VP Reaction	Citrate Use	Urease Activity	Catalase activity	Oxidase activity	TSI reaction	
Nitrosomonas europia medium	2	Small	Nil	Irregular	Entire	-	Rods	-	-	+	+	+	-	-	A/A	*Nitrosomonas* sp.
Nitrobacter medium B	2	Small	Nil	Rhizoid	Serrate	-	Rods	-	-	-	+	-	-	+	K/A	*Nitrobacter* sp.

From the available data it is evident that the mode of nitrogen removal in the aerobic treatment was nitrification.

The average concentration of NO_2-N in the influent was 2.870 ± 1.4 mg/L and 99.5-99.9% oxidized to NO3-N (74-85%) as the aerobic treatment time increased from 1-3 day (Table 2).

Conversely, following anaerobic treatment 98% of NO_3-N was reduced to N_2 (Table 2) which purely reflected process of denitrification within the system. The process of simultaneous nitrification and denitrification is usually controlled by the relative concentrations of carbon, nitrogen and phosphorus ratios. By adjusting the ratio C:N:P to 100:5:1 and a high total nitrogen removal efficiency of up to 88.3% has been reported by various researchers (Singh and Srivastava, 2010; Ghehi, *et al.*, 2014). Generally, decline in concentration of NO_2 and raise in NO_3 under aerobic conditions was attributed to the NO_2 oxidizing activity of nitrifying bacteria *Nitrosomonas* sp. and *Nitrobacter* sp. (Sakuma, *et al.* 2008).

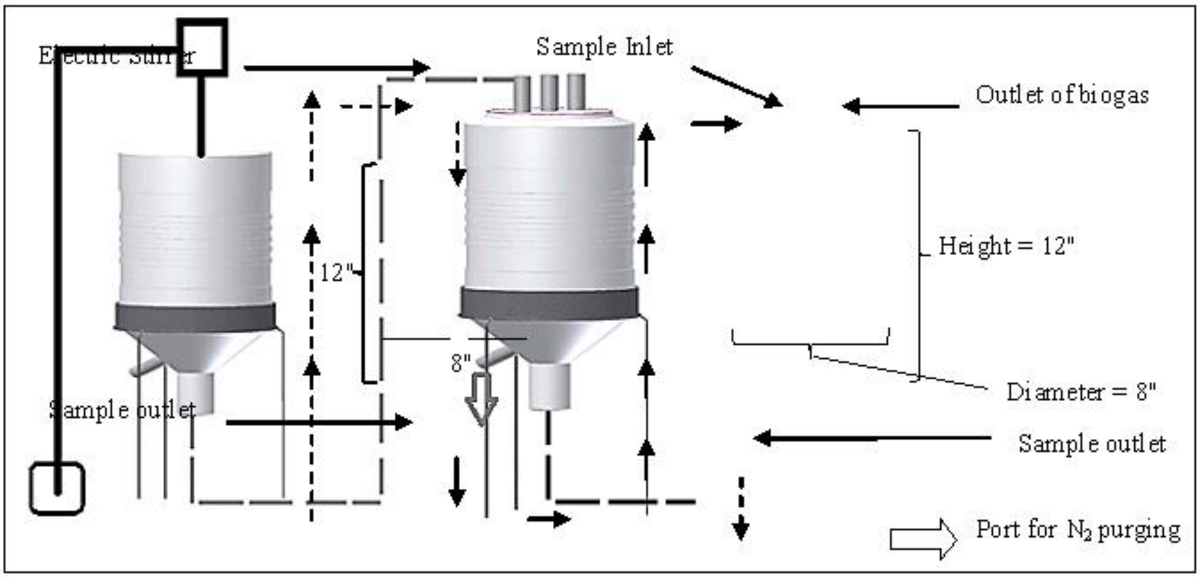

Fig. 1. Schematic diagrame of labscale sequential aerobic-anaerobic treatment system.

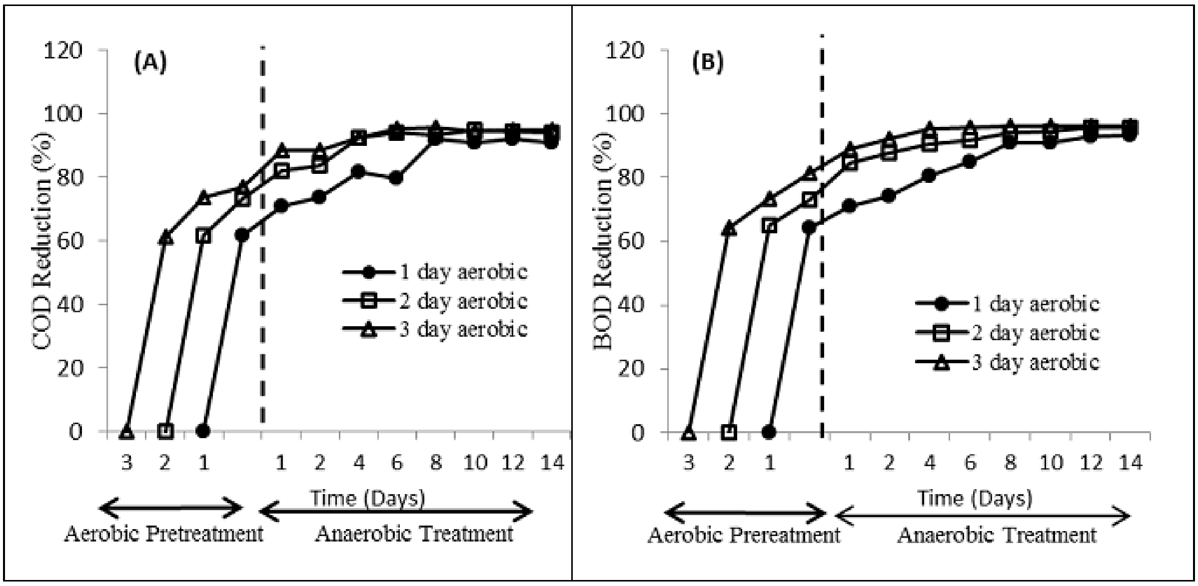

Fig. 2. COD (A) and BOD (B) reduction rates (%) of municipal waste water treatment under different aerobic pretreatment time.

In the present study, fecal coliforms were chosen as an important indicator to monitor the quality of raw and treated wastewater. The organisms existing in the untreated samples were deduced using information of all these microbiological analyses. These included *Klebsiella pneumonia*, *Proteus vulgaris*, *Escherichia coli*, *Pseudomonas fluorescens*, *Micrococcus luteus*, *Streptococcus lactis*, *Bacillus subtilus*, *Enterobacter aerogenes*, *Shigella dysenteriae*, *Alcaligenes faecalis*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Staphylococcus aureus* and *Salmonella typhimurium* (Table 3). According to WHO (2006) and USEPA (2004) guidelines, the fecal coliform count must not increase from log value of 10^3 fecal coliforms per 100 ml in water used for irrigational purposes (Messenger, 1993). After aerobic treatment under different retention time, bacterial count reduced by more than half i.e. 200-240 MPN/100 ml (MPN index of 200) and 2×103 CFU ml-1 water. However, it was reduced to undetectable limits (MPN index = < 3) (CFU = <30), when water was given 8 days anaerobic treatment (Table 4).

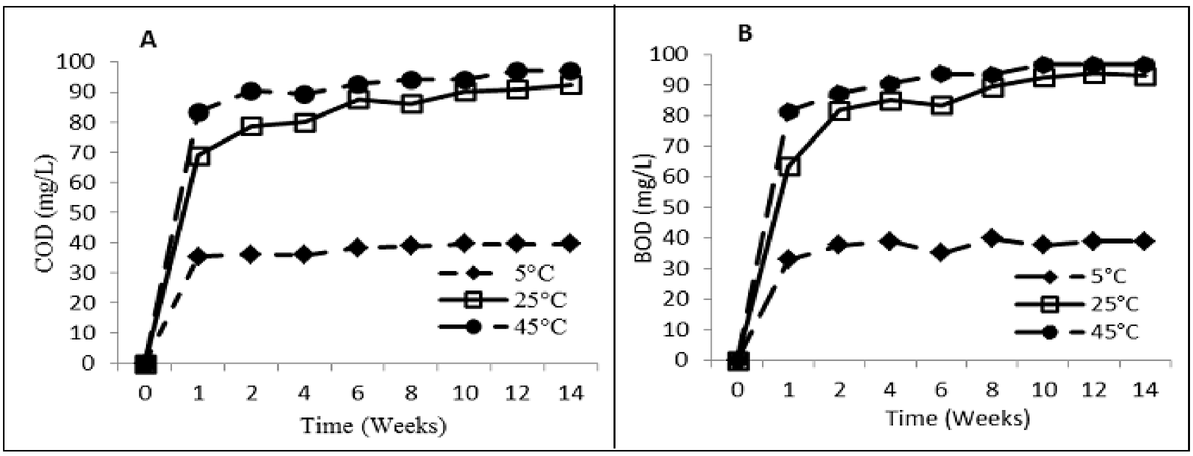

Fig. 3. COD (A) and BOD (B) reduction rates (%) of domestic waste water treatment under different temperatures (5, 25 and 45°C) regimes.

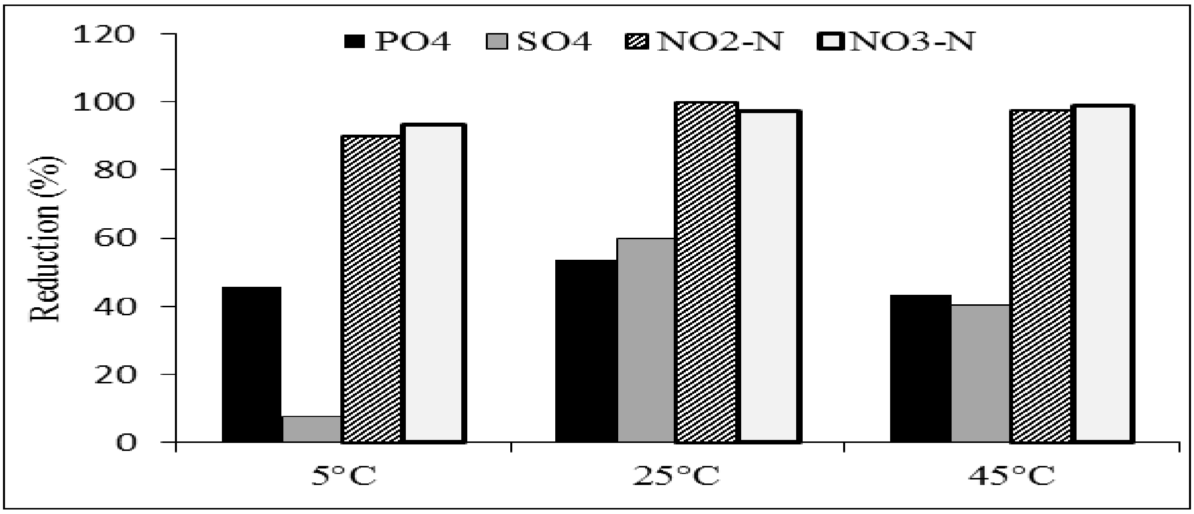

Fig. 4. Phosphate, sulphate NO_2-N and NO_3-N reduction rates (%) of domestic waste water treatment under different temperatures regimes (5, 25, 35 and 45°C).

This was in accordance to (Zhang, *et al.*, 2004) where they reported complete stabilization and removal of pathogens takes place in the anaerobic step in the dual digestion process. Our findings showed that the production of sludge along with treated wastewater remained comparatively low i.e 0.5-1 g/l, besides it represented as nutrients rich chiefly free of specific pathogenic bacteria.

According to a study by von Sperling and Chernicharo (2005) aerobic treatment provided pathogenic bacterial removal log of about 1–2. Hence, the integrated aerobic and anaerobic digestion proved to be efficient in reduction of fecal coliforms as compared to independent digestion process. These results were in conformity with the previous studies on sequential aerobic and anaerobic digestion and SBR (Singh and Srivastava, 2010).

Besides, these declining microbial counts after dual aerobic and anaerobic treatment might be related to depleting of nutritional sources in water (Zhang, *et al*., 2004).

Effect of the operational temperature on wastewater treatment efficiency of the sequential aerobic-anaerobic digestion system

Domestic wastewater was treated under three different temperatures viz. 5, 25, and 45°C regimes. Under aerobic condition of 2 days, ≥92% efficiency (P value < 0.05) was achieved in most of the parameters like COD (91.9 ± 0.7%), BOD (93.1 ± 0.8) and turbidity (98%) whereas; it was only 45.8, 47, 53.9, and 59.8 % in parameters like TDS, TSS, PO_4 and SO_4, respectively.

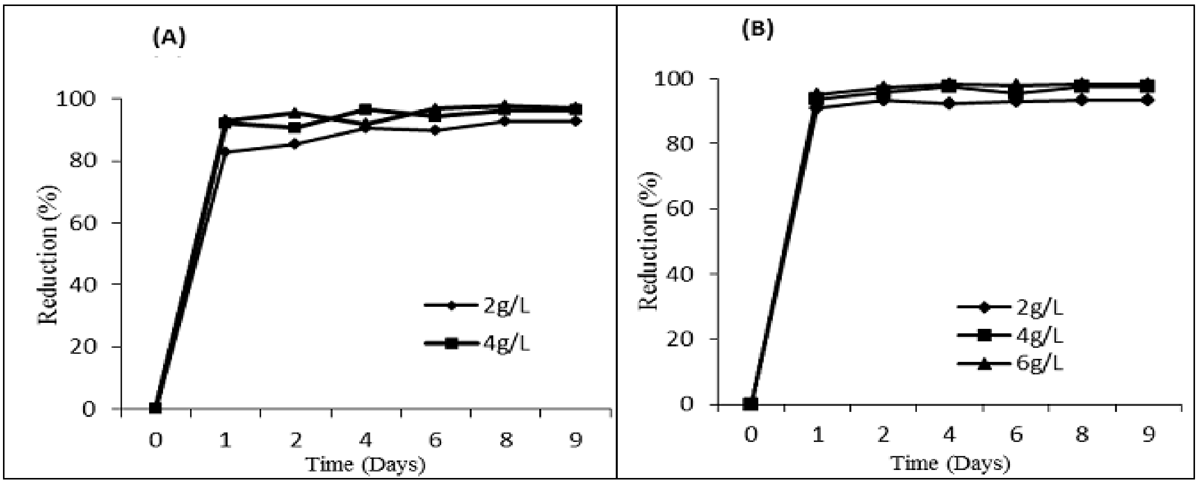

Fig. 5. Change in the concentration of COD (A) and BOD (B) reduction rates under different concentrations (2, 4 and 6g/L) of activated sludge.

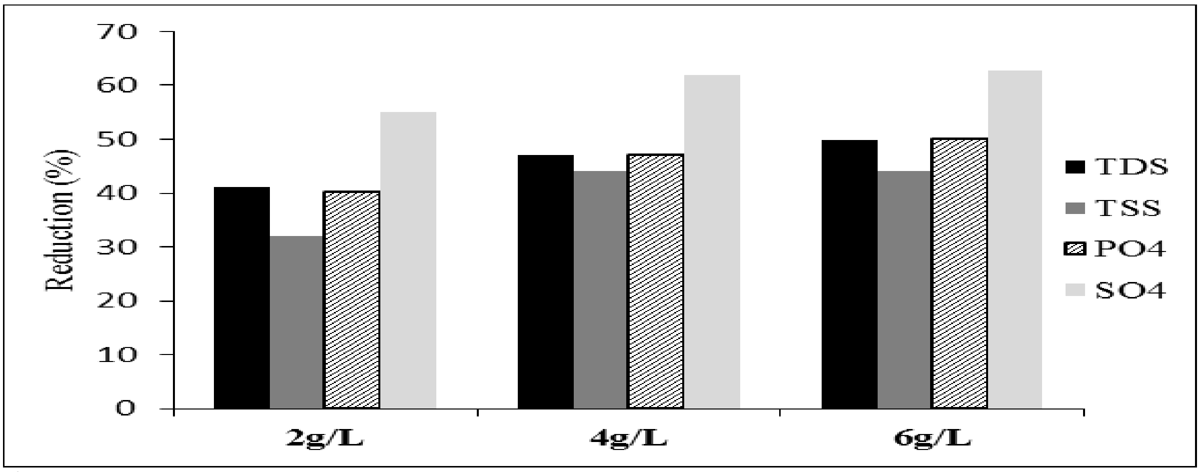

Fig. 6. TDS, TSS, phosphate and sulphate reduction rates (%) of domestic waste water treatment addition of activated sludge (inocula) at 25°C.

Treatment efficiency was increased by 40-60% in terms of parameters like COD/BOD and turbidity when temperature increased from 5-25°C. These reductions signified that the degradation of organic and inorganic matter was highly effected by temperature.

However, further increase in temperature up to 45°C caused slight increase (2-6%) in the reduction COD (96.1 ± 1.7%) and BOD (97 ± 0.4%) as shown in the Fig. 3. In other words, the group of microorganisms involved in the aerobic process worked at its maximum capability at ambient temperature (25°C) and the treatment efficiencies not significantly increased as the temperature increased.

Aerobic thermophilic pretreatment of wastewater for 1-2 day proved to be reducing the anaerobic retention time by around 30% (Borowski and Szopa *et al.*, 2007). Low temperature treatment generally reduces the substrate utilization pattern to minimum and microbial physiology mostly works only for survival patterns (Lettinga *et al.*, 2001). In addition, specific microbes like nitrifiers quantitatively reduced to minimum at low temperatures which are highly required for the digestion of the organic material in wastewater (Twafik *et al.*, 2005). Contrarily, removal of NO_2 and NO_3 even kept 89.8-93.2% at low temperature condition but it remained only 39% for COD/BOD, 60 ± 1% for turbidity and 5-7% other parameters (Fig. 4).

Treatment of domestic wastewater has always been found reducing microbial counts specifically pathogens. Total viable counts of pathogenic bacteria (*Salmonella* sp., *Shigella* sp., *Klebsiella* sp., *Proteus sp.*, *E. coli*, *Pseudomonas* sp., *Streptococcus* sp., *Staphylococcus* sp., and *Bacillus* sp.,) in raw wastewater was considerably high i.e., 150-4800 (MPN index of 1100) as shown in Table 3 and 4. After aerobic treatment it reduced to more than half (MPN index of 200). However, it was significantly declined to minimum i.e., 9 (MPN index of < 3) (99.5 ± 0.5%) when wastewater was given 8 days anaerobic treatment (Table 3 and 4).

This illustrated that anaerobic treatment comparatively gave higher wastewater treatment efficiency in the removal of pathogenic bacteria. In the present research, CFU per ml of the wastewater before treatment was 2×10^9 and was reduced to 2.5×10^4 after aerobic treatment and became too less to count after anaerobic treatment (Table 4).

Effect of the activated sludge inocula as a bioaugmentation strategy on wastewater treatment efficiency of the sequential aerobic-anaerobic digestion system

For the effective function of any biological treatment for wastewater the role of microbial community within it is crucial.

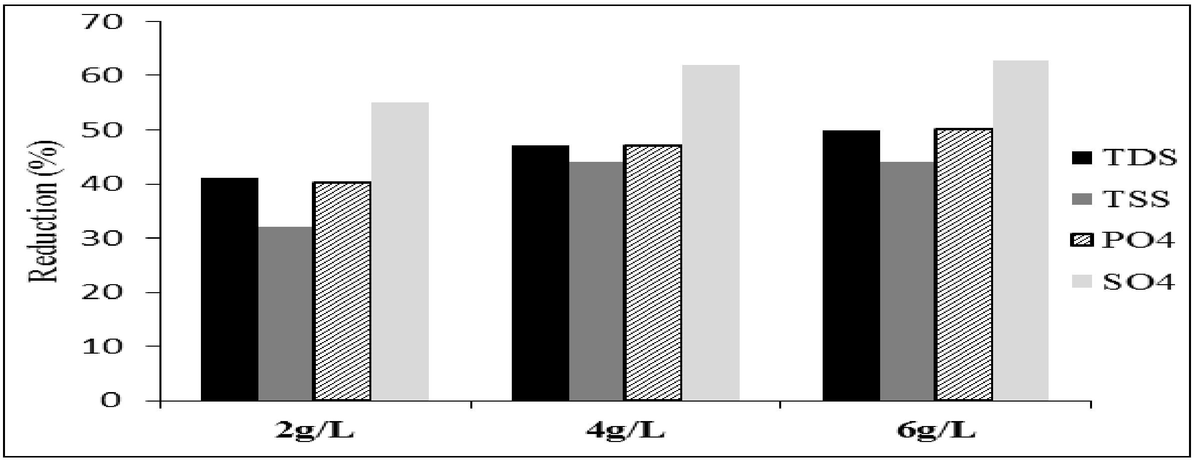

Fig. 7. COD (A) and BOD (B) reduction rates (%) of domestic waste water treatment addition of activated sludge (inocula) at 5°C.

Bioaugmentation has been one of the strategies for such systems in introducing and more actively growing bacterial community in order to improve the efficiency (Stephenson and Stephenson, 1992). This technique has frequently been applied to aerobic systems after multiple upsets from uncontrolled biomass loss, fluctuations in pH, toxic events, or temperature decrease (Abeysinghe *et al.*, 2002). Further aeration of this system helped reducing the organic substrates by biological oxidation (Rojas *et al.*, 2010). When activated sludge was used as a seed/inoculum in the aerobic reactor for domestic wastewater treatment, it decreased the overall treatment time to 4-6 days (2 days aerobic, 6-8 days anaerobic) as shown in the Fig. 5.

The overall treatment efficiency remained almost same and remained 90-100 % for most of the parameters except TDS (41, 47, and 50 %), TSS (32, 44, and 44.5%), PO_4 (40, 47 and 50 %) and SO_4 (55, 62 and 63 %) at 2, 4 and 6g/L sludge inocula (Fig. 6). COD reduction (96.5-97%) was significantly higher than the previously established data by Dhouib *et al.* (2006). He reported the improvement in COD reduction by 65% and better detoxification of wastewater treated with activated sludge containing white rot fungi in a batch aerobic reactor in 7 days. Similarly, Li-ping, *et al.*, (2000) reported enhanced reduction of quinolone rich domestic wastewater by bioaugmenting the aerobic system with 43g/L inoculum of *Burkholderia picketti* W2 (a quinoline degrader) in 5 hours. Further Jianlong *et al.*, (2002) suggested that the oxic-reactor/phase was the best situation for bioaugmentaion in the sequential anaerobic-anoxic-oxic treatment, where COD removal increased by more than 55% within HRT of 35 hours at 28°C.

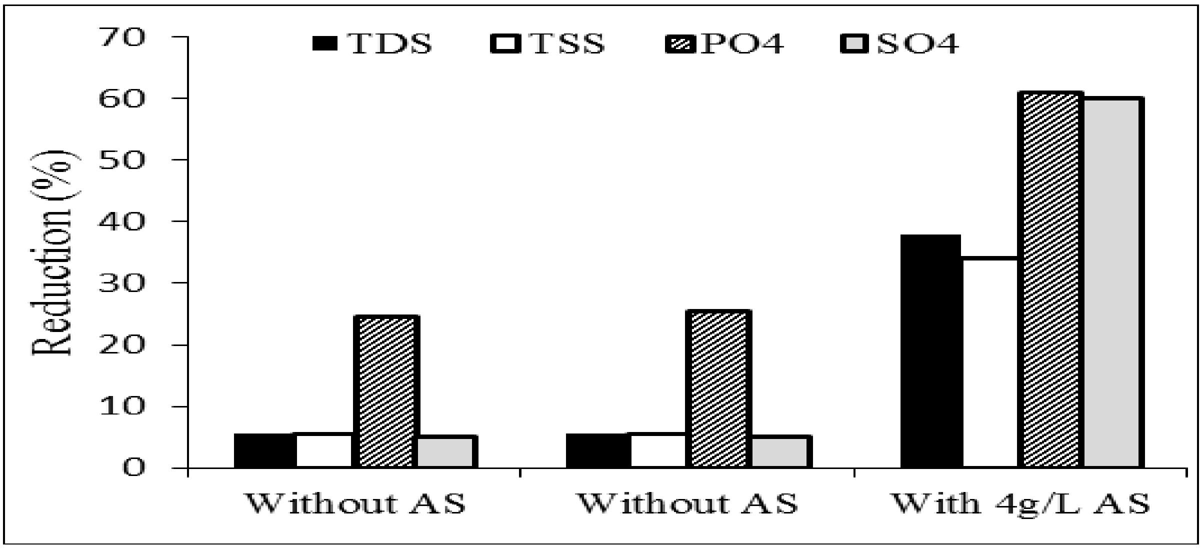

Fig. 8. TDS, TSS, phosphate and sulphate reduction rates (%) of domestic waste water treatment addition of activated sludge (inocula) at 5°C.

In order to increase the treatment efficiency at 5°C the aerobic treatment time was kept at 2 and 3 days in subsequent experiments without the addition of inocula. However, there was observed no substantial increase in wastewater treatment efficiency (Fig. 7). This is because of the fact that in winters the nitrifiers and many *Pseudomonas* sp. are in low quantity (Twafik *et al.*, 2005) Most studies on the effect of temperature on anaerobic digestion showed a strong negative effect on the metabolic activity of mesophilic anaerobic methanogenic bacteria at lower temperature (Lettinga *et al.*, 2001).

To increase the treatment efficiency at low temperature (5°C), additional sludge i.e. 4g/L was used during aerobic treatment and it resulted in a significant improvement (P value < 0.05) in removal of COD (97.8 + 1.2%), BOD (95.3 + 0.9%), turbidity (61 + 1.2%), NO_2 (99.2%), NO_3 (99.9%) and 38-70% removal of parameters like TDS, TSS, PO_4 and sulphate (Fig. 8). Nitrification rate (99.8%) was significantly higher than previously established data by Cui *et al.*, (2014).

They demonstrated the improvement in nitrification efficiency by 85% with cold adapted ammonia and nitrite oxidizing bacteria in sequential batch reactors (SBRS). Studies also highlighted the impact of sludge seeding for improving; process performance, stability, BOD removal, prevention of sludge bulking, and removal of specific pollutants by specially adapted bacteria (Stephenson and Stephenson, 1992).

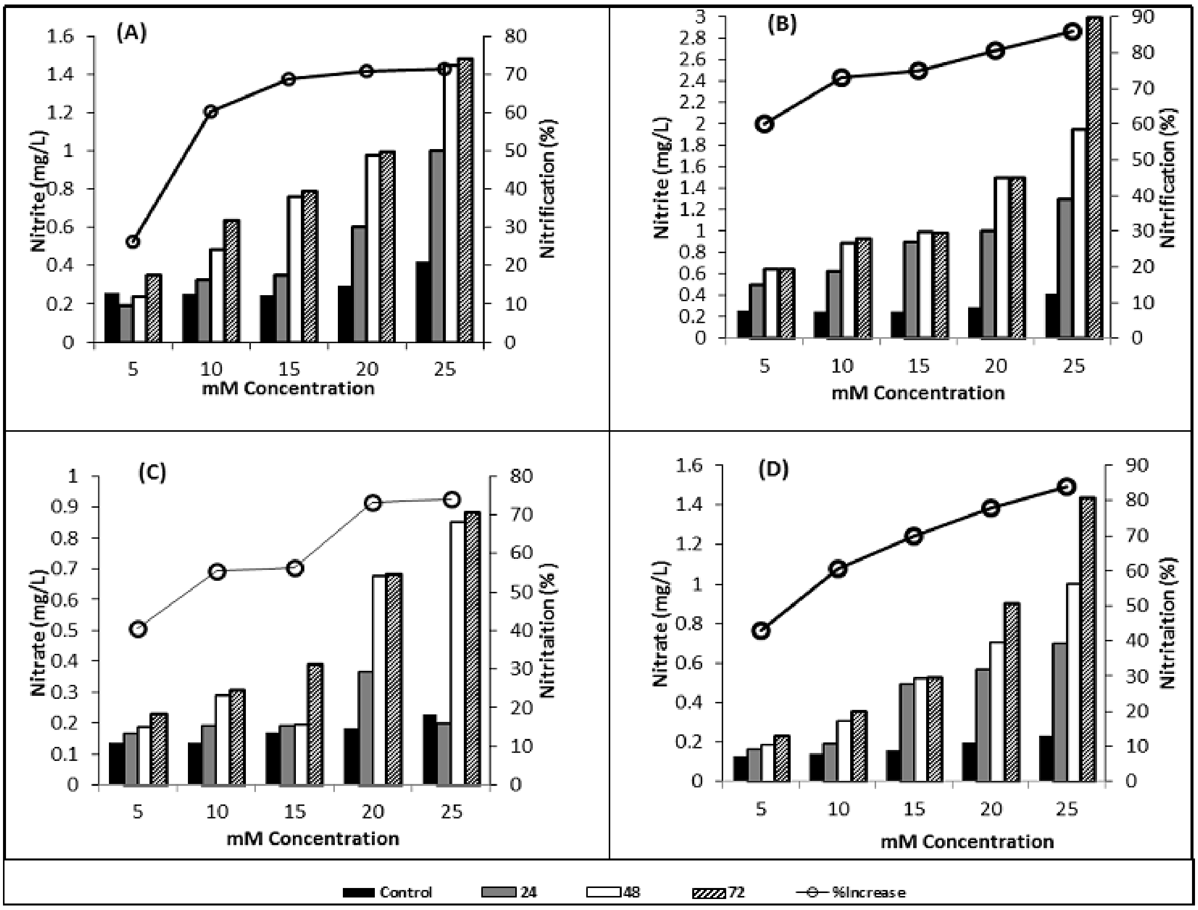

Fig. 9. Activity analysis of ammonia oxidizers from wastewater (A) and activated sludge (B); Nitrite oxidizers from wastewater (C) and activated sludge (D).

Isolation and identification of nitrifiers from wastewater and activated sludge inocula used in sequential aerobic-anaerobic digestion

The present research also emphasized on the culturing of nitrifying bacteria by their enrichment in the specific growth media (*Nitrosomonas* europia medium and *Nitrobacter* medium B). According to cultural, microscopic and biochemical characteristics, the enriched bacterial strains were identified as *Nitrosomonas* and *Nitrobacter* sp. (Table 5). Both of these strains were gram negative rods have negative H_2S production, indole production and catalase activity confirmatory tests. However *Nitrosomonas* sp. was identified by their positive response to MR-VP reaction, citrate utilization, urease activity and production of yellow color in TSI reaction. On the other hand, *Nitrobacter* sp. has shown negative MR-VP reaction, urease activity and positive citrate utilization and oxidase activity tests. A red coloration was also noticed in its TSI test (Table 5).

Assessment of nitrifying abilities of enriched nitrifying bacteria

Batch experiments were inoculated with the nitrifying bacterial isolates of municipal wastewater (MWW) and activated sludge inocula (AS) and then activity analysis were performed according to their substrate utilization rate. The nitrifying species *Nitrososmonas* was tested for the oxidation of ammonium nitrogen (NH_4-N) to nitrite nitrogen (NO_2-N) and *Nitrobacter* for nitrite (NO_2-N) to nitrate (NO_3-N) in their specific liquid growth media. The conversation rates of ammonium to nitrite varied from 20-40% per 48 hrs at 5, 10, and 15 mM by Nitrosomonas. However, it increased up to 71% at 20 mM of ammonium (Fig. 9A). While, *Nitrosomonas* strain isolated from activated sludge also showed maximum rate of nitrification (85.8%) at 25 mM concentration after 72 hrs (Fig. 9B). This revealed that the time to degrade substrate depends on the concentration of feed i.e. higher the concentration, longer the time is required for degradation.

These observations clearly indicate the newly enriched *Nitrosomonas* sp. has the capability to tolerate against high ammonia load. These findings also correspond to various previous reports, that *Nitrosomonas* europia/eutropha is primarily found in the environment with high concentration of NH_3 (Bollmann and Laanbroek, 2001; Koops and Pommererning-Roser, 2002).

Correspondingly, maximum nitratification was verified by calculating increase in nitrates level in the specific growth medium (*Nitrobacter* medium B). Investigations revealed significant decline in the concentration of NO_2-N with increased levels of NO_3-N in all media amended with different concentrations of Na_2NO_2 (5, 10, 15, 20 and 25mM) while incubated with enriched strains of *Nitrobacters* sp. This showed maximum degradability (73%) at 20mM concentration NO2-N after 72hrs (Fig. 9C). While, in case of *Nitrobacters* sp. isolated from activated sludge, there was observed a thorough linear increase in nitratification rate at 5 and 10 mM concentration. However, an exponential increase in concentration of NO3-N (77.88 and 77.98%) was found in media having 20 and 25mM of NO3-N (Fig. 9D).

Conclusion

The performance of locally designed sequential aerobic-anaerobic digesters varied under the effect of different temperature regimes, aerobic treatment time and activated sludge inocula (Bioaugmentation). The optimal aerobic treatment of 2 days up graded the overall treatment efficiency of dual digestion system by contributing 60-70% reduction in physicochemical and microbiological parameters. Overall treatment efficiency of about 90-99.8% was achieved in the parameters like COD, BOD, Turbidity NO_2-N and NO_3-N under the combined effect of aerobic and anaerobic treatments. The existence of the enriched nitrifying bacteria was the underlying reason why the process was efficient and was further improved to about 10-20% nitrification and 60-80% reduction in other physicochemical parameters at 5°C when 4g/L activated sludge in the system (Bioaugumentation)

Thus sequential aerobic-anaerobic digestion system can be scaled up because of its low energy cost and maintenance requirements, sustainability and environmental compatibility for the treatment of domestic wastewater.

Acknowledgement

This research work is a part of a project "Small Scale Sewage Treatment and Wastewater Reuse System for Pakistan" funded by Higher Education Commission under Pak/US academic research program from 2009 to 2011.

References

Abeysinghe DH, De Silva DGV, Stahl DA., Rittmann BE. 2002. The effectiveness of bioaugmentation in nitrifying systems stressed by a washout condition and cold temperatures. Journal of Water Environment Research **74 (2)**, 187-199.

Ali I, Hadi F, Bano A. 2012. Microbial assisted phytoesxtraction of metals and growth of soy bean (*Glycine max* L. Merril) on industrial wastewater contaminated soil. Pakistan Journal of Botany **44**, 1593-1599.

Atlas RM. 2005. Handbook of media for environmental microbiology. CRS press.

Azbar N, Dokgoz FT, Keskin T, Eltem R, Korkmaz KS, Gezgin Y, Akbal Z, Oncel S, Dalay MC, Gonen C, Tutuk F. 2009. Comparative evaluation of bio-hydrogen production from cheese whey wastewater under thermophilic and mesophilic anaerobic conditions. International Journal of Green Energy **6**, 192–200.

Bergey DH, Holt GJ, Sneath PH, Krieg NR. 1994. Bergey's manual of determinative bacteriology, 9th Ed, Baltimore, USA: Lippincott Williams & Wilkins.

Blumenthal UJ, Mara DD, Peasey A, Ruiz-Palacios G. 2000. Guidelines for the microbiological quality by Packing Material and Hydraulic Loading Rate. Journal of Environmental Engineering **134**, 346–352.

Bollmann A, Laanbroek HJ. 2002. Influence of oxygen partial pressure and salinity on the community composition of ammonia-oxidizing in the Schelds estuary. Aquatic Microbial Ecology **28**, 239-247.

Borowski S, Szopa JS. 2007. Experiences with the dual digestion of municipal sewage sludge. Bioresourse Techonolology **98,** 1199–1207. http://dx.doi.org/10.1016/j.biortech/2006.05.017

Chan Y J, Chong MF, Law CL, Hassell DG. 2009. A review on anaerobic-aerobic treatment of industrial and municipal wastewater. Chemical Engineering Journal **155,** 1–18. http://dx.doi.org/10.1016/j.cej/2009.06.041

Crites RW, Middlebrooks EJ, Bastian RK. 2014. Natural wastewater treatment system. Second Edition.

Cui D, Li A, Qiu, T, Cai R, Pang LC, Wang J, Yang J, Ren NMF. 2014. Improvement of nitrification efficiency by bioaugmentation in SBRS at low temperature. Frontier Environmental Science Engineering **8,** 937-944.

Dhouib A, Ellouz M, Aloui F, Sayadi S. 2006. Effect of bioaugmentation of activated sludge with white-rot fungi on olive mill wastewater detoxification, Letters in Applied Microbiology **42,** 405–411. http://dx.doi.org/10.1111/j/1472-765X.2006.01858.x

Environmental Protection Agency US. 2000. Wastewater technology fact sheet trickling filter nitrification. Office of Water Cincinnati, Ohio, EPA Office of Water, Washington, DC, EPA 832-F-00-015.

Environmental Protection Agency US. 2004. Guidelines for Water Reuse, Development, **26**, 252. http://www.epa.gov/nrmrl/pubs/625r04108/625r04108.pdf

Florante AM, Pagasa DG, Joseph LA. 2009. Biological Nitrogen and COD Removal of Nutrient-Rich Wastewater Using Aerobic and Anaerobic Reactors. Journal of Water Resource and Protection **1**, 376-380

González-González A, Cuadros F. 2014. Effect of aerobic pretreatment on anaerobic digestion of olive mill wastewater (OMWW): An ecoefficient treatment. Food Bioproduction and Process, **95**, 339–345. http://dx.doi.org/10.1016/j.fbp/2014.10.005

Grady CPL, Daigger GT, Lim HC. 1999. Biological wastewater treatment, Hazardous Waste 1076. http://www.loc.gov/catdir/enhancements/fy0647/98037263-d.html.

Ghehi TJ, Mortezaeifar S, Gholami M, Kalantary RR, Mahvi AH. 2014. Performance evaluation of enhanced SBR in simultaneous removal of nitrogen and phosphorous. Journal of Environmental Health Science and Engineering **12**, 1–7. http://dx.doi.org/10.1186/s40201-014-0134-2.

Jianlong W, Xiangchun Q, Yi QLW, Hegemann W. 2002. Bioaugmentation as a tool to enhance the removal of refractory compound in coke plant wastewater. Process Biochemistry **38**, 777-781.

John T, Novak, Banjade S, Sudhir N, Murthy. 2011. Combined anaerobic and aerobic digestion for increased solids reduction and nitrogen removal. Water Research **45,** 618-624.

Kim HS, Gellner JW, Boltz JP, Freudenberg RG, Gunsch CK, Schuler AJ. 2010. Effects of integrated fixed film activated sludge media on activated sludge settling in biological nutrient removal systems. Water Research **44**, 1553-1561.

Kim J, Novak JT. 2011. Combined anaerobic/aerobic digestion: effect of aerobic retention time on nitrogen and solids removal. Water Environment Research **83,** 802–806.

Koops HP, Pommererning-Roser A. 2001. Distribution and ecophysiology of the nitrifying bacteria emphasizing cultured species. FEMS Microbiogy Physiology **37(1)**, 1-9.

Hosseini Koupaie E , Barrantes Leiva M , Eskicioglu C , Dutil C. 2014. Mesophilic batch anaerobic co-digestion of fruit-juice industrial waste and municipal waste sludge: Process and cost-benefit analysis. Bioresource Technology 152, 66–73. http://dx.doi.org/10.1016/j.biortech/2013.10.072

Kumar N, Novak JT, Murthy S. 2006. Effect of secondary aerobic digestion on properties of anaerobic digested biosolids. Water Environmental Federation 79th Annual Technical Exibition and Conference, Dallas, 6806- 6829.

Lahav O, Morgan BE. 2004. Titration methodologies of monitoring of anaerobic digestion in developin countries – a review. Journal of Chemical Technology and Biotechnology **79**, 1331-1341.
http://dx.doi.org/10.1002/jctb/1143.

Lettinga G, Rebac S, Zeeman G. 2001. Challenge of psychrophilic anaerobic wastewater treatment. Trends Biotechnology **19**, 363-370.

LI-Ping H, Jian-long W, Han-chang S, Yi Q. 2000. Bioaugmentation: a new strategy for removal of recalcitrant compounds in wastewater- a case study of quinolone. Journal of Environmental Sciences **12**, 22-25.

Makaya E, Hoko Z, Parawira W, Svotwa E. 2007. An assessment of the effectiveness of biological nutrient removal from wastewater: A case for Hatcliffe Sewage Treatment Works in Zimbabwe. EJEAF Chemistry **6**, 2409-2419

Martins AM, Pagilla K, Heijnen JJ, van Loosdrecht M. 2004. Filamentous bulking sludge-a critical review. Journal of Water Research **38**, 793-817.

Messenger JR, Villiers H A, Laubscher SJA, Kenmuir K, Ekama GA. 1993. Evaluation of dual digestion system: Part1: Overview of Milnerton experience. Journal of Water **19**, 185-191.

Metcalf E, Eddy H. 2004. Waste water Engineering, Treatment and reuse / Metcalf & Eddy, Inc McGraw-Hill New York.

Naz I, Saroj DP, Mumtaz S, Ali N, Ahmed S. 2014. Assessment of biological trickling filter systems with various packing materials for improved wastewater treatment. Environmental Technology. http://dx.doi.org/10.1080/09593330.2014.951400

Operating Procedures Wastewater Sampling. 2013. SESDPROC-306-R3.
E:/literature/wastewater/Wastewater-Sampling.pdf

Pant A, Mittal AK. 2007. Monitoring of pathogenicity of effluents from the UASB based sewage treatment plant. Environmental Monitoring and Assessment **133**, 43-51.

Peng YZ, Wu CY, Wang RD, Li XL. 2011. Denitrifying phosphorus removal with nitrite by a real time step feed sequencing batch reactor. Journal of Chemical Technology and Biotechnology **86,** 541-546.

Rojas J, Burke M, Chapwanya M, Doherty K, Hewitt I, Korobeinicov A, Meere M, Mc Carty S, O'Brien M, Tuoi VTN, Winstanely H, Zhelev T. 2010. Modelling of autothermal thermophilic aerobic digestion. Mathematics-in-Industry Case studies Journal **2**, 34-63.

Sakuma T, Jinsiriwanit S, Hattori T, Deshusses MA. 2008. Removal of ammonia from contaminated air a biotrickling filters: Denitrifying bioreactor combination system. Water Research **42**, 4507-4513.

Shalaby IM, Altalhy AD, Mosallam, HA. 2008. Preliminary field study of a model plant for sewage water treatment using gravel bed hydroponics method. World Applied Science Journal **4(2),** 238-243.

Shi X, Lefebvre O, Kwang Ng K, Yong Ng H. 2014. Sequential anaerobic–aerobic treatment of pharmaceutical wastewater with high salinity. Bioresource Technology **153,** 79–86.

Singh M, Srivastava RK. 2010. Sequencing batch reactor technology for biological wastewater treatment: a review. Asia-Pacific Journal of Chemical Engineering **6,** 3-13.

Standard methods for the examination of water and wastewater. 2005 20th edn, American Public Health Association/American Public Health Association., Washington, DC, USA.

Stephenson D, Stephenson T. 1992. Bioaugmentation for enhancing biological wastewater treatment. Biotechnology Advance **10,** 549-559.

Strom PF. 2006. Technologies to Remove Phosphorus from Wastewater. Rutgers University, p. 1–6.
http://www.water.rutgers.edu/Projects/trading/p-trt-lit-rev-2a.pdf.

Twafik A, Klapwijk B, El-Gohary F. Lettinga G. 2005. Potential of using rotating biological contracter for the post-treatment of anaerobically pretreated domestic wastewater. National Research Centre, Water Pollution Control Department, Cairo, Egypt.

United Nation. 2003. Waste water treatment technologies: A general review. New York, United nations. Economic and Social Commission for Western Asia.
http://igemportal.org/Resim/Wastewater%20Treatment%20Technologies_%20A%20general%20rewiev.pdf

Environmental Protection Agency US. 2000. Wastewater technology fact sheet trickling filter nitrification. Office of Water Cincinnati, Ohio, EPA Office of Water, Washington, DC, EPA 832-F-00-015.

Von Sperling M, Chernicharo CAL, Soares AME, Zerbini AM. 2005. Coliform and helminth eggs removal in a combined UASB reactor-baffled pond system in Brazil: performance evaluation and mathematical modeling. Water Science and Technology **5,** 237-242.

Warakomski A, Swope B. 1967. Evolution of ATAD/Dual Digestion and Recent Process Developments, p. 391– 407.

World Health Organization. 2006. Guidelines for the Safe Use of Wastewater Excreta and Greywater, 3.

Yenigun O, Demirel B. 2013. Ammonia inhibition in anaerobic digestion: a review. Process Biochemistry **48**, 901–911.

Zhang RH, Yang P, Pan Z, Wolf TD, Turnbull JH. 2004. Treatment of swine wastewater with biological conversion, filtration, and reverse osmosis: A laboratory study. American Society of Agriculture Engineers **47**, 243– 250.

Zupancic GD, Ros M. 2008. Aerobic and two-stage anaerobic–aerobic sludge digestion with pure oxygen and air aeration. Bioresource Technology **99**, 100–109.