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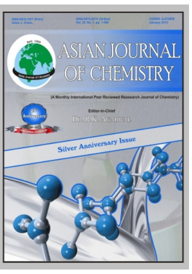

Cycle Time Minimization During Renewable Methane Production from H_2O_2 Enhanced Anaerobic Co-Digestion of Petrochemical Wastewater in Continuous Stirred Tank Reactor

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The latent of per-oxidation pretreatment to petrochemical wastewater prior to anaerobic co-digestion process was explored in continuous stirred tank reactor continually with dairy cattle and beef cattle manure. Hydrogen peroxide oxidation, elevated biodegradability index (BOD/COD) up to 35 %. While continuous stirred tank reactor operated with non-oxidation by hydrogen peroxide petrochemical waste water system was failed at organic loading of 6.5-12.99 kg COD/m^3/d due to vigorous volatile fatty acid accumulation. Inversely, oxidation by hydrogen peroxide petrochemical waste water rendered sustainable superior total COD removal at 6.03-11.7 kg COD/m^3/d organic loading with durable process stability at co-digestion period. As methane production is considered to be inhibited due to volatile fatty acid accumulation leading to instability of reactor operation during anaerobic digestion, co-digestion of oxidation by hydrogen peroxide pretreated petrochemical waste water resulted in exaggerated methane yield, followed by 98 ± 0.5 %, 95 ± 0.05 % and79 ± 0.06 % COD reduction at 9, 6 and 4 days hydraulic retention time. The concrete data revealed that prolonged hydraulic retention time and abridged cycle time caused continuous stirred tank reactor aggrandized total COD removal efficiency and methane yield.

Key Words: Anaerobic degradation, Petrochemical waste water, Hydraulic retention time, Cycle time, Oxidation by hydrogen peroxide, Methane yield.

INTRODUCTION

Investigations explored that specifically in case of recalcitrant substrates; the hydrolysis is a rate-defining stage in anaerobic digestion[1]. To perk up biochemical degradation competence of numerous refractory materials, a quantity of pretreatment might be helpful[2]. Such pretreatments can transpose various refractory organic matters to biodegradable organic ones, which can be easily degraded afterwards in biological reactor[3]. Several pretreatments have been performed including thermal hydrolysis, UV treatment, particle size diminution, ultrasonic effect, ozonization, oxidation by hydrogen peroxide *etc*. Oxidation by hydrogen peroxide (OHP), a defensible technology appeared to cover the gap between wastewater technologies treating the effluents with moderate to high refractory organic matter content and low biodegradability[4]. Additionally, this technology is opposite to treat all kinds of organic wastewater[5]. Compounds such as halogenated organics, chloramines, adsorbable organic halide, fats and greases, phenols, mono and polycyclic aromatics, naphthalene, polychlorinated biphenyls, dyes, pesticides and herbicides *etc*. are effectively treated in the industrial oxidation by hydrogen peroxide plants. Oxidation by hydrogen peroxide is principally interesting for petrochemical plants, production of dyes, fine and general chemical manufacturing plants, plastics, rubbers and similar industries, in addition to other low biodegradable wastewater, like landfill leachate and wastewater form waste processing plants[6].

Anaerobic co-digestion, a convincible technology, broadly applied to various waste treatments, particularly animal manure. Retaining elevated buffering capacity and ample multiplicity of nutrients essential for optimum bacterial growth, manure is an exceptional co-substrate[7]. Hence, at an apt C: N ratio and a sustainable pH and equilibrium of nutrients, necessary for enhanced methane production, might be achieved from co-digestion with manure[8]. Accordingly, food industrial waste[9], potato tuber industrial by-products[10] and energy crops with residues have already been productively treated through co-digestion technology using manure[11].

The continuous stirred tank reactor is equivalent to a closed-tank digester equipped with mixture facility[12]. Over and above basic reactor design, the filling of most anaerobic digesters are mixed to assure competent transfer of organic

TABLE-1
CONTINUOUS STIRRED TANK REACTOR OPERATION PARAMETERS AND FEEDING STRATEGY

Run	Specification	HRT (d)	CT (h)	Pretreatment	OL (kg COD/m^3/d)	OLC (kg COD/m^3/cycle)
1	H9C12	9	12	-	5.99 ± 0.1	2.99 ± 0.04
2	H6C12	6	12	-	8.98 ± 0.15	4.42 ± 0.07
3	H4C12	4	12	-	12.99 ± 0.25	6.5 ± 0.12
4	H9C12OHP	9	12	OHP	6.03 ± 0.4	3.02 ± 0.02
5	H9C24 OHP	9	24	OHP	6.03 ± 0.4	6.03 ± 0.4
6	H9C48 OHP	9	48	OHP	6.03 ± 0.4	12.06 ± 0.8
7	H6C12 OHP	6	12	OHP	8.36 ± 0.4	4.18 ± 0.2
8	H6C24 OHP	6	24	OHP	8.36 ± 0.4	8.38 ± 0.4
9	H6C48 OHP	6	48	OHP	8.36 ± 0.4	16.76 ± 0.8
10	H4C12 OHP	4	12	OHP	11.7 ± 0.59	5.85 ± 0.29
11	H4C24 OHP	4	24	OHP	11.7 ± 0.59	11.7 ± 0.59
12	H2C48 OHP	4	48	OHP	11.7 ± 0.4	23.4 ± 1.18

material for the active microbial biomass, to discharge gas bubbles trapped in the medium and to prevent sedimentation of denser particulate material[13]. Regardless of these advantages, anaerobic degradation in continuous stirred tank reactor is not practiced in petrochemical industries widely due to problems of slow reactions, which oblige longer hydraulic retention time and particularly poor process stability in conventional reactor designs[14]. Investigation during the operation flashed that those parameters had gone below recommended levels. Consequently, after increasing the organic loading rate to the level of 12 kg COD $m^{-3}day^{-1}$, the whole experimental operation had failed. The failure was countervailed by a sudden drop of pH and increasing concentration of volatile fatty acid. It is well known that these two factors are limiting to the anaerobic digestion process, especially to the sensitive methanogen group of bacteria. Thereupon, insufficient buffering control and disruption of microbial population balance between non-methanogen and methanogen to convert carbonaceous organic to CH_4, were identified to be the main reason of operational failure[15].

It has become increasingly important to minimize time length in continuous stirred tank reactor operation for economic feasibility. The main factors affecting total cycle time combines degradation kinetics, mass transfer fluxes of sub-startes to microbses and settleability of solids in the reactor as well. All these will direct about the continuous stirred tank reactor operation. Latif *et al.*[16] observed that high volatile fatty acids accumulation during excess-load conditions reactor became unstable at the end of 15 days. Longer filling time would be beneficial due to lower total volatile fatty acids concentration in reactor by slowing down acidogenesis rate, especially for rapidly acidified substrates. Nevertheless, the extracellular polymer formation in the reactor with a radical drop in COD removal efficiency in lengthy fill cycle was reported by Ratusznei *et al.*[17]. Accommodating immobilized biomass in continuous stirred tank reactor, stability could not be achieved at feeding time above 30-min in a 180-min total cycle time. Therefore, much research is still to be focused in this field indeed.

The current research was carried out with the vision to assess the effect of oxidation by hydrogen peroxide, cycle time and hydraulic retention time on the performance of continuous stirred tank reactor treating petrochemical waste water.

EXPERIMENTAL

Sample collection and characterization: A 100 L petrochemical waste water sample was collected in plastic containers at the point of discharge of petroleum refinery Tarranganu, Malaysia. Approximately 100 kg of partially digested beef cattle manure (BCM), dairy cattle manure (DCM) were collected directly from ejection of cow fatteners in a medium-scale farm in Gambang, Malaysia. The petrochemical waste water, beef cattle manure and dairy cattle manure were placed in compact ice boxes and transported from origins within 1-2 h. Then, they were aliquot into 1 kg zip-lock bags and stored at -20 ºC until used. The pH of the effluent was adjusted to 6.5, using 6 N sodium hydroxide solution. The alkalinity was adjusted to 1500-1700 mg $CaCO_3$/L using sodium bicarbonate. Supplementary nutrients like nitrogen (NH_4Cl) and phosphorous (KH_2PO_4) were added to yield a COD: N: P ratio of 250:5:1. Table-1 explains composition and characteristics of petrochemical waste water, beef cattle manure, dairy cattle manure and inoculum.

Pretreatment (oxidation by H_2O_2): Four waste water samples of equal volume (150 mL) were placed in conical flasks. Thereafter, each of the three volume of waste water was treated with 50 mL of standard volume of H_2O_2 solution of 30 % concentration and 1.0 Mm Fe^{3+}. To find the optimal dose of H_2O_2 solution for better degradation, percentage of H_2O_2 addition was gradually increased (*ie*. 0. 5%, 1 %, 1.5 %). The liquid content of effluent with H_2O_2 was agitated for 0.5 h with a mechanical device. The residual H_2O_2 was scavenged by catalase activity. The optimal H_2O_2 dosing (1 %) was predetermined by preliminary study aiming to obtain high biodegradability in terms of BOD/COD ratio. Application of 2.5-h oxidation by H_2O_2 (OHP) achieved approximately 35 % enhancement in biodegradability (from 0.51 ± 0.11 to 0.69 ± 0.05) accompanied by 20.5 % COD reduction (Table-2).

Segment 1: Role of oxidation by hydrogen peroxide pretreatment on digestibility of petrochemical wastewater: To achieve pH 7.5 a solution of 5N Na_2CO_3 was added to the non-oxidation by hydrogen peroxide petrochemical waste water. Influent pH adjustments were performed in the operation of three continuous stirred tank reactors at hydraulic retention time 9, 6 and 4 days under cycle time 12 h. All three reactors were found in stable condition for approximately 190 days where variation in removal efficiency and biogas production were less than 10 % consistently. Subsequently, reactors were fed non-oxidation by hydrogen peroxide petrochemical waste water without pH adjustment in order to check the comparison of oxidation by hydrogen peroxide and non-OHP petrochemical waste water treatments in the same operating condition. Afterwards, results revealed that pH adjustment was required for unbeaten operation. Hence, pH adjusted non-OHP petrochemical waste water was resumed according to runs 1, 2 and 3 (Table-1). Unless stable condition was achieved, reactors were run. Then, in a view to measure the effects of oxidation by hydrogen peroxide on biodegradability of petrochemical waste water, reactors were fed oxidation by hydrogen peroxide petrochemical waste water under the same hydraulic retention times according to treatments 4, 7 and 10. Due to the cycle plus input of the influent, the organic loadings (OL) operated were in a range of 6.4-13.5 kg COD/m^3/d, while the organic loading per cycle (OLC) ranged from 3.2-24 kg COD/ m^3/d. Water displacement was used to measure the biogas production.

TABLE-2
PHYSICOCHEMICAL CHARACTERISTICS OF PETROLEUM WASTEWATER

Parameter	Non-OHP	OHP	Non-OHP-pH*
BOD_5	7650 ± 130	8338 ± 90	8950 ± 98
TCOD	15000 ± 530	11911 ± 230	16650 ± 400
SCOD	7942 ± 170	8354 ± 200	10324 ± 300
SS	2200 ± 80	1892 ± 60	2970 ± 90
BOD_5/ TCOD	0.51 ± 0.15	0.69 ± 0.05	0.65 ± 0.04
pH	6.12 ± 0.04	6 ± 0.04	7 ± 0.11
Alkalinity	440 ± 48	382 ± 39	10120 ± 900

*All units are in mg/L except pH

Segment 2: Role of cycle time and hydraulic retention time on digestibility of petrochemical waste water: Oxidation by hydrogen peroxide petrochemical waste water was employed in this part. Each reactor was run at three cycle time/one hydraulic retention time. At different combinations of hydraulic retention time and cycle time, process efficiencies were evaluated in terms of organic removal and methane production. Complete fractional experimental design was undertaken with hydraulic retention time 9, 6 and 4 days and cycle time 12, 24 and 48 h (Run 4-12 in Table-1). Unless stable condition was achieved all systems were run. Data at stable condition were considered to inscribe performance parameters.

Pretreatment of manures: Thermal pre-treatments of manure are effective at increasing methane production by 20 %, reducing fibrous particle sizes[18]. This is ascribed to thermal hydrolysis. Thus, solid fraction of mixed cattle and pig manure was heated to 100-140 ºC prior to anaerobic degradation to improve methane production and volatile solids reduction according to Mladenovska *et al.*[19] procedure.

Batch test studies: 1.5 L capacity plastic bottles were selected as digesters. This set up was a modified form of compact system digester that digests small volume of manure to generate biogas. In order to measure the temperature a thermometer was inserted in each digester. The gas pressure was measured by AU tube while pH at 6.5 was maintained by a digital pH meter using 1 N H_2SO_4 and 1 N NaOH solution. The mean value of 3 readings has been finalized. The maximum methane yield was found at petrochemical waste water: beef cattle manure: dairy cattle manure (50: 25: 25) ratios (Table- 4).

TABLE-3
DAIRY CATTLE MANURE AND BEEF CATTLE MANURE MIXING RATIO AND METHANE PRODUCTION

Mixing ratio	C/N ratio	pH	Mean methane yield (mmH_{20})/day
0:100	14/1	7.55 ± 0.20	45
25:75	22/1	7.33 ± 0.20	104
50:50	27/1	7.26 ± 0.20	176
75:25	38/1	7.19 ± 0.20	54
100:0	42/1	7.05 ± 0.20	22

TABLE-4
PETROLEUM WASTEWATER (PWW), DAIRY CATTLE MANURE (DCM) AND BEEF CATTLE MANURE (BCM) MIXING RATIO AND METHANE PRODUCTION

Mixing (%)			C/N ratio	pH	Mean methane yield (mL/g)
PWW	DCM	BCM			
25	37	38	20/1	7.51 ± 0.50	84
40	30	30	26/1	7.45 ± 0.30	89
50	25	25	30/1	7.26 ± 0.20	101
60	20	20	34/1	7.19 ± 0.10	99
75	12	13	63/1	6.90 ± 0.30	93

Inoculum maturation and reactor operation: Single-stage anaerobic co-digestion of petrochemical waste water, beef cattle manure and dairy cattle manure was performed in a 4.5 L (total volume) (working volume 2.7 L) continuous stirred tank reactor was equipped with on-line temperature, pH and oxygen concentration measurement. The continuous stirred tank reactor was armed with a stirrer of 2 impellers to provide even mixing in the continuous stirred tank reactor as well, whereby the rotational speed can be adjusted between 0 and 1250 rpm. In order to provide heating up to 60 ºC for the system a heating plate attached to the fermenting flask. pH adjustments in the system were carried out with two dosing pumps connected to acidic and alkaline buffers. In a view to prevent loss of liquid from the continuous stirred tank reactor through evaporation during operation at thermophilic condition, the biogas was cooled with a condenser installed at the gas stream outlet of the continuous stirred tank reactor. Water displacement system was used to measure the biogas volume.

The continuous stirred tank reactor was started up using partially digested ratio beef cattle manure: dairy cattle manure of 1:1 as sole substrate. The reactors were operated at 37 ºC for 30 days during inoculum development. After reaching steady state the slurry was slowly replaced by at petrochemical waste water: beef cattle manure: dairy cattle manure (50: 25: 25) as substrate at a strategic organic loading rate, hydraulic retention time and cycle time for approximately 160 days. When acclimatization of the microbes was completed the performance of the continuous stirred tank reactor was monitored continuously through reactor performance parameters.

Analysis: Gas composition was determined with a Shimadzu, Japan (Class-GC 14B) gas chromatography equipped with a porapak N column and thermal conductivity detector. Helium was used as carrier gas at a flow rate of 30 mL/min. The oven, injector and detector temperatures was maintained at 70,120, 120 ºC respectively. pH, TS, VS, total

COD (TCOD), soluble COD (SCOD), BOD, TOC, volatile fatty acid, alkalinity, sludge concentration in the reactor (VSS), TKN and P both for feed and reactor effluent were regularly analyzed according to standard procedures (APHA, 1995)[20]. The volatile fatty acid concentrations (acetic acid, propionic acid, isobutyric acid, butyric acid) were analyzed using Shimadzu, Japan (Class-GC 14B) gas chromatography equipped with a flame ionization detector (FID) and Carbowax B-DA column. The oven, injector and detector temperatures was maintained at 170, 200, 200 ºC respectively. Helium was used as carrier gas at a flow rate of 50 mL/min and nitrogen was used as a makeup gas at a flow rate of 50 mL/min.

Operational and performance parameters: Operational and performance parameters constituted cycle time (tc or cycle time), organic loading per cycle (OLC), solid retention time (SRT), Px, hydraulic retention time (HRT), organic loading rate (OLR), food to microorganism ratio (F/M) and volatile fatty acid/alkalinity ratio of the reactor. The ratio of total biomass within the reactor to biomass wasted per given time is defined as solid retention time. Contaminant concentration and microbial mass and is the mass of pollutant applied to a unit mass of microbial mass per unit time (*e.g.* g COD/ g VSS day) is accommodated by F/M. The parameters were determined using the relationship has given below:

$$t_c = t_f + t_r + t_{sd} \tag{1}$$

$$\text{Organic loading per cycle (OLC)} = \frac{V_f COD_{in}}{V_s t_f} \tag{2}$$

$$\text{Solid retention time (SRT)} = \frac{V_r X}{Q_w X_r} \tag{3}$$

$$P_x = Q_w X_r \tag{4}$$

$$\text{Hydraulic retention time (HRT)} = \frac{V}{Q} \tag{5}$$

$$\text{Organic leading rate (OLR)} = \frac{COD_{in}}{HRT} \tag{6}$$

$$\text{Food to microorganism ratio } \left(\frac{F}{M}\right) = \frac{COD_{in}}{VSS * HRT} \tag{7}$$

where, t_c stands for total treatment cycle time, t_f stands for the duration of the feed period, t_r stands for the duration of the react period, t_{sd} is the duration of the settle and draw period, OLC is the organic loading per cycle. V_f is the feed volume, COD_{in} is the COD concentration in feed, V_s is the volume of sludge at beginning of the cycle, t_f is the full time, V_r is volume of reactor, X is mixed-liquor suspended solids (MLSS) with activated sludge reactor, Q_w waste sludge flow rate from the return line, X_r is the concentration of sludge in return line, Px is the net waste activated sludge produced each day, Q is the influent flow rate (L/day), V is the volume of the reactor (liters), VSS is the sludge concentration in the reactor.

RESULTS AND DISCUSSION

Oxidation by hydrogen peroxide oxidation petrochemical waste water: Oxidation by hydrogen peroxide oxidation effect was measured on oxidation by hydrogen peroxide and non-OHP petrochemical waste water physicochemical characteristics (Table-2). It can be noticed that alkalinity and pH was not affected by oxidation by hydrogen peroxide, at the same time has an obvious influence on organic constituents. Moreover, BOD_5 and SCOD enhancement was coupled with TCOD and SS diminution. Oxidation by H_2O_2 caused broken down of larger constituents in to smaller ones. The biodegradability index, BOD_5/COD was enhanced approximately by 35 % from 0.51 ± 0.15 to 0.69 ± 0.05. Parilti in 2010[21] investigated 38 % enhancement in BOD_5/COD ratio by adopting solar oxidation by hydrogen peroxide process. Babu *et al.*[22] reported 35 % and 48 % depletion in BOD_5 and COD respectively by electro-fenton process resulting in 16 % rise in BOD_5/COD ratio. According to our treatment strategy, oxidation by hydrogen peroxide time and dose were kept low intentionally to facilitate subsequent biological degradation in a view to achieve a plenty of enhancement in BOD_5/COD ratio and tiny depletion in TCOD. Fenton pretreatment of palm oil shell transforms high molecular weight fatty acids in to smaller weight fatty acids such as acetic acid, formic acid as observed by Mae *et al.*[23]. The continuous stirred tank reactors were operated for a period of 190 days with pH adjusted non-OHP petrochemical waste water, earlier than experimental runs. volatile fatty acid rapidly increased after the run of non-OHP petrochemical waste water without pH adjustment (Fig. 1). An obvious enhancement of volatile fatty acid/alkalinity ratio was observed at hydraulic retention time 4 days without much change in alkalinity concentration (Fig. 1). Consequently, relaying upon the hydraulic retention time, to < 4 within only 2 days, sharp decrease of pH from 7.00 was observed. Microbial activities inhibited and biogas production depleted at such low pH levels. In all hydraulic retention times the system was found to be failed. Under all applied loadings, in a view to get stable continuous stirred tank reactor operation pH adjustment is a must. Hence, pH adjustment was adapted to the non-OHP petrochemical waste water in runs 1, 2 and 3 (Table-1). Table-2 illustrates the effects of oxidation by hydrogen peroxide on petrochemical waste water for the similar set of samples described in 'non-OHP' and 'oxidation by hydrogen peroxide' columns. However, it can be noticed that pH adjustment had a strong influence increasing alkalinity and biodegradability of petrochemical waste water, which will be discussed afterwards.

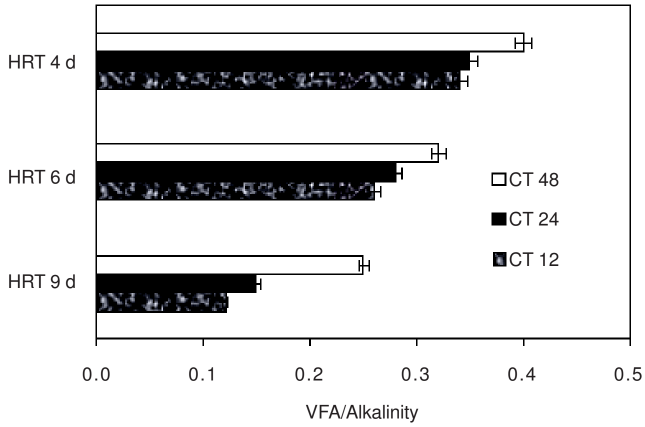

Fig. 1. Effects of co-digestion on oxidation by hydrogen peroxide pretreated petrochemical waste water in terms of volatile fatty acid/ alkalinity ratio

Run of continuous stirred tank reactor in terms of oxidation by hydrogen peroxide and non-OHP petrochemical waste water operation: The performance of continuous stirred tank reactor at cycle time 12 h receiving oxidation by hydrogen peroxide and non-OHP petrochemical waste water were compared at hydraulic retention times 9, 6 and 4 days. Then, non-OHP petrochemical waste water was undergone pH adjustment (runs 1-3 in Table-1). pHs were found to be quite steady in a range of 6.8-6.98 for all hydraulic retention time tested for the non-OHP petrochemical waste water (Fig. 2) as revealed from the results at steady condition.

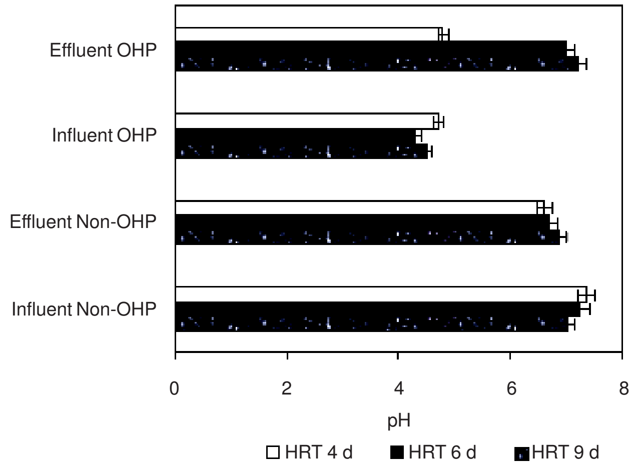

Fig. 2. pH of effluent when operating continuous stirred tank reactor with oxidation by hydrogen peroxide and non-OHP petrochemical waste water at cycle time 12 h and hydraulic retention time 9, 6 and 4 days

Due to the superfluous alkalinity added to the feed, the continuous stirred tank reactor could uphold buffer capacity in the system in check even with the amplified volatile fatty acid concentration (Fig. 3) to as high as 7000 ± 130, 6800 ± 110 and 6500 ± 100 mg/L as acetic acid at hydraulic retention times 9, 6 and 4 days respectively.

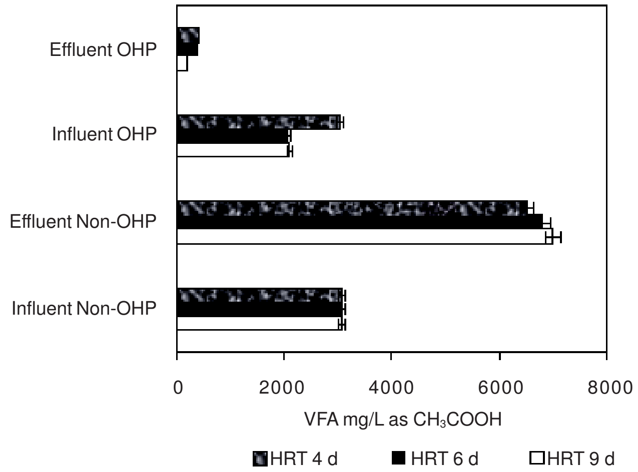

Fig. 3. Volatile fatty acid of effluent when operating continuous stirred tank reactor with oxidation by hydrogen peroxide and non-OHP petrochemical waste water at cycle time 12 h and hydraulic retention time 9, 6 and 4 days

It can be observed that volatile fatty acid/alkalinity ratio remains up to 0.4 indicating clear process stability. This is not only due to oxidation by hydrogen peroxide but also for the combined effect of co-digestion as well. Process instability, drastic drop in pH, sludge washout and inhibitory effect on methanogenesis will take place at volatile fatty acid/alkalinity ratio above 0.8 (WPCF 1987)[24]. Even though the volatile fatty acid to alkalinity ratios could be set aside within a range of 0.29-0.31, TCOD removal was found radically lower than the oxidation by hydrogen peroxide petrochemical waste water treatments. Khanal[25] reported that volatile fatty acid toxicity to the methanogenic organisms above 2000 mg/L as acetic acid is inhibitory. Mentionable that even at pH 4.10-4.59 of the oxidation by hydrogen peroxide petrochemical wastewater feed, continuous stirred tank reactor could stably run producing effluent at pH 7.2 ± 0.09 and 7 ± 0.05 and volatile fatty acid of 180 ± 40 and 350 ± 50 mg/L as acetic acid at hydraulic retention times 9 and 6 days, respectively. Due to the pre-hydrolysis of huge refractory molecules in to simple forms which are fit for biological conversions, oxidation by hydrogen peroxide pretreatment of petrochemical waste water might help the subsequent anaerobic degradation. Table-2 proves the evidence in terms of enhancement of BOD_5/COD ratio and SCOD/TCOD ratio. Nevertheless, at an abridged hydraulic retention time of 4 days, an organic loading of 12.99 kg COD/m^3d was too high for the continuous stirred tank reactor system. The recommended range at this stage is in the range of 1.2-2.4 kg COD/m^3d as reported by Metcalf and Eddy[26] and 11.3 kg COD/m^3d according to Luo *et al.*[27]. Still the continuous stirred tank reactor system was strongly stable due to subsequent co-digestion of petrochemical waste water, beef cattle manure and dairy cattle manure.

Effect of mixing proportion petrochemical wastewater and (beef cattle manure: dairy cattle manure): Table-3 illustrates the effect of different beef cattle manure and dairy cattle manure mixing ratio on methane production. The 100 % beef cattle manure produced higher methane compared to 100 % dairy cattle manure. Hashimoto[28] found 0.328 m^3/kg VS biogas production from beef cattle manure while, 0.148 m^3/kg VS in dairy cattle manure due to low biodegradable material. However, the higher biogas yield from the BMC might be due to the presence of native micro flora in it[29,30]. The maximum methane produced at mixing proportion beef cattle manure: dairy cattle manure (50:50).

This specific proportion was selected for reactor operation. The higher production from the mixtures could be due to a proper nutrient balance, which is attained by mixing as suggested by Fulford[31].

Table-4 summarizes the conclusive mean methane potentials of different petrochemical waste water, beef cattle manure and dairy cattle manure mixtures. At mixing ratio of 50: 25: 25 of petrochemical waste water, beef cattle manure and dairy cattle manure respectively, pH 7.26 ± 0.20, C:N ratio 30/1, the mean methane yield was the maximum (101 mL/g). In contrast, other mixtures provided less methane yield although C: N ratio was increased. Hence, co-digestion was carried out maintaining mixing ratio of 50: 25: 25 of petrochemical waste water, beef cattle manure and dairy cattle manure respectively, C: N ratio 30/1 subsequently.

Co-digestion effect on total COD removal and biogas yield: To authenticate the bilateral effect of organic loading rate, cycle time and hydraulic retention time on reactor performance receiving oxidation by hydrogen peroxide petrochemical waste water (Tables 5-7), total COD removal and biogas production was taken into account. At hydraulic retention time 9 and 6 days total COD removals of 98 ± 0.05 % and 95 ± 0.05 % were achieved while only 30 ± 2.5 % and 33 ± 2.2 % in case of non-OHP petrochemical waste water treatments. Lg/Lr/d (liter of gas per liter of reactor per day) and Lg/ Lww (liter of gas per liter of wastewater) were used as the units of Biogas production calculation. The degree of completeness of degradation process can strongly be indicated by COD, as any undigested material will require oxygen to complete degradation[13]. Radical enhancement in biogas production and methane yield were achieved in oxidation by hydrogen peroxide treatments (Table-5), which is compliant with the amplified total COD removal efficiency. Elevated rate of volatile fatty acids degradation was attained by the capability to uphold low volatile fatty acid concentration in the oxidation by hydrogen peroxide petrochemical waste water treatments (Fig. 3). Considerably minor total COD removal along with the accumulation of volatile fatty acid was found in case of non-OHP petrochemical wastewater treatments, as seen in Fig. 4. As the dissemination of each species of fatty acids produced were dissimilar in oxidation by hydrogen peroxide and non-OHP petrochemical wastewater, fatty acids assimilation was not executed. Long chain fatty acids such as oleic acid, a C_{18} with one double bond, in petrochemical waste water might cause time-consuming degradation.

TABLE-5
CSTR BIOREACTOR PERFORMANCE OPERATING WITH OHP AND NON-OHP PWW AT CT 12 h AND HRT 9, 6 AND 4 DAYS

Run	HRT (d)	Parameter			
		$L_g/L_r/d$	L_g/L_{ww}	CH_4 fraction (%)	CH_4yield (L_{CH4}/gT $COD_{removed}$)
Non-OHP	9	0.26 ± 0.02	2.67 ± 0.25	23.47	0.03 ± 0.002
	6	0.43 ± 0.02	3 ± 0.14	19.6	0.03 ± 0.003
	4	0.53 ± 0.02	2.68 ± 0.11	16.45	0.02 ± 0.004
OHP	9	2.75 ± 0.10	27.51 ± 1.09	67.8	0.433 ± 0.02
	6	3.8 ± 0.04	26.21 ± 0.22	60	0.44 ± 0.03
	4	0.55 ± 0.04	2.75 ± 0.1	38.60	0.097 ± 0.02

*Values are the mean ± S.D. of 3 dimensions

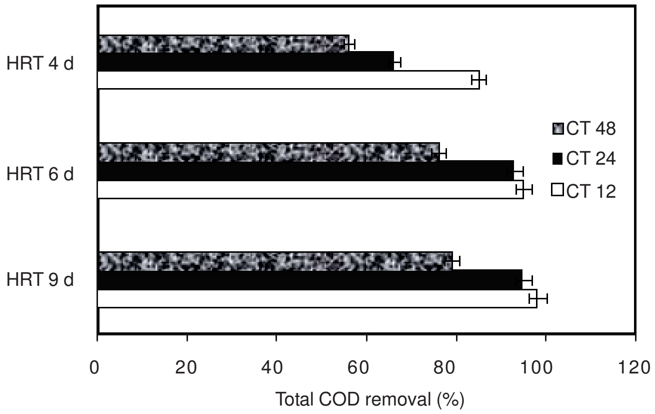

Fig. 4. Effects of co-digestion on oxidation by hydrogen peroxide pretreated petrochemical waste water in terms of total COD removal

Inhibitory effect of unsaturated long-chain fatty acid to acetogens and methanogens were widely reported[32]. Oxidation by hydrogen peroxide could facilitate anaerobic degradation increasing hydrolysis. Bulky molecule of long-chain fatty acids could be broken down into smaller ones.

Allocation of volatile fatty acid species generated from acidogenesis stage might have also been shifted to the small chain fatty acids because of the existing simpler molecules as the preliminary materials. A significantly superior methane composition in the formed biogas was assumed to be outcome of such simpler volatile fatty acid species intermediates. As petrochemical waste water accommodates phenolic compounds[33,14], their toxicity to microbial consortium could also has been the reason for inadequate degradation. oxidation by hydrogen peroxide could reportedly destroy more than 75 % of polyphenols, facilitating the successive anaerobic digester to operate at elevated loading[3]. As the generation and accumulation of volatile fatty acids still developed in our non-ozonated POME treatments, the phenolic inhibition definitely affected sensitive methanogens at a greater extent compared to acidogens. Noticeable that nearly similar methane yields have been achieved at 9 and 6 days hydraulic retention time. Short hydraulic retention time will cause hydraulic overload which will prevent the methane-forming bacteria from reproducing fast enough to avoid washout of alkalinity. Moreover, the short hydraulic retention time will reduce the contact time between substrate and microorganisms[16]. While treating petrochemical waste

TABLE-6
CSTR BIOREACTOR PERFORMANCE WITH OHP PWW AND CO-DIGESTION PROPORTION PWW: DCM: BCM (50:25:25) AT STABLE CONDITION IN TERMS OF PH, VFA, ALKALINITY AND VFA/ALKALINITY

HRT (d)	CT (h)	Parameters				
		pH	VFA	Alkalinity	VFA/alkalinity	Total COD removal (%)
9	12	7.2 ± 0.09	180 ± 40	1500 ± 90	0.12 ± 0.01	98 ± 0.05
	24	7.5 ± 0.05	230 ± 50	1533 ± 90	0.15 ± 0.01	96 ± 0.05
	48	4.58 ± 0.34	280 ± 40	1120 ± 40	0.25 ± 0.01	85 ± 1.00
6	12	7 ± 0.05	350 ± 50	1346 ± 40	0.26 ± 0.01	95 ± 0.05
	24	6.96 ± 0.07	345 ± 40	1232 ± 40	0.28 ± 0.02	93 ± 0.04
	48	4.80 ± 0.06	390 ± 40	1218 ± 40	0.32 ± 0.02	66 ± 0.06
4	12	4.78 ± 0.69	398 ± 40	1170 ± 40	0.34 ± 0.02	79 ± 0.06
	24	4.82 ± 0.01	410 ± 40	1171 ± 40	0.35 ± 0.02	76 ± 0.06
	48	4.81 ± 0.02	476 ± 40	1190 ± 40	0.4 ± 0.02	59 ± 0.06

*VFA and alkalinity are in mg/L as CH_3COOH, *Values are the mean ± S.D. of 3 water using anaerobic UFFR, Patel *et al.*[14] reported 0.37-0.43 m^3/kg COD/d methane yield at hydraulic retention times 3-9 days. Results showed that methane compositions in the biogas were at 69.1 % and 63.8 % at hydraulic retention time 9 and 6 days, respectively, which were higher than the non-OHP treatment. Inversely, total COD removal at 4 days hydraulic retention time in case of oxidation by hydrogen peroxide treatment was a little lesser than the non-OHP treatment.

The reason might be due to drastic drop of pH in oxidation by hydrogen peroxide petrochemical waste water reactor to 4.78 ± 0.69 compared to 6.84 ± 0.03 of the non-OHP treatment whose alkalinity was supplemented in ample supplied by pH adjustment. Oxidation by hydrogen peroxide might not help to perk up the organic havoc in this condition, but still provide an approximately 2.3-fold higher in methane content in the biogas (Table-5) and the superior methane yield. For non-OHP petrochemical waste water treatments, methane yields among hydraulic retention time 9, 6 and 4 days were not appreciably unlike and minor than that of oxidation by hydrogen peroxide petrochemical waste water. Even so, it is noticeable that at highest OLC (23.4 ± 1.18 kg COD/m^3 cycle), while F/M ratio was 0.6 g COD/g VSS d and volatile fatty acid/alkalinity ratio was 0.4, the system was stable (Table-6). Thus, reveals co digestion of petrochemical waste water with beef cattle manure and dairy cattle manure can successfully sustain sufficient buffering capacity without external addition of nutrients and buffering agents limited by the lack of nitrogen. Inversely, beef cattle manure contains high amount of ammonia, which may inhibit methanogenesis. Hence, co-digestion of petrochemical waste water with dairy cattle manure and beef cattle manure could reduce both chemical usage and operating costs for nitrogen and buffering agent supplementation for biomethanation from petrochemical waste water. Likewise, digestate from co-digestion incorporated lower amounts of ammonium nitrogen than that from digestion of petrochemical waste water alone. This might resolve the inhibitory effects of ammonia on anaerobic digestion and make a post treatment system for nitrogen removal simpler and cheaper. Thereupon, co digestion could accomplish possible more cost-effective and ecological biogas plants. This could be due to effect of co-digestion with beef cattle manure and dairy cattle manure which provides appropriate buffering capacity and optimum C/N ratio during methanogenesis[34]. Consequently, the risk of volatile fatty acid accumulation and sludge wash-out was avoided.

Co-digestion effect on continuous stirred tank reactor in terms of cycle time and hydraulic retention time: Results revealed that oxidation by hydrogen peroxide petrochemical waste water assured a gratification to the successive anaerobic degradation in excess of the non- OHP petrochemical waste water. According to our pre-designed methodology (Run 4-12), oxidation by hydrogen peroxide petrochemical waste water without pH adjustment as feed was employed. The aim was to investigate the effects of organic loading and cycle time on the performance of the continuous stirred tank reactor.

pH, volatile fatty acid, alkalinity:

Total treatment cycle time can be defined as the summation of the duration of the feed period, the duration of the react period, the duration of the settle and draw period. Prolonged cycle time indicates that a feeding frequency is abridged. Even though the general organic loading of the process operated from 6.03 to 11.7 kg COD/m^3/d, the immediate crest organic loading per cycle (OLC) was as high as 23.4 kg COD/m^3/cycle, a two fold of the supposed daily loading when ran in a cycle time 48-h regimen (Table-1). Abridged cycle time poise some benefit as the organic load was disseminated more uniformly providing the process minor and tranquil instantaneous load.

It is worth mentionable that when cycle time goes to zero, the system will move toward a continuous feed motif and no profit from batch settling will be achieved. While the continuous stirred tank reactor was run at cycle time 12 and 24 h only at hydraulic retention time 9 and 6 days, it was observed that pH could be maintained above 7. Under such conditions, organic loading (OL) and cyclic organic loading (OLC) were remained within 10 kg COD/m^3/d (Table-1). Noticeable that when a cycle time increased to 48 h, the system acquired OLC 12.06 ± 0.8 and 16.76 ± 0.8 kg COD/m^3/d at hydraulic retention time 9 and 6 days respectively. In case of hydraulic retention time 4 days, even if OLC was reduced to only 5.85 ± 0.29 kg COD/m^3/d at cycle time 12 h, the elevated ostensible organic loading at 11.7 ± 0.59 kg COD/m^3/d still revert the system to minor degradation of TCOD. Moreover, volatile fatty acids concentration also bounced to a level above 1170 ± 40 mg/L as acetic acid in react to the elevated cycle time and lower hydraulic retention time, which is inhibitory to methanogenesis.

At the identical hydraulic retention time and organic loading, longer cycle time reduced the volatile fatty acid degradation; leading to volatile fatty acid accumulation in the effluent (Table-6). Integrated these into a whole, the ultimate instantaneous load to the continuous stirred tank reactor inversely affected the TCOD removal efficiency. Table-6 illustrates, volatile fatty acid/alkalinity ratios in were evidently within 0.4, which implies stability of the anaerobic degradation. The concrete data recommend that the success of continuous stirred tank reactor operation depends upon not only the nominal organic load but also the cyclic organic load or the cycle time employed.

Total COD removal and biogas production: The determination of optimum hydraulic retention time indispensable for complete anaerobic degradation is still remains a big challenge. The cycle time affects total COD removal efficiencies also. Results revealed that total COD removal was reduced due to longer cycle time (Table-6). When cycle time was extended from 12 to 48 h, a loss of approximately 14 % was resulted at hydraulic retention time 9 days. This tendency was monitored to be identical at hydraulic retention time 6 and 4 days while loss was approximately 30 and 25 % respectively (Fig. 5).

The reason may be microorganisms experienced repeated high peak organic loading due to operating at higher OLC, which enforced a superior stress to the system. Ndegwa *et al.*[35] and Oliveira *et al.*[36] reported the improved removal efficiency with longer cycle time, but our findings are not similar to them. They treated swine wastewater and surfactant containing wastewater at organic loads of 0.6-1.2 kg COD/m^3/d and 0.25-0.752 kg COD/m^3/d, respectively. In our case, at higher organic loadings of the more recalcitrant wastewater, the advantage of load normalization by shortening the cycle time was expressed.

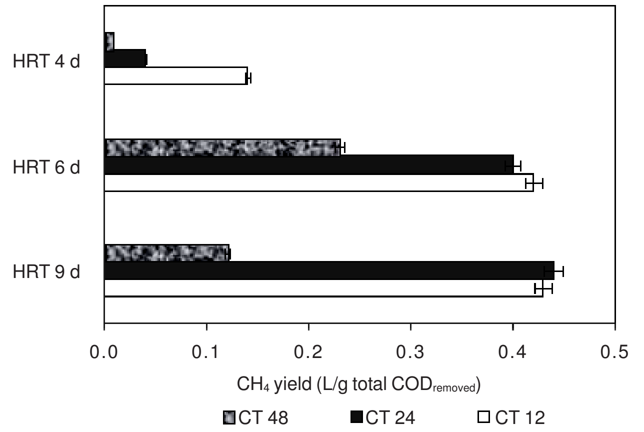

Fig. 5. Effects of co-digestion on oxidation by hydrogen peroxide pretreated petrochemical waste water in terms of CH_4 yield

Similarly, abridged hydraulic retention time resulted in a reduction in total COD removal (Table-6), since the contact time between wastewater and microorganisms in the reactor was reduced. Regardless of how the highest load was distributed, all continuous stirred tank reactor systems operated at a nominal organic load at 11.7 kg COD/m^3/d (hydraulic retention time 4 days) unsuccessful to remove the total COD efficiently. It suggests a limit of organic load for continuous stirred tank reactor under the operations tested.

Petroleum hydrocarbon, microbial cell production and MLSS: Fig. 6 explains that total petroleum hydrocarbon concentration was gradually decreased with the increase of solid retention time. Under these testing conditions, the MLSS concentrations are proportional to petroleum hydrocarbon effluent concentrations over the entire range of solid retention time values. Moreover, as solid retention time values exceed beyond 10 days, relatively small incremental changes formed for both petroleum hydrocarbons and MLSS concentrations.

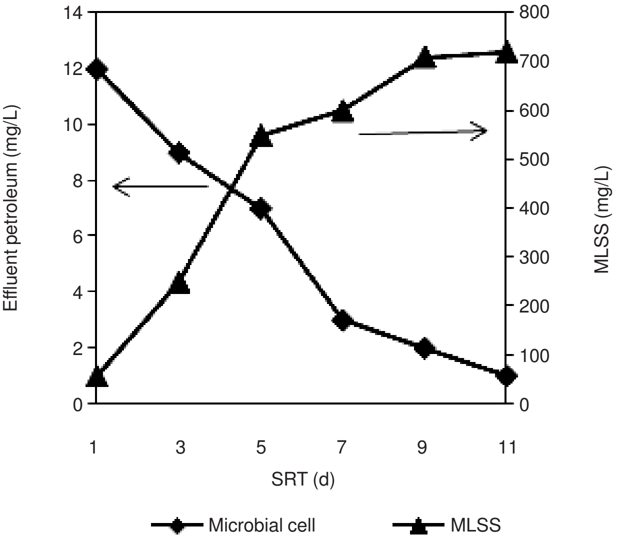

Fig. 6. Effluent petroleum concentration *vs.* solid retention time for co-digestion process

During anaerobic degradation, it is a must to measure the quantity of sludge produced per day due to its effect on process stability and disposal facilities. In order to prevent excess accumulation of microbial cell in the system, the quantity of sludge produced/day must be wasted. As anaerobic degradation incorporates cell recycle, the additional importance of cell decay and sludge production at increasing solid retention time can be seen by examining solid retention time with respect to MLSS and microbial cell production, as illustrated by Fig. 7. This plot illustrates the net synthesis in the system broadly, the mass of cells formed less those lost by decay. The highest daily production of 110 g/d was found at 3 d solid retention time. It reflects the necessary time frame and accumulative production at which a great deal of the dissolved petroleum substrate has been degraded and transformed to cells while decay is not yet a major factor. Nevertheless, sludge production started to turn down steadily through the remaining solid retention time values with the extended solid retention time values. A shift from cell synthesis to cell decay due to the increasing solid retention time values and the attainment of complete stabilization of the petroleum hydrocarbons might be suggested by the declination of the curve.

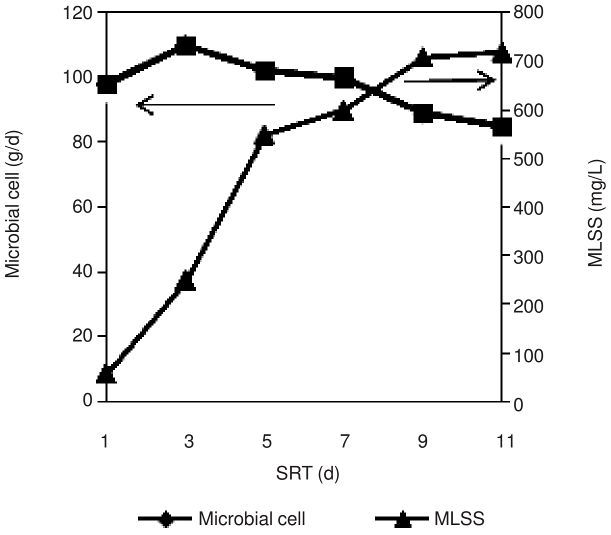

Fig. 7. Effluent microbial cell concentration *vs.* solid retention time for co-digestion process.

Conclusion

The current study demonstrates the biodegradability and methane producing potential of oxidation by hydrogen peroxide pretreated petrochemical waste water, dairy cattle manure and beef cattle manure as the effects of a mixture ratio on the performance and stability of the continuous co-digestion process. oxidation by hydrogen peroxide pretreatment of the petrochemical waste water ensured an improvement to the subsequent anaerobic digestion as its digestibility was augmented. Moreover, pre-OHP treatment to petrochemical waste water, not only an enhanced continuous stirred tank reactor performance in terms of organic removal but also superior process stability could be achieved devoid of pH adjustment. The anaerobic bio-degradability of petrochemical waste water, dairy cattle manure and beef cattle manure was more or less identical despite their combinations were largely tainted. As petrochemical waste water comprehend greater concentrations of merely degradable organic matter, an enhancement in the proportion of petrochemical waste water in co-digestion recovered the reactor performance in terms of methane yield and solids reduction. Yet, when the reactor ran with feedstock comprising an unsuitable C: N ratio, the lofty level of easily degradable fraction in feedstock might influence the reactor stability extremely. Preceding studies recommended that the defilement of the reactor at a high petrochemical waste water ratio was evoked by radical volatile fatty acid accumulation and inadequate buffering capacity. Co-digestion enhances the buffering capacity and produces a nitrogen resource for microbial synthesis propagating a balanced anaerobic degradation process. At high organic loading applied, abridged cycle time and longer hydraulic retention time were preferred as the continuous stirred tank reactor could remove total COD more effectively while producing the biogas in higher quantity and methane composition.

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