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Anaerobic co-digestion of oil refinery wastewater and chicken manure to produce biogas, and kinetic parameters determination in batch reactors

E. Mehryar[1,2], W.M. Ding[1,*], A. Hemmat[2], M. Hassan[1,3], J.H. Bi[4], H.Y. Huang[4] and J. Kafashan[5,6]

[1]College of Engineering, Nanjing Agricultural University, CN210031 Nanjing, Jiangsu, China
[2]Department of Bio-systems Engineering, College of Agriculture, Isfahan University of Technology, IR84156-83111 Isfahan, Iran
[3]US-Pakistan centre for advanced studies in Energy, National University of Science and Technology, PK44000 Islamabad, Pakistan
[4]Institute of Agricultural Resources and Environment, Jiangsu Academy of Agricultural Science, CN210014 Nanjing, Jiangsu, China
[5]Division of Mechatronics, Biostatistics and Sensors (MeBioS), KULeuven, Kasteelpark Arenberg 30, B-3001, Belgium
[6]Department of Mechanical Engineering in Agro Machinery & Mechanisation, Agricultural Engineering Research Institute, Agricultural Research Education and Extension Organisation, P.O. Box 31585-845, Karaj, Iran
[*]Correspondence: wmding@njau.edu.cn

Abstract. In order to improve the anaerobic fermentation of oil refinery wastewater (ORWW) via an appropriate nutrients pool for microbial and buffer capacity growth, a study was carried out on related anaerobic co-digestion (AcoD) with a rich organic carbon source, namely chicken manure (CM). The kinetic parameters were investigated (including cumulative biogas production, bio-methane content, retention time, and soluble chemical oxygen demand stabilisation rate) of batch AcoD experiments related to six ORWW:CM-ratio treatments (5:0, 4:1, 3:2, 2:3, 1:4, and 0:5) under mesophilic conditions. The highest soluble chemical oxygen demand removal rate was obtained for the 4:1-ratio treatment. However, the highest biogas production and bio-methane contents were achieved for the 1:4-ratio treatment. When taking into consideration the highest oil refinery wastewater portion in the AcoD mixtures and the statistical test results ($LSD_{0.05}$) for the kinetic parameters, it can be seen that the 4:1-ratio treatment provided the maximum biogas production levels.

Key words: anaerobic co-fermentation, bio-methane production, oil refinery wastewater, ammonia accumulation.

INTRODUCTION[1]

With the remarkable growth in energy demand and the large amount of wastewater production from industrial areas, today's world has been facing these two challenging problems for some time (Tommasi et al., 2012). The refining process for crude oil consumes a bulk quantity of water. As a consequence, a significant volume of wastewater is generated. The volume of petroleum refinery effluents generated during processing is between 0.4–1.6 times the volume of the crude oil processed (Diya'uddeen et al., 2011). This means that when taking into consideration the importance of crude oil products in today's word, millions of barrels of oily sludge and effluents are being generated each day. Therefore, an efficient and economical approach should be developed in order to make it possible to manage petroleum refinery effluents, and to try to introduce new areas of technology for oil refinery wastewater (ORWW) recycling. Treatments for ORWW have been the focus of a good many studies, and several areas of technology have been proposed such as absorption, microwave-assisted catalytic wet air oxidation, photo degradation, ultrasound, photo-Fenton oxidation, and biodegradation (Diya'uddeen et al., 2011; Rastegar et al., 2011; Siddique et al., 2014; Siddique et al., 2015; Choromanski et al., 2016; Haak et al., 2016; Roy et al., 2016; Wang et al., 2016).

As is known, anaerobic digestion (AD) is a biological process which is used to biodegrade organic substrates in the absence of oxygen (Zhang et al., 2016), although the petroleum derivatives can be used as an additional carbon source in the fermentation process. However, the presence of heavy metals, ammonia, sulphides, and chlorophenols has a negative effect on AD process efficiency. On the other hand, previous researchers illustrated the fact that the negative effects of petroleum products on tested bacterial groups can be decreased by means of providing proper nutrients in the bio-digester (Choromanski et al., 2016). Therefore, providing a nourished-enriched substrate to carry out co-digestion with ORWW can provide the supporting conditions for microbial adaptation.

As a reliable natural methodology, anaerobic co-digestion (AcoD) has been used extensively for the treatment of large amounts of waste materials (Siddique et al., 2015). The main achievement of AcoD for substrates with manure is that it shows an increase in bio-methane yields through the means of creating an appropriate C:N ratio, robust pH levels, and consistent nutrient contents (Abouelenien et al., 2014; Li et al., 2014).

As one of the main livestock manure products in China when it comes to biogas production, chicken manure (CM) has a higher fraction of biodegradable organic material than other livestock manures. However, due to an accumulation of ammonia and volatile fatty acids (VFAs) which lead to fermentation inhibition, CM has not been fully utilised so far (Abouelenien et al., 2014).

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[1] CM: chicken manure; ORWW: oil refinery wastewater; AcoD: anaerobic co-digestion; TS: total solid; VS: volatile solid; TOC: total organic carbon; TN: total nitrogen; C:N: carbon: nitrogen ratio; TCOD: total chemical oxygen demand; CODs: soluble chemical oxygen demand; LSD: least significant difference test; BGP: cumulative biogas production; BMP: cumulative bio-methane production; TAN: Total ammonia nitrogen; FAN: free ammonia nitrogen.

Although AD technology had been applied to treat ORWW in several recent research studies (Choromanski et al., 2016; Haak et al., 2016; Roy et al., 2016; Wang et al., 2016; Mehryar et al., 2017), very limited studies have been reported on AcoD in ORWW with agricultural and organic wastes. Three examples that can be given are reports on AcoD in petrochemical wastewater with activated manure in a continuously-stirred tank reactor (Siddique et al., 2014; Siddique et al., 2015), AcoD in ORWW with molasses in an up-flow anaerobic sludge blanket reactor (Rastegar et al., 2011), and AcoD in ORWW with sugarcane bagasse in a batch digester (Mehryar et al., 2017). The majority of past studies have focused on the effects of toxin elements in ORWW on the AD process or microbial community analysis during the fermentation period. That is an area that can be evaluated by surveying the AcoD process performance.

In this study, the areas that proved to be assets were providing an appropriate environment for a microbial community to self-adapt in petroleum derivatives, and increasing the stability and performance of the AcoD process for ORWW (as a nutrient-low substrate) with CM (as a nutrient-rich substrate and one of the most widely-available products to be generated by livestock in China). To this end, the main objective of this study was to evaluate the anaerobic co-digestion process of oil refinery wastewater and chicken manure. In order to investigate the optimum ORWW:CM ratio for treatment, four different kinetic parameters (KPs) including cumulative biogas production (BGP), bio-methane content of the BGP, retention time, and a soluble chemical oxygen demand (CODs) stabilisation rate were experimentally measured and pertinently discussed.

MATERIALS AND METHODS

Utilising substrates and seed sludge for anaerobic co-digestion processes

The ORWW was collected from the Jinlin SINOPEC oil refinery factory, Nanjing, Jiangsu Province, China. The CM was obtained from a local livestock farm located near the College of Engineering, Nanjing Agricultural University (NJAU), which itself is located in Pukou District, Nanjing, Jiangsu Province, China. The inoculum of anaerobically-digested sewage sludge was taken from a wastewater treatment plant at Yangzi Petrochemical Co Ltd, Nanjing, China. In order to remove the dissolved methane and easily-degradable organic matter from the collected sewage sludge, the activation stage set out by Xi et al. (2014) was utilised, and was further used as an inoculum (Xi et al., 2014).

Experimental set-up for bio-methane production (BMP)

Batch lab-scale experiments of simulated practical anaerobic digestions were conducted under mesophilic conditions (37.0 ± 1.0) °C (Xi et al., 2014; Hassan et al., 2016; Mehryar et al., 2017) using lab-scale anaerobic digesters fabricated from 1 L glass bottles (Fig. 1). Batch reactors were used to survey the AcoD in ORWW which had been mixed with CM, as an external organic carbon source. The working volume of each digester was kept at 800 mL, including 400 mL inoculum, which was an optimised amount of the inoculum required to co-digest oil refinery wastewater with activated manure (Siddique et al., 2015).

The contents of each digester were smoothly shaken manually for two minutes a day before biogas volume measurements were taken. In order to be able to obtain the optimum portion of ORWW and CM from the AcoD process, six different mixing ratios of ORWW:CM were designed; including 5:0, 4:1, 3:2, 2:3, 1:4, and 0:5-ratio treatments. Each treatment was conducted in triplicate. The CM with inoculum (the 0:5-ratio treatment), and ORWW with inoculum (the 5:0-ratio treatment) were also anaerobically digested as controls. To be able to estimate the background biogas production from inoculum, three digesters containing unmixed inoculum were used as blank assay digesters.

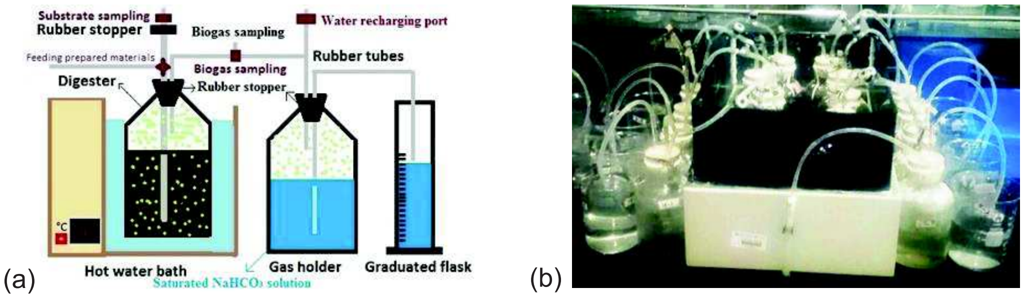

Figure 1. (a) Schematic description of batch digesters (b) manufactured batch digesters used in the anaerobic co-digestion experiments.

Analytical methods

According to the Standard Methods of the American Public Health Association, the characteristics of the substrates and inoculum, including pH, total solids (TS), volatile solids (VS), total organic carbon (TOC), total nitrogen (TN), carbon: nitrogen ratio (C:N ratio), total chemical oxygen demand (TCOD), and CODs were determined prior to the experiments (APHA, 2006). The pH value was directly measured from the liquid samples using a digital pH meter (FE20K, Mettler-Toledo, Switzerland). The total content of volatile fatty acids (TVFA) was determined via gas liquid chromatography (Model GC-2014, Shimadzu, Japan), equipped with a thermal conductivity detector (TCD), which had a column (DA, 30m × 0.53mm × µm Stabilwax) and flame ionisation detector, while injector and detector temperatures were set at 150 °C and 240 °C, respectively. The CODs stabilisation was calculated by Eq. (1) (Hassan et al., 2016; Mehryar et al., 2017):

$$\text{CODs (\%)} = \frac{\text{CODs}_\text{i}(mg \cdot L^{-1}) - \text{CODs}_\text{f}(mg \cdot L^{-1})}{\text{CODs}_\text{i}(mg \cdot L^{-1})} \times 100 \qquad (1)$$

where *i* and *f* are the initial and the final CODs (mg L^{-1}) values respectively during the AcoD process. The total ammonia nitrogen (TAN) content was also measured by means of an ammonia meter (Lianhua Tech Co, China) (Hassan et al., 2016). The free ammonia concentration figure (FAN) was calculated by Eq. (2) (Chen et al., 2014):

$$FAN\ (mg \cdot L^{-1}) = \frac{TAN(mg \cdot L^{-1}) \times K_a}{C_H \times (\frac{K_a}{C_H} + 1)} \qquad (2)$$

where *FAN* is the free ammonia nitrogen concentration (mg L^{-1}); *TAN* is the total ammonia nitrogen content (mg L^{-1}); K_a is the temperature-dependent dissociation constant (1.097×10^{-9} for the mesophilic condition); and C_H is the concentration of hydrogen ions.

During the digestion process, the volume of biogas production from each digester was recorded daily by using the liquid displacement method, for which the saturated $NaHCO_3$ solution was utilised as a displacement liquid (Xi et al., 2014). Biogas composition was determined by means of gas chromatograph (GC 9890A, Renhua, China), equipped with the TCD and a column (Φ4 mm × 1 μm, Shimadzu, Japan) and with hydrogen gas being used as a carrier gas. The oven, injector, and TCD temperature were recorded at 150 °C, 100 °C, and 120 °C respectively. The flow rate of carrier gas and the injected sample were at 50 mL min^{-1} and 0.5 mL respectively.

Statistical analysis procedure

To be able to examine the effect of various mixing ratio treatments for ORWW with CM on the KPs for the AcoD process, a completely randomised design was utilised. All of the data obtained were analysed by looking at the variance procedure (ANOVA) using the *Statistix 8.1* software (Tallahassee, Florida, USA). When the *F-test* indicated a statistical significance at the $P = 0.05$ probability level, the treatment means were separated by using the least significant difference test ($LSD_{0.05}$).

RESULTS AND DISCUSSION

Substrate characteristics

The estimated characterisations of the applied substrates are shown in Table 1. The experimental determined characteristics for ORWW were in line with previous studies (Hu et al., 2013; Siddique et al., 2014; Siddique et al., 2015). And the characteristics of the CM conferred its fermentation ability, which was in agreement with previous reports (Abouelenien et al., 2014; Li et al., 2014). As Table 1 demonstrates, the initial C:N ratios of substrates were 10.41:1, 151.40:1, and 18.37:1 for the CM, ORWW, and inoculum respectively. While the reported range for a preferred C:N ratio in previous research lies between 15–30 (Abouelenien et al., 2014), the C:N ratio for ORWW and CM are outside this range. One of the main purposes of applying the AcoD technique in several studies was the C:N ratio adjustment in the bio-digesters (Ahn et al., 2010; Zhang et al., 2013; Abouelenien et al., 2014), which is one of the objectives of this fresh research exercise. Through experimentally utilising ORWW:CM AcoD mixtures, the C:N ratio and nutrient content of the feed substrate were improved. The physicochemical characteristics of the different AcoD treatments are presented in Table 2.

Table 1. Characteristics of the applied substrates and inoculum

Characteristics	Substrates		
	CM	ORWW	Inoculum
pH	7.89 ± 0.10*	7.49 ± 0.10	7.55 ± 0.10
TS (%)	33.86 ± 2.50	1.73 ± 0.03	2.51 ± 0.10
VS (% of TS)	65.35 ± 0.60	2.69 ± 0.70	49.35 ± 1.00
TOC (g.kg of TS^{-1})	440.22 ± 3.50	30.28 ± 2.10	368.13 ± 4.30
TN (g.kg of TS^{-1})	42.27 ± 0.10	0.20 ± 0.01	20.04 ± 0.20
TCOD (mg L^{-1})	530 ± 180	18,500 ± 300	5,200 ± 100
C:N ratio	10.41:1	151.40:1	18.37:1

* mean ± standard deviation.

Table 2. Physicochemical characteristics of the different ratio treatments' substrates

Characteristics	Treatments [*]					
	5:0	4:1	3:2	2:3	1:4	0:5
pH	7.75 ± 0.00	8.17 ± 0.11	8.52 ± 0.04	8.21 ± 0.02	8.03 ± 0.02	8.12 ± 0.25[**]
CODs (g L^{-1})	1.60 ± 0.20	4.96 ± 0.15	5.20 ± 0.10	5.60 ± 0.23	10.00 ± 0.50	11.84 ± 0.40
TAN (g L^{-1})	2.26 ± 0.01	1.15 ± 0.11	1.38 ± 0.08	1.88 ± 0.07	2.17 ± 0.14	2.34 ± 0.10
TN (g kg of TS^{-1})	0.19 ± 0.08	0.63 ± 0.17	1.07 ± 0.31	1.51 ± 0.16	1.98 ± 0.24	2.24 ± 0.43
TOC (g kg of TS^{-1})	3.81 ± 0.32	8.29 ± 1.01	12.79 ± 0.1	17.28 ± 1.16	22.22 ± 3.15	25.03 ± 3.71
C:N ratio	19.5:1	13.5:1	12:1	11.5:1	11.25:1	11:1

[*] Oil refinery wastewater: chicken manure; [**] mean ± standard deviation.

BMP

The daily BMP for different treatments are shown in Fig. 2 (a) which illustrated the success of AcoD for ORWW and CM. The BMP started on the first day after fermentation onset, and kept increasing until reaching a peak on the seventh to tenth days of the digestion period for different treatments. After the main peaks had been reached there was no continuous decrease in the BMP kinetic trends for the different AcoD treatments and, indeed, one or two more small BMP peaks were experience later on. In order to be able to explain this observation, the interaction between pH and TVFA may lead to an 'inhibited steady state' representing a lower methane yield (Zhang et al., 2013). The 5:0-ratio treatment (ORWW alone) did not produce any considerable amounts of biogas, although the presence of the different hydrocarbon profiles can lead to an uncertain level of influence on petroleum products in biological systems (Choromanski et al., 2016). However, the presence of sulphur compound as a highly toxic material which existed in the form of hydrogen sulphide (H_2S) (Diya'uddeen et al., 2011) could be the main reason for the current observation. On the other hand, a high concentration of petroleum hydrocarbons (PHCs) and malnourished bacteria could be other reasons for lower levels of performance. Similarly, Choromanski et al. (2016) demonstrated that the spent engine oil had a significant effect on the BGP performance levels during the initial days of the digestion period (Choromanski et al., 2016). They reported that, thanks to the adaptation by the micro-organisms to the components in the spent engine oil, the output BGP in the second week of the digestion period was increased (Choromanski et al., 2016). Although the 0:5-ratio treatment produced the highest BMP volume and the 5:0-ratio treatment did not produce any BGP; the present study discussion focuses on the different AcoD treatments and their KPs so that the different AcoD treatments can be evaluated and the optimum conditions discovered. The results obtained clarified the fact that the KPs could be improved by means of a dilution of toxic substances and the buffering capacity development of the feedstock, which were the benefits of AcoD using livestock manures (Siddique et al., 2014).

As shown in Table 3, the 1:4-ratio treatment produced the second highest BGP volume, this being (301.27 ± 4.02) mL g VS^{-1} BGP during a period of 35 days after the 0:5-ratio treatment (with CM alone). The 2:3-ratio treatment produced biogas with a (54.20 ± 2.02)% bio-methane content and the highest quality of BGP over the course of a period of 34 days. However, the most feasible treatment was the one which consumed the higher volumes of ORWW and produced biogas with a high bio-methane content and a shorter retention time, this being the 4:1-ratio treatment. During a period of 36 days, its BGP and BMP were at (194.02 ± 3.42) mL g VS^{-1} and (85.24 ± 4.92) mL g VS^{-1}

respectively. These experimental findings showed that by increasing the ORWW ratio in AcoD mixtures, the BGP and BMP levels were more stable. In order to explain this, focusing on the estimating pH, TAN, and FAN to evaluate the ammonia accumulation levels can be more beneficial.

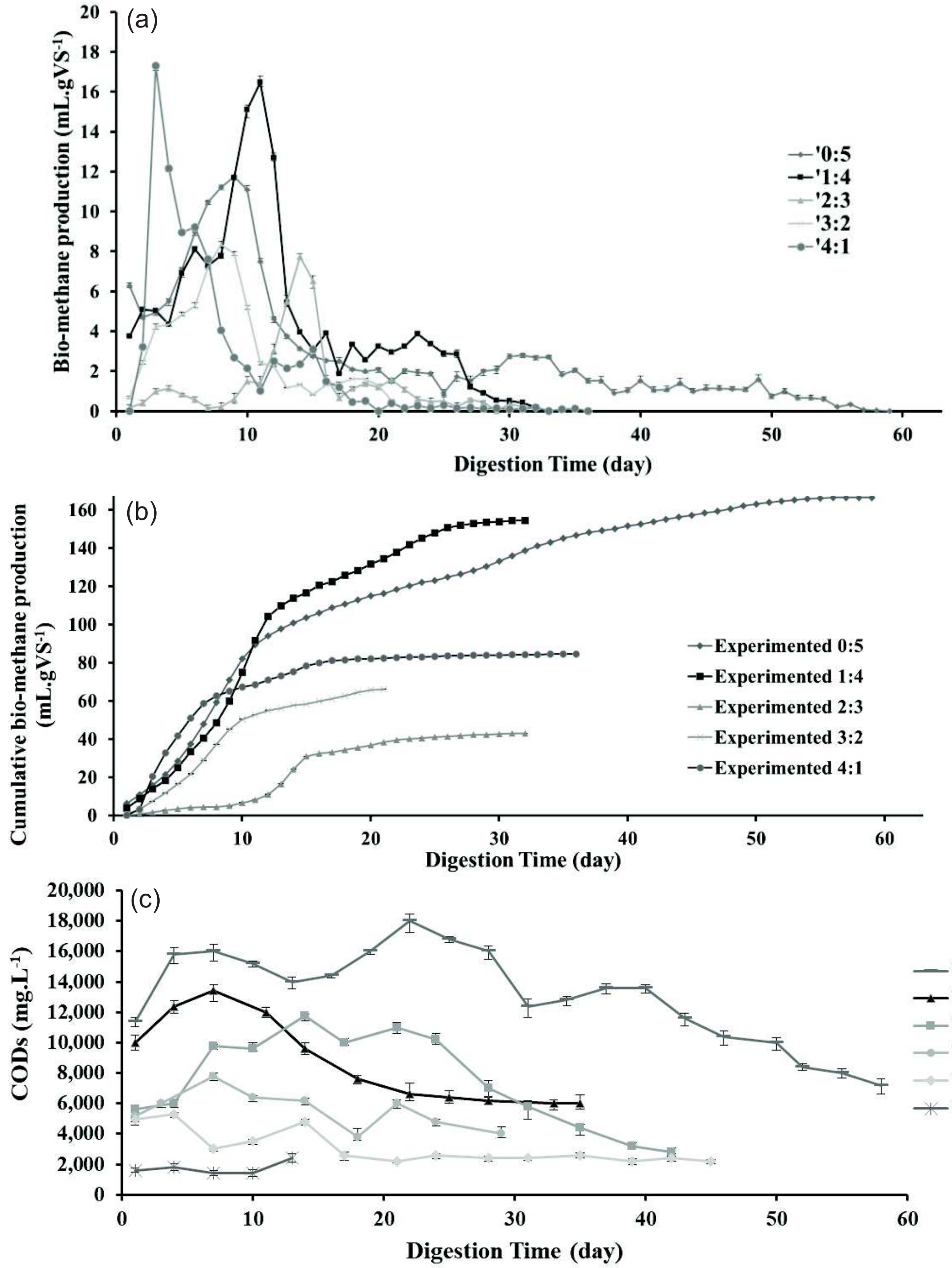

Figure 2. (a) Daily bio-methane production yields, (b) cumulative bio-methane production for the different AcoD treatments (ORWW:CM ratio), and (c) temporal CODs variation during digestion time.

CODs stabilisation profile during AcoD

The chemical oxygen demands a (COD) concentration of the wastewater, especially since CODs are one of the most important parameters when it comes to analysing the AD and AcoD process (Hassan et al., 2016), which was measured every three days in the present study. The CODs variation trends for various treatments are depicted in Fig. 2 (c). The first stage of the AD process is hydrolysis, which leads to CODs increasing in the initial days of the digestion period. During this stage, bacteria transform the particulate organic substrate into liquefied monomers and polymers, ie proteins, carbohydrates, and fats are transformed into amino acids, monosaccharides, and fatty acids respectively, which directly or indirectly takes part in the methane and carbon dioxide production process (Fang et al., 2014). By consuming the components produced, biogas will be produced and CODs will be reduced. The estimated CODs variation graphs depict the same trends. The estimated CODs variation graphs (Fig. 2 (c)) depicted the fact that, by decreasing the CM ratio in the ORWW:CM mixtures, the initial CODs were reduced, which is a factor that is related to the total levels of organic matter in the feedstock. Generally, the observed CODs variation trends in the present study showed that the results were consistent with what other investigators had previously observed (Ahn et al., 2010; Choromanski et al., 2016; Hassan et al., 2016).

To be able to evaluate the quality of the anaerobic fermentation process and discuss different effects upon it, various KPs have been mentioned by many authors such as BGP, bio-methane content, CODs stabilisation rate, ammonia removal, microbial communities' variations, VS removal, AD process retention time, TVFA removal, and so forth (Haak et al., 2016; Hassan et al., 2016; Li et al., 2016; Siddique et al., 2015). Based on the research objectives and research limitations, one or more of these parameters have been focused upon in different studies. The main parameters in previous research can be listed as including BGP, bio-methane content, CODs stabilisation rate, ammonia removal, TVFA stabilisation, and retention time (Abouelenien et al., 2014; Hassan et al., 2016; Xi et al., 2014). The results of a mean comparison ($LSD_{0.05}$ *test*) for the KPs – including the percentage of CODs stabilisation in all ORWW:CM mixtures with their AcoD process retention time, BGP and bio-methane content, and its percentage for different AcoD treatments – are represented in Table 3.

Table 3. Results of the $LSD_{0.05}$ analysis of the kinetic parameters for the different AcoD treatments

Treatments*	Parameters				
	Retention time (day)	CODs reduction (%)	Cumulative BGP (mL g VS^{-1})	Cumulative BMP (mL g VS^{-1})	Bio-methane content of BGP (%)
5:0	0.00[d]	0.00[e]	0.00[e]	0.00[e]	0.00[d]
4:1	36.67 ± 4.51[b**]	55.65 ± 3.00[a]	194.02 ± 1.08[b]	85.24 ± 1.08[c]	43.95 ± 2.34[c]
3:2	22.00 ± 1.53[c]	16.67 ± 2.00[d]	151.11 ± 3.42[c]	81.44 ± 3.42[c]	53.76 ± 4.92[a]
2:3	34.00 ± 2.65[b]	50.00 ± 2.00[b]	77.82 ± 2.59[d]	42.19 ± 2.59[d]	54.20 ± 2.02[a]
1:4	35.33 ± 1.53[b]	40.00 ± 3.00[c]	301.27 ± 4.02[a]	143.15 ± 1.97[b]	47.53 ± 1.75[b]
0:5	58.66 ± 2.52[a]	42.57 ± 1.50[c]	302.87 ± 7.41[a]	166.40 ± 5.39 [a]	54.98 ± 3.20[a]

[*] Oil refinery wastewater: chicken manure; [**] mean ± standard deviation. While different letters within the same column present the significantly different values at $P = 0.05$ probability level by $LSD_{0.05}$ *test.*

In general, a shorter retention time and higher values for the BGP, the bio-methane content, and the CODs stabilisation rate are preferred. As shown in Table 3, the shortest retention time was obtained (22.00 ± 1.53)d for the 3:2-ratio treatment, while the CODs stabilisation rate was (16.67 ± 2.00)%, as the lowest value in comparison with other AcoD treatments. The results of the $LSD_{0.05}$ analysis showed that during non-significantly different retention times, the 4:1-ratio treatment produced a lower BGP and its resultant bio-methane content than the 1:4-ratio treatment (which demonstrated the greatest KPs values). The highest CODs stabilisation rate was related to the 4:1-ratio treatment. On the other hand, the fraction of ORWW in the mixture for the 4:1-ratio treatment was four times higher than that of the 1:4-ratio treatment. Therefore the use of the 1:4-ratio treatment means treating a greater ORWW proportion in the mixture, which was one of the present study's main aims. Thanks to this discovery, the experimental results showed that the 4:1-ratio treatment is the optimum one.

pH and TVFA profiles

With regard to monitoring the AD process, pH and TVFA values can provide a proper and applicable dataset. The temporal variations in pH versus TVFA during the digestion period for the various AcoD treatments are presented in Fig. 3 (a and b). The estimated TVFA values demonstrated that the highest TVFA concentration observed was for the 0:5-ratio treatment, while the 5:0-ratio treatment had the lowest TVFA concentration levels (≤ 300 mg L^{-1}). This low performance was due to low ammonia nitrogen concentration levels (≤ 500 mg L^{-1}) which can cause a loss of biomass and a decrease in acetoclastic methanogenic activity (Rajagopal et al., 2013). Although TVFA production of the 0:5-ratio treatment produced the highest resultant figures of all AcoD treatments, its bio-methane production trends and volume during the first and second weeks of the digestion period was lower than the others. The estimated peaks of TVFA production led to a partial drop of pH levels and an increase in BMP, with these findings being similar to those of previous studies that presented evidently contrasting trends between TVFA and pH (Zhang et al., 2013; Hassan et al., 2016). The TVFA peak for the 4:1-ratio treatment was taking place sooner than for the others, which led to its higher BMP rate and volume during the first week of the digestion period (Fig. 2 (a and b)) when comparing it to other AcoD treatments. The main intermediate acid products during the AD process for organic wastes included volatile fatty acids such as acetic, propionic, butyric, and valeric acids (Zhang et al., 2014). These are not toxic – in fact they can be produced and normally consumed by bacteria in the form of nutrients. However, their rapid increase or accumulation can lead to lower pH levels and increase the acidogenic bacteria which can have an effect on methanogens (Kwietniewska & Jerzy, 2014; Liu et al., 2015). On the other hand, acetate degradation and the production of bio-methane will be reformed (Abouelenien et al., 2014) with the same trends being observed after the initial ten days of the digestion period. Then the pH values were stable to the end of the experiments. This pH stability confirms that the AcoD process reached the methanogenesis phase (Zhang et al., 2013). As an important index when it comes to showing the VFAs conversion to bio-methane, the TVFA stabilisation rate for different AcoD treatments ranged between (87.04–97.38)%. This confirmed that the successful fermentation systems were successfully carried out. The measured pH levels and TVFA results clarified the fact that AcoD in ORWW when combined with CM improved VFAs accumulation by balancing the C:N ratio and producing ammonia nitrogen, with these results being in line with those of previous studies (Abouelenien et al., 2009; Zhang et al., 2013; Choromanski et al., 2016). However, focusing on the TAN and FAN can provide a good description of BMP trends and the AcoD process.

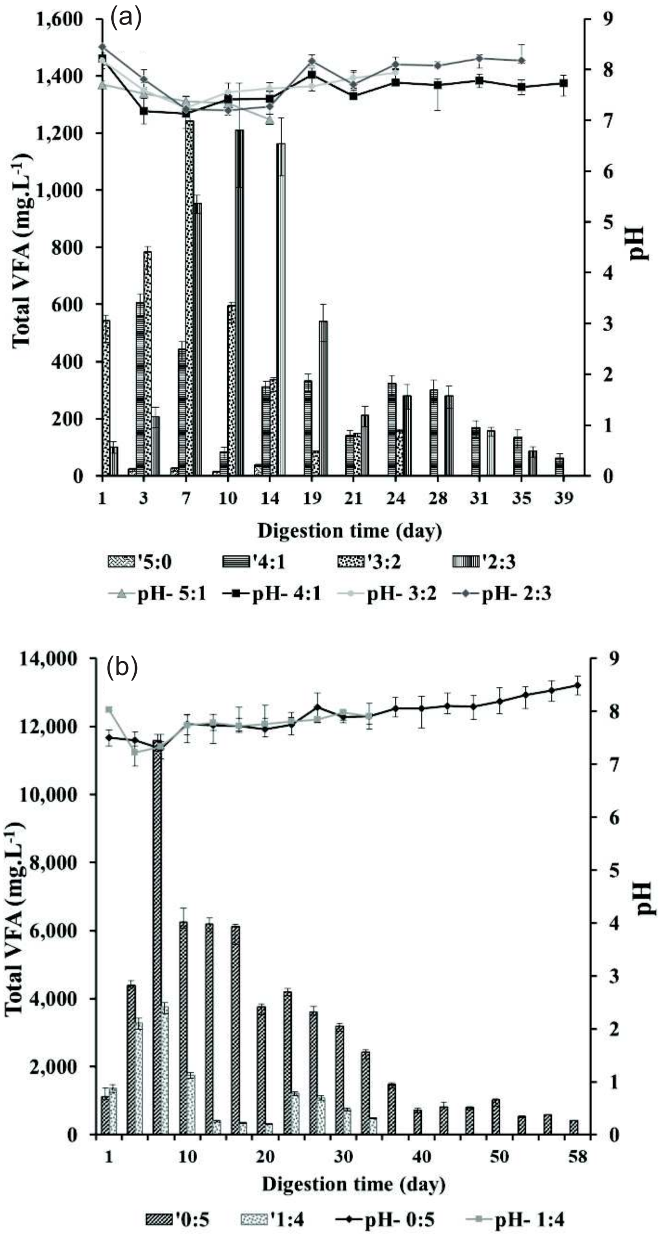

Figure 3. The temporal variations of the pH vs. TVFA during digestion period for the different AcoD treatments (ORWW:CM ratio) with TVFA values (a) less than 2,000 mg L^{-1}, and (b) more than 2,000 mg L^{-1}.

TAN and FAN

Ammonia is the end-product of the anaerobic digestion of proteins, urea, and nucleic acids (Rajagopal et al., 2013). The TAN and FAN have been considered as the effective agents in all bioprocesses, with their variation trends during the batch digestion period for different AcoD treatments being shown in Fig. 4 (a and b). The low TAN concentration levels (≤ 500 mg L^{-1}) can result in a low methane yield, a loss of biomass, and limiting aceticlastic methanogenic activity (Rajagopal et al., 2013). In contrast, a high concentration of TAN may inhibit the anaerobic process and decrease the BMP.

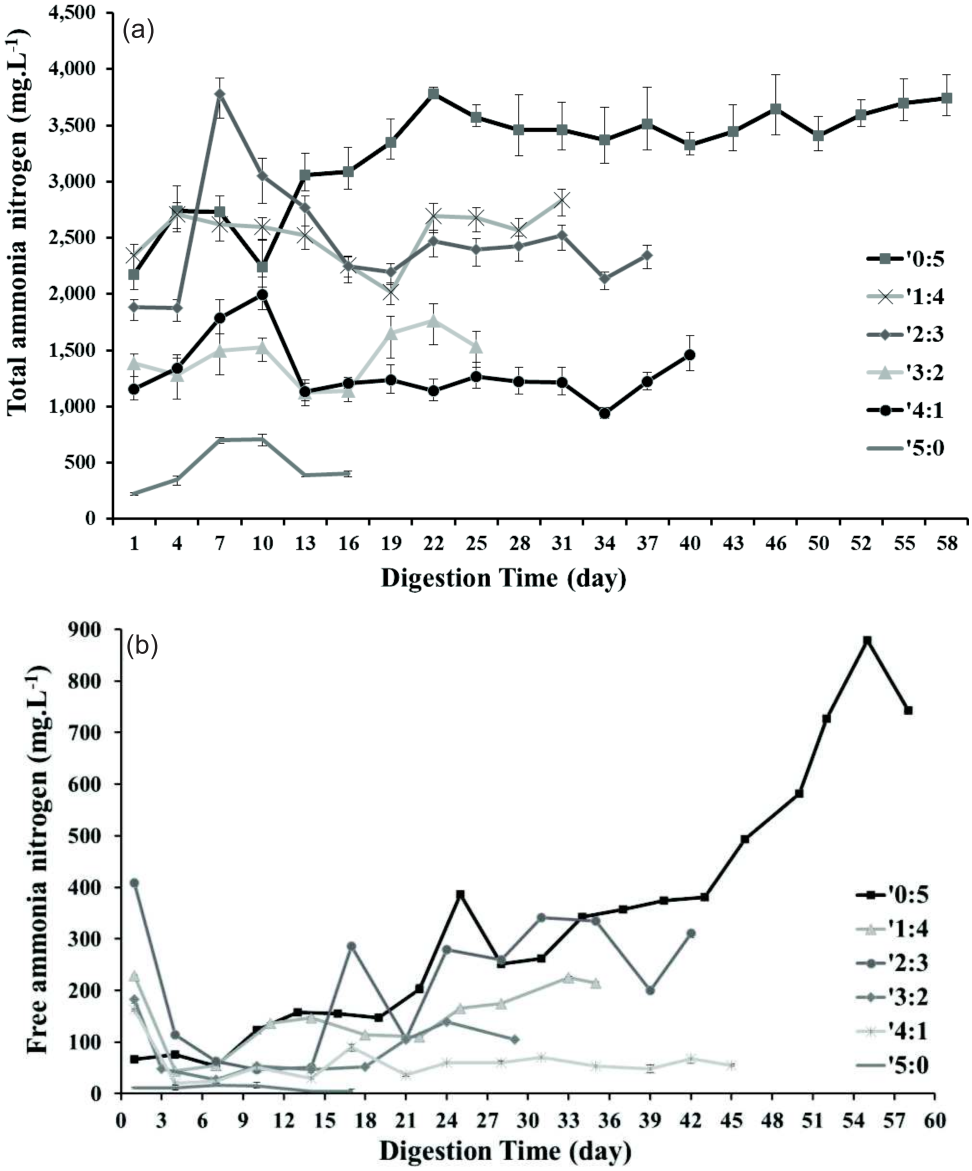

Figure 4. (a) total ammonia nitrogen (TAN), and (b) free ammonia nitrogen (FAN) variation trends for different AcoD treatments during fermentation process.

As the estimated results showed, the initial TAN content in the 5:0-ratio treatment (ORWW alone) was (407.5 ± 21.0) mg L^{-1}, which was insufficient when it came to preparing a good growth of bacterial agents for BMP. The results for AcoD treatments confirmed that when CM was added to ORWW, the TAN accumulation process was improved and the resultant values increased in comparison to that of the 5:0-ratio treatment. Generally in line with the reported previous studies, the experimental TAN data clarified the fact that bio-methane inhibition for various treatments occurred during the TAN production process at more than 2,000 mg L^{-1}. The previous studies explained the fact that AD inhibition can occur in a rather wide range between 1,700 mg L^{-1} and 14,000 mg L^{-1} for TAN, which is a function of the differences in feedstock, inoculums, operational conditions (such as temperature and pH), and acclimatisation periods (Zhang et al., 2014). In addition to TAN, the estimated results for FAN proved that higher levels of FAN concentration led to a reduction in the BMP. Just as for TAN, the wide range of between 200 mg L^{-1} and 800 mg L^{-1} was reported by previous researchers as being the critical range for FAN concentration (Chen et al., 2014). Between AcoD treatments, the 0:5 and 2:3-ratio treatments faced a high concentration of FAN (> 200 mg L^{-1}), with the resultant biogas and BMP output being inhibited. Chen et al. (2014) reported that releasing levels of FAN concentrations were the result of AD in livestock, which affected the activities of methanogenic bacteria and led to low levels of methane production (Chen et al., 2014). As may be seen in Fig. 4 (a and b), the FAN concentration levels increased along with a pH increase which expressed the fact that pH played the most effective role in FAN production and variation trends. Rajagopal et al. (2013) reported that with the pH value increasing from seven to eight, the free ammonia will increase by a factor of eight, which makes the biogas process a more sensitive one (Rajagopal et al., 2013). In this research project, the CM was a nutrient-rich substrate for bacterial growth. The results observed served to confirm the fact that decreasing FAN and TAN accumulation could improve the AcoD for ORWW:CM through the process of diluting toxic components in those treatments which had a lower CM ratio.

CONCLUSIONS

As part of the research for this study, evaluations were carried out on AcoD for ORWW with a rich organic carbon source, namely CM for bio-methane production. The significant and positive effect of AcoD for ORWW with CM in biogas production was confirmed by the experimental data that has been recorded. The data observed presented a picture of decreasing FAN and TAN accumulation due to a lower CM ratio which could improve AcoD for ORWW:CM. The improvement which was realised in terms of bio-methane production was due mainly to a dilution in toxic compounds for substrates, especially FAN. Besides this, it was also thanks to preventing ammonia accumulation in the bio-digester and ensuring the presence of proper nutrients to support microbial activities, although they had different areas of impact upon biogas production. It was the FAN values that had an impact upon biogas production rather than those from the TAN. In addition, in order to maximise ORWW usage and to produce acceptable bio-methane, a ratio treatment of 4:1 of ORWW:CM is recommended when designing the AcoD process for the continuous stirrer tank reactor (CSTR) digesters.

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