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Effects of hydrocarbon degrading inoculum for carwash effluent treatment in a UASB reactor

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ABSTRACT

In the present study, car wash wastewater was treated in up flow anaerobic sludge blanket (UASB) reactor. In the first step reactor was seeded with 1.5 L of anaerobic sludge and in the second step when this reactor stopped its performance 100 mL hydrocarbon degrading culture was added and operated for further one month. Samples from service station and effluent were analyzed for chemical oxygen demand (COD), electrical conductivity (EC), total dissolved solids (TDS), biogas, oil and grease. During both process the reactor was operated at 4, 3 and 2 d hydraulic retention time (HRT). UASB reactor showed good efficiency at 4 d HRT than 2 and 3 d HRT while no change in bioaugmented UASB reactor was observed. In the first phase, the reactor showed 80% and 73% removal of COD and oil/grease respectively. The biogas production was 1.45 $m^3\,kg^{-1}$ COD removed and volatile suspended solids (VSS) was 20.69 g L^{-1}. In bioaugmented reactor, 96%, and 96.8% removal of COD and oil/grease respectively was observed. The average biogas production was 2.137 $m^3\,kg^{-1}$ COD removed and VSS was 40.5 g L^{-1}. It is concluded that bioaugmentation proved as a good practice and showed better results than sludge process alone in UASB reactor.

Keywords: UASB; Carwash wastewater; Oil and grease; Biogas; Sludge; Bioaugmentation

1. Introduction

In Pakistan car wash service stations are increasing with an increase in the growth of transportation. The effluents from car wash service stations cause air, water and soil pollution, these pollutants include oil, diesel, petrol, greases, salt, clay, and animal dung and contribute in chemical oxygen demand (COD), biological oxygen demand (BOD) and suspended solids [1]. To find low cost treatment solution for the car wash wastewater, other treatment processes like filtration and advance oxidation process are not affordable for car wash service station's owners in developing countries like Pakistan. Many researchers have used UASB reactor as low investment for the treatment of domestic wastewater with COD ranges between 400–500 mg/L [2,3].

Anaerobic treatment process is a promising tool that offers benefits as compared to the conventional aerobic treatment. It has great ability for degrading concentrated wastes, produces a little amount of sludge with less energy consumption [4]. The operation and efficiency of a UASB reactor depends upon the highly compact and flocculated sludge aggregates known as granules [5,6]. These sludge granules commonly develop in the start-up of UASB reactors due to self-immobilization of anaerobic bacterial cells [7].

In a UASB reactor anaerobic species of pure cultures or consortia are much more metabolically useful than earlier believes [8]. Under stress environmental conditions problem associated with the degradation of the organic matter

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by indigenous microbes can be overcome by bioaugmentation which results in the enhancement of process efficiency. It is appropriate in occasions where slow degradation of pollutants occurs due to insufficiency and/or slow adaptation of the native microorganisms [9].

The main objectives of the present study were to accelerate the car wash wastewater treatment by means of bioaugmentation with hydrocarbon degrading bacterial culture and to achieve energy in the form of biogas.

2. Materials and methods

2.1. Sampling of wastewater

Car wash station selected for this study was a well-known in Abbottabad, Pakistan. In this facility approximately 30 cars are washed daily which discharges 5310 L wastewater into the main waterways. Grab sampling method was used during peak hour around 11:00 am to 12:00 pm and initial wastewater quality parameters were checked (Table 1). This study was focused on two comparative approaches, in first approach, the wastewater was treated in UASB rector seeded with sludge while in the second approach, same UASB reactor was bioaugemented with anaerobic bacterial culture and the efficiency of both approaches were compared. The biogas production was measured by using water displacement method [10] with help of 60 mL syringes. A small pipe of 2 mm diameter placed inside the inverted syringe, which was placed in filled water container, and the other side of the pipe was connected with UASB. As the gas bubbles occupied the place inside the syringe, water displayed, which was measured by reading the scale on the syringe. Gas produced in $m^3\,kg^{-1}$ of COD removed was calculated by using following equation:

$$Flow\ rate = \frac{Volume(l)}{HRT(d)} \tag{1}$$

HRT = hydraulic retention time, Volume = volume of the reactor

$$Organic\ Loading\ rate = \frac{Flow\ rate\left[{}^{l}\!/_{d}\right] \times Influent\ COD\left({}^{kg}\!/_{l}\right)}{Volume(l)} \tag{2}$$

Gas produced in m^3 per kg of COD removed was calculated by dividing per day gas produced with organic loading rate [11].

Table 1
Initial wastewater quality parameters

Parameter	Concentration (mg·L^{-1})
Temperature (°C)	20
TDS	282
pH	7.5
COD	699
BOD	454
VSS	10
O/G	539

$$= \frac{{}^{gas\ produced(l)}\!/_{(d)}}{{}^{kg}\!/_{l \cdot d}} = \sqrt{l^2}\!/_{kg} = {}^{l}\!/_{kg} = 0.001m^3\!/_{kg} \tag{3}$$

2.2. UASB reactor set up

In the present study UASB reactor was made with 5 mm thick transparent acrylic material with 43.5 cm length, 11.7 cm internal diameter and 4 L capacity. This reactor had 5 valves, two on the left side used for effluent collection, one on the right side used for influent feed and one at the top of the reactor for gas collection (Fig. 1).

Sludge 1.5 L collected from a local domestic septic tank used to seed the UASB, with initial VSS values of 11.28 g L^{-1}. This sludge was screened through 0.6 mm sieve before seeding into the reactor to remove the fibers, sand, stones and big size debris. At the start, the reactor was fed with synthetic influents solution containing micro nutrients, macro nutrients, trace elements and glucose as source of food and energy for the growth of anaerobic bacterial biomass (Tables 2, 3) [12], to achieve sludge granulation.

2.3 Physicochemical analysis of wastewater treatment

The wastewater was analyzed before and after treatment for different water quality parameters according to the standard methods for water and wastewater analysis [13]. The pH and temperature of the influents and the effluent (treated sample) was checked by using digital desktop pH meter of Jenway Company (Model 520). Volatile suspended solids were measured by gravimetric method, in which 10 ml of well shaken sludge sample was taken in crucible and then kept in dry oven for 1 h at 105°C, cooled and final weight was recorded, dried sample was again placed in furnace at 550°C for 15 min. The COD was determined by closed reflux colorimetric method by using digester (HACH-LTG 082.99.40001). The readings were checked through Lovibond COD spectrophotometer set at 420 nm. GC-MS method was used to characterize the extracted petroleum oil from car wash wastewater. The extracted oil/grease (OG) during both treatments in the effluents was measured by liquid-liquid partition gravimetric method 5520 B [13].

2.4. Bioaugmentation of UASB reactor

The anaerobic bacterial culture was prepared in mineral salt media (MSM) and sterilized at 121ºC for 15 min. After sterilization, in 150 mL media 2 ml sludge from the UASB reactor was added and media was supplemented with 200 µL diesel oil for anaerobic hydrocarbon degraders [14]. The flask was sealed air tight to prevent oxygen entrance into the flask and placed in shaking incubator for 2.5 d at 30ºC to achieve the log phase of hydrocarbon degraders. Then the tubes were centrifuged for 7–10 min at 4500 rpm. The supernatants were discarded and equal volume of 0.9% saline solution was added to the tubes and mixed well. The inoculum volume was decided depending on the indigenous hydrocarbon degrader’s count and to achieve final concentration of 1×10^{10} cell/g of sludge.

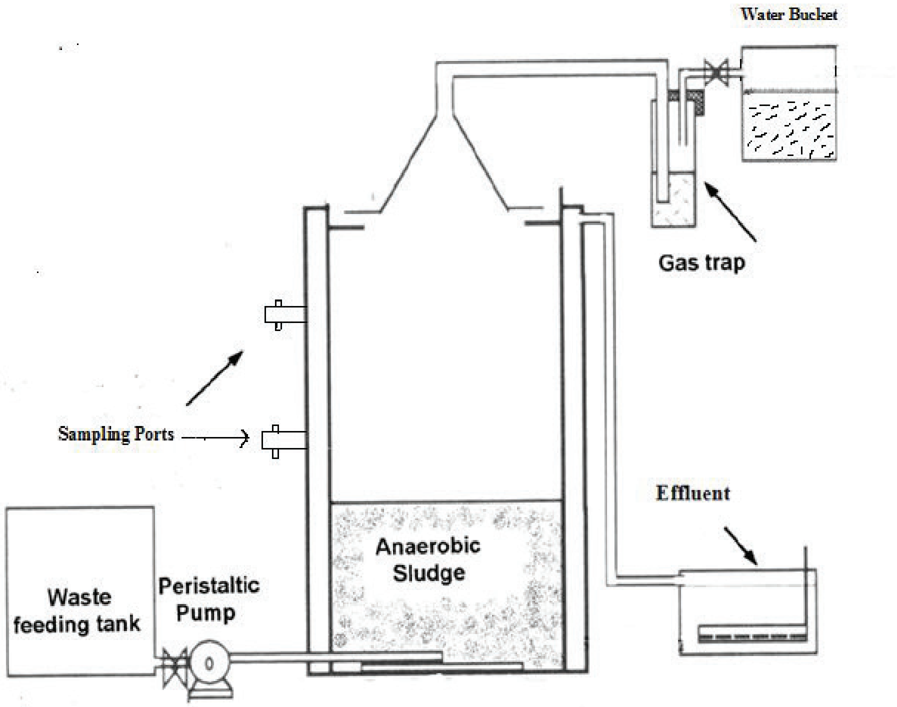

Fig. 1. Schematic diagram of reactor set up.

Table 2
Trace elements used in wastewater

Compounds	Amount ($g \cdot L^{-1}$)
Na_2EDTA	5
NaOH	11
$CaCl_2 \cdot 2H_2O$	7.34
$FeCl_2 \cdot 4H_2O$	3.58
$MnCl_2 \cdot 2H_2O$	2.5
$ZnCl_2$	1.06
$CoCl_2 \cdot 6H_2O$	0.5
$(NH_4)_6MnO_{24} \cdot 4H_2O$	0.5
$CuCl_2 \cdot 2H_2O$	0.24

Table 3
Nutrients composition of wastewater

Compounds	Amount ($g \cdot L^{-1}$)
$NaHCO_3$	1.0
Trace elements sol	1.0
$MgCl_2$	1.0
$(NH_4)_2SO_4$	0.24
Glucose	2.5

2.5. Statistical analysis

In order to check the significance difference (at $p < 0.05$) of various parameters SPSS Version 25 (IBM) was used. XLSTAT, 2017 version was used to perform descriptive statistics, correlation and principal component analysis (PCA).

3. Results and discussion

3.1. COD removal in UASB reactor

The reactor was operated with sludge process from day 1 to day 63 for wastewater treatment and 80% COD removal was observed with this process. Reactor was started with 4 days HRT and influent COD dropped from 545 mg L^{-1} to 138.3 mg L^{-1} during day 4 to 39. High fluctuation in influent COD was observed, as different types of cleaning process were performed at service station including washing of small private vehicles, public transport vehicle, trucks, poultry supply vehicle and even floor cleaning of service station. There was an increase in effluent COD values during days 45–63 when the HRT of the reactor reduced to 3 d and 2 d. This decline in COD removal may due to low retention time which was not sufficient for substrate uptake by microbes and hydrocarbon accumulation causes oily layer formation around the sludge which washed out sludge from the reactor. In another study H_2 gas production in UASB reactor from galactose was decreased when HRT was reduced [15]. After day 63 this reactor was bioaugmented with 100 mL L^{-1} hydrocarbon degrading bacterial culture (Fig. 2) and reactor operated till day 88. In the present research bioaugmentation was first time performed in UASB reactor for car wash wastewater treatment. Bioaugmentation has enhanced the biodegradation of hydrocarbon in the reactor, which can be observed by high COD removal from 1252 mg L^{-1} to 91 mg L^{-1} and 0 mg L^{-1} (below detectable limit of COD meter) at 3 and 2 d HRT respec-

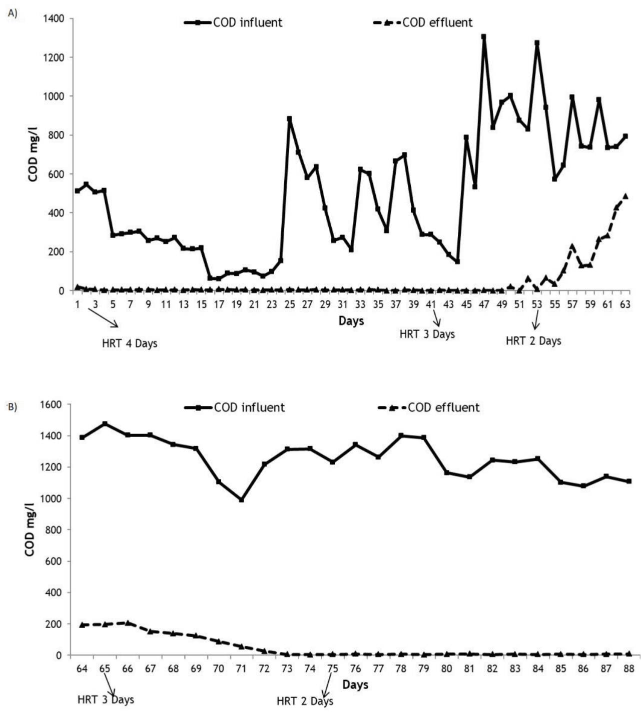

Fig. 2. COD removal in UASB reactor with A) sludge process and B) bioaugmentation at different HRT.

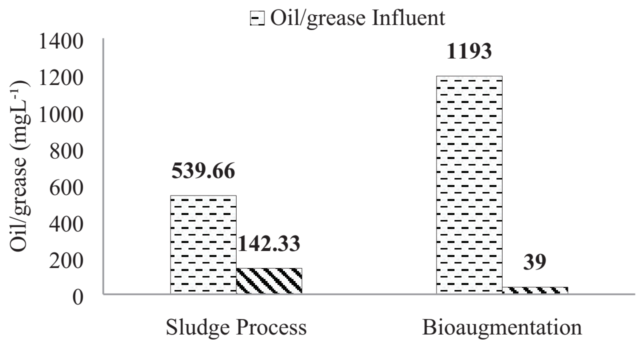

Fig. 3. Analysis of oil and grease removal between sludge and bioaugmentation processes of UASB reactor.

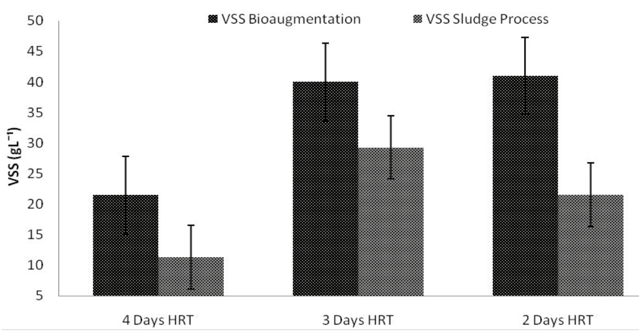

Fig. 4. VSS comparison between sludge process and bioaugmentation of UASB reactor at different HRTs.

tively. Previously, for bioremediation of contaminated environments, seeding by introduction of microorganisms has been considered a valuable tool for increasing the rate and extent of biodegradation of pollutants in the soil [16].

3.2. Oil and grease removal during sludge and bioaugmentation processes

Car wash wastewater contains heavy amount of oil and grease. In sludge process, removal of oil and grease was observed as 73.7% (from 539.66 mg L^{-1} to 142.33 mg L^{-1}) and in bioaugmented process it was 96.8% (Fig. 3). In the sludge process lower degradation was due to insufficient indigenous oil degraders population or they may not be capable of degrading the wide range of potential substrates. In this situation, bioaugmentation with autochthonous microbes was found promising low-cost technique in which indigenous bacterial consortia after enrichment are introduced to the contaminated environment because they are found to be best adopted with the contaminated environment [17].

3.3. VSS at different HRTs before and after bioaugmentation

During sludge process the VSS values were 11.28 g L^{-1}, 29.3 g L^{-1}, 21.5 g L^{-1} at 4, 3, 2 d HRTs respectively. There was a gradual increase found in VSS values and the values increased from 21.5 g L^{-1} to 40 g L^{-1} and 41 g L^{-1} at 4, 3 and 2 days HRT respectively after the bioaugmentation (Fig. 4). This increase in VSS value attributed to the higher number of hydrocarbon degrading bacteria. In most of the studies VSS is used as the indicator of the biomass growth in biological wastewater treatment system as it is degradative product of organic matter therefore directly related to the microbial activity [11].

3.4. Bioaugmented UASB reactor performance at different HRTs

The reactor reached to its full performance after bioaugmentation, as pH of the reactor remained in neutral range which favors the methanogenesis process. Single factor ANOVA at $p < 0.05$ shows, temperature and pH of effluent significantly different at 4 days HRT with 2 and 3 d HRT with and without bioaugmentation. Effluent EC and TDS were insignificantly different ($p < 0.05$) at 4 d HRT with 3 d while significantly different with 2 and 3 d HRT after bioaugmentation. COD removal was 99.7% and below detectable limit of COD meter at 3 and 2 d HRT respectively. Oil /grease removal was 95.3% and 99.2% at 3 and 2 d HRT respectively. Effluent COD has significantly difference ($p < 0.05$) on 2 and 4 d HRT with sludge treatment while 3 and 4 d HRT were significantly different after bioaugmentation. The biogas production was high (2.6 $m^3 kg^{-1}$ COD removed) at 3 d HRT (Table 4).

3.5. Biogas production with respect to organic loading rate

Organic loading rate was in the range of 0.19–0.49 kg $m^{-3}·d^{-1}$ during sludge process and 0.3–0.7 kg $m^{-3}·d^{-1}$during

Table 4
Performance of UASB reactor with bioaugmentation process against HRTs

Parameters	HRT 3 d			HRT 2 d		
	Influent	Effluent	Removal %	Influent	Effluent	Removal %
Temperature (°C)	24.9	23.9	–	26.2	25.3	–
pH	7.34	7.16	–	7.69	7.32	–
COD (mg L^{-1})	991–1451	4–205	85–99.7	1078–1400	0	100
OG (mg L^{-1})	1203	69	95.3	1183	9	99.24
VSS (mg L^{-1})	–	40	–	–	41	–
Biogas (m^3 kg^{-1} COD removed)		2.6			1,7	
EC (µS cm^{-1})	517.26	526.8	2	577.5	610	5.6
TDS (ppm)	268.7	268.8	0	295	315	6.7

bioaugmentation of UASB reactor. Biogas production was significant inversely correlated with organic loading rate, as indicated by correlation value of $r = –0.9$ ($n = 17$) and –0.8 ($n = 23$) during sludge and biaugmentation process respectively. Maximum biogas production during sludge process was 2.96 m^3 kg^{-1} COD removed on day 55 when organic loading rate was 0.19 L_{org} kg m^{-3}·d^{-1}. In case of bioaugmentation biogas production was 3.6 m^3 kg^{-1} COD removed on 72 day with 0.3 L_{org} kg m^{-3}·d^{-1} (Fig. 5). In another study by using wastewater of distilled gin production co-digested with swine wastewater in a UASB reactor, sharp decrease in the methane content of biogas from 82.9% to 65% was observed at OLR of 3.9 to 17.1 kg COD m^{-3}·d^{-1} [18].

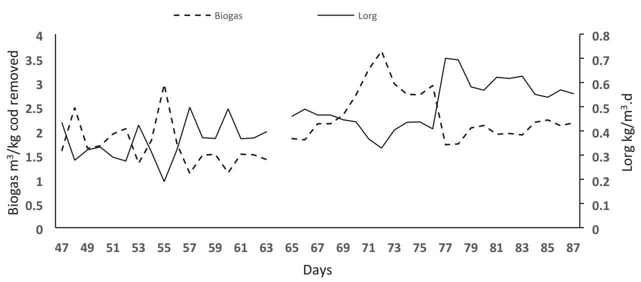

Fig. 5. Biogas production with respect to organic loading rate of the reactor.

3.6. Comparison of sludge and bioaugmentation process

The most effective treatment process was bioaugmentation with 96.4% and 96.8% COD and oil/grease removal respectively, along with an increase in average gas production (i.e. 2.137 m^3 kg^{-1} COD removed). VSS value 40.5 g L^{-1} found double as compare to the sludge process 20.69 g L^{-1} (Fig. 4), this indicates that the reactor was working properly, pH favors the methanogenesis process and due to the degradation of organic matter by inoculated hydrocarbon degraders. During sludge process the reactor showed 80.3% and 73.7% COD and oil/grease removal respectively (Table 5).

There are several factors and process conditions which can affect the efficiency of a UASB reactor. These include pH, temperature, hydraulic retention time (HRT), organic loading rate, as well as seed sludge type and sludge age [14,18]. The average range of temperature during sludge process was 19.7–20.6ºC and the gas production was also lower as compare to bioaugmentation process. This range of temperature was not favorable for methane production [19], while the range of temperature was 22–28ºC during bioaugmentation process was considerably favorable for methane production, as reported by Lettinga and Pol [20]. UASB reactor efficiency was enhanced due to rise in the digestion rate which was attributed to favorable reactor temperature and formation of well granular sludge bed [21,22].

The introduced anaerobic bacterial biomass played important role in overall reactor efficiency which was indicated by the increased in VSS values after bioaugmentation of the reactor. The introduced culture helped in sludge granulation and provided protection to other indigenous microorganisms which lead to an increased VSS values and efficient substrate removal along with higher gas production. More than 90% contents of VSS are due to active bacterial biomass, and the residual 10% are recognized to the non-biodegradable volatile solids and debris of dead cells [23]. The populations of the—specific organisms selected by the contaminant can account for 10% of the total community [24] or several orders of magnitude higher than other organisms which do not metabolize the contaminant. As selective enrichment of culture was performed in the presence of diesel in MSM therefore culture from sludge was already adapted with the hydrocarbon high concentration and tolerated the high amount of hydrocarbon in the UASB reactor.

Climenhaga and Banks also reported stable performance of reactor when anaerobic UASB reactor used for food industry wastewater was fed with constant organic loading rate (OLR) with different HRT [25]. Removal of COD symbolizes the substrate utilization during anaerobic digestion and production of biogas denotes the methanogenic activity [26]. Principal component analysis shows that COD of influent and effluent were the major components of the reactor variability, these two components contribute 41% of the total variability, with 36.5% of variability of influent COD alone (Fig. 6).

Table 5
Comparison of sludge and bioaugmentation treatments in UASB

Parameters	Sludge process			Bioaugmentation process		
	Influent	Effluent	Removal %	Influent	Effluent	Removal %
Temp	20.63	19.7	–	25.5	24.4	–
pH	7.5	7.4	–	7.4	7.3	–
COD	699.78	92.49	80	1252	45	96
OG	539.66	142.3	73.7	1193	39	96.8
VSS	–	20.69	–	–	40.5	–
Gas (m^3 kg^{-1} COD removed)	–	1.45	–	–	2.137	–
EC (µS cm^{-1})	543.8	577	61	392	423.6	80.6
TDS (ppm)	282	285	10.6	234	208	11

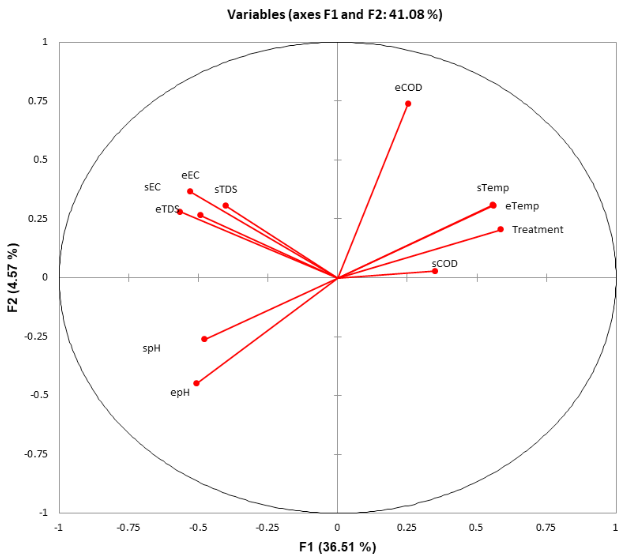

Fig. 6. PCA analysis of various operating parameters of UASB reactor showing % variability.

3.7. GCMS analysis of car wash wastewater before and after bioaugmentation

Microbial degradation of hydrocarbons is natural primary mechanism in wastewater. These microbes having appropriate metabolic activity in presence of other nutrients and pH support to degrade hydrocarbon in anaerobic condition [28]. It becomes ideal situation when number of degrading microorganism present sufficiently. The enzymes produced by oleophilic microbes are hydrocarbon-specific, though some are physiologically flexible and can degrade a wide variety of hydrocarbons [29]. The GC-MS analysis of this wastewater showed 40 different hydrocarbons compounds.

3.7.1. Anaerobic sludge treatment

During anaerobic sludge treatment, 7 compounds from alkanes group were completely degraded and not found in effluent (Table 6). Five ester compounds were identified in raw wastewater, out of them two (sulfurous acid, octadecyl 2-propyl ester andbenzenacetic acid, 2-tetradecyl ester) were degraded and not detected in the effluent. Alcoholic compounds not degraded and found after sludge treatment process including behenyl chloride, bis(2-ethylhexyl) phthalate and 1-pentacontanol. This shows that anaerobic species may not successfully remove these alcoholic compounds.

3.7.2. Bioaugmentation

Out of three alcoholic group of compounds only one compound bis(2-ethylhexyl) phthalate was degraded. In hydrocarbons degradation, nine saturated compounds were not degraded. In ester group, only two compounds were identified sulfurous acid, pentadecyl 2-propyl ester and sulfurous acid, butyl octadecyl ester. It means insufficient microbial population fail to completely degrade hydrocarbon.

In bioaugmented process five new alkane compounds were identified 1) tetratriacontane, 17-hexadecyl- 2) pentadecane, 2,6,10-trimethyl-, 3) heptadecane, 2,6,10,15-tetramethyl-,4) heptadecane, 2,6-dimethyl-and 5) tridecane, 6-cyclohexyl-. Surprisingly these compounds were absent in raw wastewater which proves that these are the byproducts of hydrocarbon biodegrdation.

4. Conclusion

The current result shows the high influence of HRT on the reactor efficiency during the sludge process. It showed good efficiency at 4 d and 3 d HRT but when the HRT decreased to 2 d there was a gradual increase observed in effluent COD. The reactor also showed comparatively low gas production, less oil/grease and COD removal and showed less VSS values.

The reactor had achieved stability after bioaugmentation with anaerobic hydrocarbon degrading culture and showed good performance at 3 and 2 d HRTs. Bioaugmentation of the reactor has increased the COD and oil/grease removal and VSS values therefore, proven as a good practice as compared to a UASB reactor with sludge process alone. The bioaugmentation in a UASB reactor needs further metagenomic study to identify the functionally active anaerobic bacterial species.

Table 6
GCMS analysis of different hydrocarbon compounds found in wastewater sample and their percentage removal after treatment with sludge and bioaugmentation

Compounds identified		Anaerobic sludge process	Bioaugmented process
Groups of compounds	MW*	Removal %	Removal %
Hydrocarbons			
Spiro[bicyclo[2.2.1]hept-5-ene-2,1'-cyclopropane]	120	NF	NF
Nonane, 4,5-dimethyl-	156	46	NF
Tetradecane	198	91	NF
Pentadecane	212	52	NF
Eicosane	282	52.3	4.2
Octadecane, 1-(ethenyloxy)-	296	NF	NF
4-methyldocosane	324	52	100
Hentriacontane	436	49.6	3.6
Tetracosane, 1-bromo-	416	84	NF
Tricosane, 2-methyl-	338	35.5	NF
Heneicosane, 11-(1-ethylpropyl)-	366	26	100
Eicosane, 7-hexyl-	366	35.2	NF
Eicosane, 9-cyclohexyl-	364	0	NF
Heptacosane, 1-chloro-	414	49.4	NF
Triacontane, 1,30-dibromo-	578	36.3	4.2
Triacontane, 1-bromo-	500	0	NF
Dotriacontane, 1,32-dibromo-	606	94	NF
Tetracosane, 11-decyl-	478	51.1	6.5
Octatriacontane, 1,38-dibromo-	690	25	NF
Tetrapentacontane, 1,54-dibromo-	914	23.9	NF
Tritetracontane	604	0	9
Docosane, 9-octyl-	604	34	NF
Tetratetracontane	618	49	NF
Tritetracontane	604	32.9	9
Tetratriacontane, 17-hexadecyl-	702	0	NF
Dodecane, 2,6,11-trimethyl-	212	92.2	NF
Hexadecane, 1-chloro-	260	84.5	13.2
Hexadecane, 3-methyl-	240	72	NF
Tridecane, 7-cyclohexyl-	266	0	NF
Hexadecane, 2,6,10,14-tetramethyl-	282	69.5	NF
Heptadecane, 2,6,10,14-tetramethyl-	296	70	5.2
Esters			
Sulfurous acid, pentadecyl 2-propyl ester	334	63	6
Sulfurous acid, octadecyl 2-propyl ester	376	0	NF
Benzeneacetic acid, 2-tetradecyl ester	332	0	100
Sulfurous acid, butyl octadecyl ester	390	62	3.2
Sulfurous acid, butyl octadecyl ester	390	22.2	NF
Behenyl chloride	344	83.3	21
Bis(2-ethylhexyl) phthalate	390	44	0
1-pentacontanol	718	95.5	14.6
Others			
Tetratriacontane, 17-hexadecyl-	702	NF	NF
Pentadecane, 2,6,10-trimethyl-	254	NF	4.6
Heptadecane, 2,6,10,15-tetramethyl-	296	NF	5.2
Heptadecane, 2,6-dimethyl-	268	NF	NF
Tridecane, 6-cyclohexyl-	266	NF	NF

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