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Pathways of 3-biofules (hydrogen, ethanol and methane) production from petrochemical industry wastewater via anaerobic packed bed baffled reactor inoculated with mixed culture bacteria

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ABSTRACT

Simultaneous production of 3-biofuels (hydrogen, ethanol and methane) as by-products of the biodegradation of petrochemical wastewater containing MEG via anaerobic packed bed baffled reactor (AnPBBR), was extensively investigated. A four-chambered reactor supported by polyurethane sheets, was operated at a constant hydraulic retention time (HRT) of 36 h and different organic loading rates (OLRs) of 0.67, 1, 2 and 4 gCOD/L/d. The maximum specific H_2 and CH_4 production rates of 438.07 ± 43.02 and 237.80 ± 21.67 ml/L/d were respectively achieved at OLR of 4 gCOD/L/d. The residual bio-ethanol significantly increased from 57.15 ± 2.31 to 240.19 ± 34.69 mg/L at increasing the OLR from 0.67 to 4 gCOD/L/d, respectively. The maximum MEG biodegradability of 98% was attained at the lowest OLR. Compartment-wise profiles revealed that the maximum H_2 and ethanol production were achieved at HRT of 9 h (1st compartment), while the CH_4 production was peaked at HRTs of 27 and 36 h (last two compartments). Kinetic studies using Stover–Kincannon and completely stirred tank reactor (CSTR) in series models were successfully applied to the AnPBBR overall and compartment-to-compartment performance, respectively. The economic evaluation strongly revealed the potentials of using AnPBBR for simultaneous treatment and bio-energy production from petrochemical wastewater as compared to the classical anaerobic baffled reactor (ABR). Microbial analysis using Illumina MiSeq sequencing showed a diversity of bacterial community in AnPBBR. *Proteobacteria* (36.62%), *Firmicutes* (20.85%) and *Bacteroidetes* (3.44%) were the most dominant phyla.

1. Introduction

The global industrial development, during last decades, caused depletion of fossil fuel reserves, which states essential concerns regarding sustainability [1]. Anaerobic digestion has been well considered as a promising solution for biofuels (hydrogen, ethanol and methane) production from degradation of wastewater containing biodegradable organics [2,3]. Regarding the industrial effluents, one of the basic compounds in the petrochemical industries is ethylene with global demand about 115 million tons per year. Among the ethylene derivatives, mono-ethylene glycol (MEG) has the highest demand, which is forecast to be increased from 18.93 million tons in 2009 to 34.09 million tons in 2020 [4]. MEG is the main ingredient in the production of polyester fibers and film, polyethylene terephthalate (PET) resins, engine coolants and deicing fluids for airplanes and runway deicers [5]. Furthermore, MEG is widely used as antifreeze, as well as a dewatering agent in the natural gas industry, also with respect to the industry to generate MEG. Huge amounts of industrial wastewater containing MEG (500–7000 m^3/d) are generated in Egypt, which negatively affect on the environment and undoubtedly contaminate the surface water streams. Hence, an affordable and sustainable solution for valorization of the petrochemical wastewater is urgently required.

Nano-filtration, vacuum membrane distillation and photocatalytic and direct oxidation have been investigated for the treatment of MEG contaminated wastewaters [6–8]. However, these technologies still face economic and environmental drawbacks for the treatment of huge flow rates of industrial wastewater. Concerning the biological treatment strategies, the anaerobic processes have several advantages over the aerobic systems, such as

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the low capital and operating cost, low excess sludge production, design simplicity, easy operation, application of high organic loading rates and bio-energy production in the form of hydrogen, ethanol and methane [9–11]. Formerly, Marin et al. [12] found that the anaerobic baffled reactor (ABR) achieved a COD removal efficiency of 75% while treating aircraft de-icing fluid (ADF) wastewater containing MEG at 33 °C. A higher COD removal of 85–98% was registered at an OLR exceeding 10 gCOD/L/d in an up-flow anaerobic sludge blanket (UASB) reactor [13]. Komisar et al. [14] used a pilot-scale anaerobic fluidized bed reactor for treatment of ADF wastewater based on propylene glycol and 95% COD removal efficiency was attained at 33 °C. Meanwhile, the anaerobic degradation of petrochemical wastewater containing MEG for biofuels production at ambient temperature has not yet been investigated; whereas, it would reduce significantly the costs incurred during mesophilic and thermophilic operations [15,16]. This is of special concern since Egypt is a subtropical country with an average low-to-high ambient temperature of (9.5–17 °C) to (23–32 °C) during winter and summertime, respectively on the northern coast. Moreover, the weather changes very much farther in the interior and south where average temperatures easily soar over 40 °C during summer time.

The anaerobic metabolism of MEG follows the path of producing sequentially ethanol, hydrogen, and methane as the final by-product [17]. So far, 3-biofuels (hydrogen, ethanol and methane) production from petrochemical industry wastewater containing MEG was not extensively investigated, especially using a developed anaerobic packed bed baffled reactor (AnPBBR). These beneficial by-products increased the interest to separate and optimize their related phases to achieve the desired incentive for the manufacturers to apply such technology for simultaneous wastewater treatment and bio-energy production. Furthermore, the bio-ethanol generation pathways from disaccharides, from starches, and from lignocellulosic biomass have been well investigated [18,19]; whereas, its pathway through the anaerobic decomposition of MEG contaminated wastewaters, should be examined. From this perspective, the ABR as a series of UASB reactors was recommended. Various packing materials such as glass, peat, powdered minerals, natural zeolite and expanded clay, polystyrene sheets, recycled polyethylene, fibrous carriers and porous ceramic material have been examined in the anaerobic systems [20–23]. Among the aforementioned carrier materials, the polyurethane foam (PU) showed high applicability because of its high surface area, low cost, low toxicity, reuse possibility, flexibility for scaling up and good mechanical resistance [24,25]. To the best of our knowledge, polyurethane foam (PU) foam has been successfully investigated for treatment of a wide range of industrial effluents in anaerobic systems [24,26]. However, using PU as packing bed media, in the ABR configuration has not been yet studied, particularly for simultaneous treatment and bio-energy production from the petrochemical wastewaters.

Therefore, the main objectives of this study are to: (1) assess the performance of anaerobic packed bed baffled reactor (AnPBBR) for conversion of petrochemical wastewater containing MEG into affordable 3-bio (hydrogen, ethanol and methane) at different OLRs, at ambient temperature, with emphasis on the metabolite products, (2) investigate the pathway of the 3-bio (hydrogen, ethanol and methane) production through the AnPBBR compartments, (3) determine the appropriate kinetic coefficients to fit the AnPBBR outputs for both the overall and compartment-to-compartment performance, (4) identify the adapted microbial community responsible for the degradation of MEG and (5) highlight the economic benefits of using AnPBBR compared to the classical ABR treating petrochemical wastewater.

2. Materials and methods

2.1. Seed sludge and feed composition

The anaerobic packed bed baffled reactor (AnPBBR) was inoculated with mixed culture bacteria harvested from the thickener of sewage treatment plant situated in Al-Agamy, Alexandria, Egypt. The sludge was initially screened (<2 mm, sieve) in order to remove the large particles and debris. The pH, total solids (TS) and volatile solids (VS) of the sludge were 7.09 ± 0.17, 32.98 ± 0.93 g/L and 22.35 ± 0.66 g/L, respectively. The AnPBBR was inoculated uniformly with mixed culture bacteria with VS concentration up to 15.48 g/L. Among the petrochemical industries, the polyethylene terephthalate (PET) polyester, ethylene glycol/oxide industries and the coolant liquid discharges showed that MEG was the sole contaminant, with trace amounts of aldehydes (<0.5%) [27–30]. In accordance, these effluents were characterized as odorless and colorless liquid with a COD range from 500 to 30,000 mg/L; negligible amounts of suspended solids and less than 1–2 mg/L of TKN, NH_4-N and PO_4-P. As a result, synthetic wastewater, maintaining different OLRs based on MEG contamination, was efficiently proposed in this study in order to assess the AnPBBR performance under a controlled environment. Moreover, several studies to deal with such effluents were conducted using synthetic wastewaters supplemented with different MEG concentrations [29,31–33]. Hence, the reactor was continuously fed with synthetic petrochemical wastewater containing different concentrations of MEG ($C_2H_6O_2$). Nutrients addition, using ammonium chloride (NH_4CL) and potassium dihydrogen phosphate (KH_2PO_4), was adjusted to obtain a COD/N/P ratio of 400/7/1 [34,35]. The synthetic feed was prepared and diluted with tap water every 2 days from a preserved stock solution. The buffer and trace elements concentrations that supplemented to the feed were as follow (mg/L) [36]: $NaHCO_3$, 326; $CoC1_2{\cdot}6H_2O$, 1.2; $FeC1_3$, 5.0; $CuSO_4{\cdot}5H_2O$, 5.0; $MgSO_4{\cdot}7H_2O$, 39.0; $MnSO_4{\cdot}4H_2O$, 13.9; $CaCl_2{\cdot}2H_2O$, 36.8; $ZnC1_2$, 5.0.

2.2. Reactor configuration, operation and packing media

Fig. 1 shows a schematic diagram of the anaerobic packed bed baffled reactor (AnPBBR) used in this investigation. The working volume of the reactor was 15 L and consisted of four identical compartments (1st, 2nd, 3rd and 4th compartment). The compartments of the reactor were separated using intermediate baffles to increase the contact time between the anaerobic bacterial community and the substrate. The total dimensions of the reactor were 55 cm in length, 14.2 cm in width, and 27 cm in height. The working dimensions for each compartment are 13 × 13 × 22.5 cm. The AnPBBR was manufactured from Perspex material and was continuously supplied with synthetic petrochemical wastewater using a peristaltic pump (Masterflex – USA, Cole-Parmer Instrument Company). Polyurethane (PU) foam sheets were used as packing bed media in the reactor. The PU sheets were distributed uniformly in each compartment of the reactor and their dimensions and characteristics are shown in Table 1. The reactor was operated at organic loading rates (OLRs) of 0.67, 1, 2 and 4 gCOD/L/d by increasing the MEG concentrations (1000, 1500, 3000 and 6000 mgCOD/L, respectively). A HRT of 36 h, (9 h for each compartment) and flow rate of 10 L/d were kept constant, and the ambient temperature was varied from 15 to 30 °C.

2.3. Analytical methods

The influent and treated effluents were sampled two times a week for analysis of chemical oxygen demand (COD), total volatile

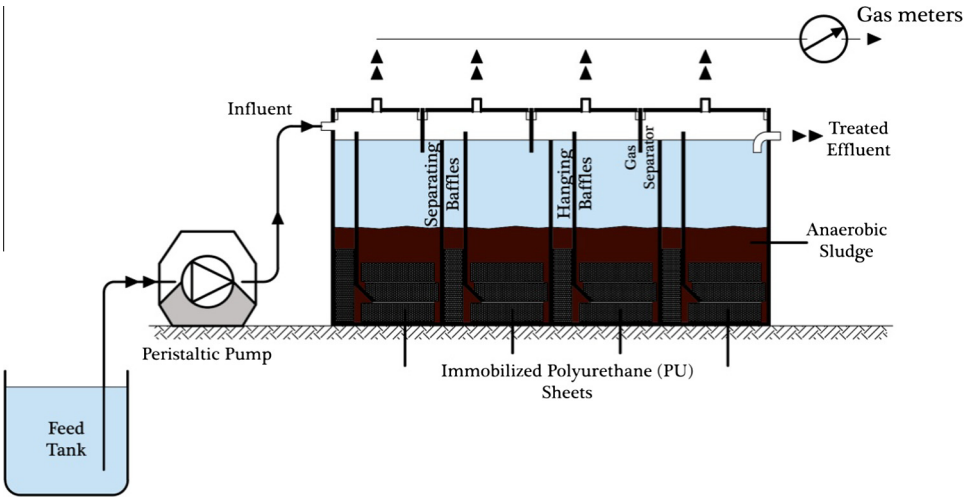

Fig. 1. Schematic diagram of the anaerobic packed bed baffled reactor (AnPBBR) for treatment of petrochemical industry wastewater.

Table 1
Dimensions and characteristics of the polyurethane (PU) sheets used for the immobilization of anaerobic bacteria in AnPBBR.

Criteria	Value
Length × width (cm × cm)	17 × 9
Thickness (cm)	2.5
Number of sheets/compartment	4
Gross volume/compartment (L)	0.3825
Working/total volume of the reactor (%)	98.77
Media	
Density (kg/m^3)	23 ± 2
Surface area (m^2/m^3)	1150.44
Total surface area in the reactor (m^2)	1.76
Voids ratio (%)	⩾97

fatty acids (TVFAs), pH and alkalinity. All analyses, including total solids (TS) and volatile solids (VS) were determined according to APHA [37]. The ethanol (EtOH) and acetate (HAc) were analyzed, twice a week as well, by using high-performance liquid chromatography (HPLC) (LC-10AD, Shimadzu, Japan). The temperature of the column oven was 40 °C and a mobile phase of 4 mM H_2SO_4 was considered with a flow rate of 0.5 ml/min for 22 min followed by 0.4 ml/min for 8 min. The volumetric gas production was measured using wet gas meter. The gas compositions (H_2, CH_4 and CO_2) were analyzed by a gas chromatography (GC-2014, Shimadzu, Japan) with a thermal conductivity detector (TCD) and a 2.0 m stainless column packed with Porapak TDS201 (60/80 mesh). The used carrier gas was helium, with a flow rate of 25 ml/min. In addition, the effluent from each compartment of the AnPBBR was also characterized in terms of bio-ethanol, volatile fatty acids (VFAs) and COD. Statistical analysis using paired student's *t*-test was considered according to a confidence level of 95% (*P*-value < 0.05) using SigmaPlot 10 software.

2.4. Calculations

The anaerobic biodegradability, methanisation and COD mass balance were calculated according to the following equations (Eqs. (1)–(3)):

$$\text{Biodegradability}\ (\%) = \text{COD removal efficiency}\ (\%) + (\text{COD}_{\text{VFA}}/\text{COD}_{\text{inf}}) * 100 \quad (1)$$

$$\text{Methanisation}\ (\%) = (\text{COD}_{\text{CH4}}/\text{COD}_{\text{inf}}) * 100 \quad (2)$$

$$\text{COD mass balance}\ (\%) = [(\text{COD}_{\text{eff}} + \text{COD}_{\text{CH4}} + \text{COD}_{\text{H2}})/\text{COD}_{\text{inf}}] * 100 \quad (3)$$

where COD_{inf} and COD_{eff} are the influent and effluent COD concentrations, respectively. The COD_{VFA}, COD_{CH4} and COD_{H2} were calculated based on 1.066 gCOD/gVFA as acetate, 4 gCOD/gCH_4 and 8 gCOD/gH_2, respectively [38].

2.5. Kinetic modeling

2.5.1. Stover–Kincannon model

The Stover–Kincannon model is the most widely applied model to assess the kinetic coefficients in the attached growth processes treating wastewater [39]. The substrate utilization rate (dS/dt) in the anaerobic process is a function of the substrate concentration and retention time. The model is derived as follow in Eq. (4) [40];

$$\frac{dS}{dt} = \frac{Q(S_i - S_e)}{V} = \frac{\mu_{max}(Q \times S_i/V)}{K_B + (Q \times S_i/V)} \quad (4)$$

where dS/dt is the substrate utilization rate (gCOD/L/h), S_i and S_e are the influent and effluent substrate concentrations (gCOD/L), respectively, μ_{max} is the maximum utilization rate constant (g/L/h), V is the reactor working volume (L), K_B is the saturation constant (g/L/h) and Q is the flow rate (L/h). Thereafter, the model equation was formed to represent a simple linear form ($Y = aX + b$), as shown in Eq. (5) [41]. Then, K_B and μ_{max} can be obtained from the equation's slope and intercept ($a = K_B/\mu_{max}$ and $b = 1/\mu_{max}$).

$$\left(\frac{dS}{dt}\right)^{-1} = \frac{V}{Q(S_i - S_e)} = \frac{K_B \times V}{\mu_{max}(Q \times S_i)} + \frac{1}{\mu_{max}} \quad (5)$$

On the other hand, the Stover–Kincannon model showed negative results when it was applied for the compartment-to-compartment performance of the stepped anaerobic baffled (SAB) reactor [33]. Accordingly, the CSTR in series model was strongly suggested to describe the anaerobic degradation process along the reactor compartments at different OLRs.

2.5.2. CSTR in series model

Previously, this model was successfully applied for both classical and hybrid anaerobic baffled reactors, treating molasses and synthetic wastewater, respectively [42,43]. CSTR in series model deals with the AnPBBR, treating petrochemical industry wastewater, as four CSTR reactors connected in series. This assumption is considered based on the mixing by the biogas generation and the movement of the flow up and down. The CSTR in series model assumes that the substrate utilization rate (K) for each compartment of the reactor follows the 1st order kinetics, as shown in Eq. (6).

$$Q * S_{n-1}(\mathrm{COD_{inf}}) = Q * S_n(\mathrm{COD_{eff}}) + K * V * S_n(\mathrm{COD_{consumed}}) \quad (6)$$

where Q is the flow rate, S_n is the COD in the compartment (n), V is the reactor volume and k is the substrate utilization rate. The hydraulic retention time (T) = V/Q and the COD fractional conversion for each compartment (n), is represented by $X_n = 1 - (S_n/S_0)$. The final model equation for the four compartments of the reactor (n = 1, 2, 3 and 4; T = 36/4 = 9 h) can be expressed as follows in Eq. (7):

$$X_n = 1 - \frac{S_n}{S_0} = \frac{1}{(1+KT)^n} \quad (7)$$

In addition, the average kinetic constant (K) can be mathematically derived from the experimental results for each compartment at various influent COD concentrations.

2.6. Microbial community analysis

2.6.1. Sample collection and extraction of genomic DNA

Sludge sample was collected from the 1st compartment of AnPBBR at OLR of 2 gCOD/L/d. The 1st compartment's consortia was selected to be analyzed as it was the most influenced one by the MEG rich environment, prior to its metabolism through the following compartments. In addition, the maximum H_2 and ethanol production were observed at the 1st compartment, which was interestingly needed to be linked to the microbial prevalence. Total genomic DNA was extracted using Fast DNA SPIN Kit (MP Biomedicals, LLC, Solon, OH) following the manufactures instructions. 2 ml of sample was centrifuged at 9600*g* for 5 min and the resulted pellets were washed twice with 1 × PBS. The above step was repeated twice to remove all the debris from the samples. Thereafter, the DNA extraction process was performed according to the manufacturer's instructions. The quality and quantity of the extracted DNA was assessed using Nanodrop Spectrophotometer (ND-1000) and Qubit fluorometer analysis and also confirmed using agarose (1%) gel electrophoresis. The samples were thereafter stored at −20 °C for further analysis.

2.6.2. Illumina MiSeq sequencing and bioinformatics analysis

The extracted DNA was analyzed using Illumina MiSeq sequencing platform using the universal bacterial fusion primer sets derived from V3–V4 region of the 16S rRNA with adapter primers attached to its 3′ end [44]. The PCR products were sequenced using illumina (Miseq) next generation sequencing platform. The sequence obtained was analyzed using CLC main bench software. The raw sequence dataset was cleaned by removing all sequences containing ambiguous nucleotides and low quality reads using based pipeline for NGS data. The generated dataset, raw paired-end reads was trimmed by their quality with a minimum quality score of 30 ($Q > 30$) and a minimum of read length of 150 bp [45]. The remaining clean reads were used for further analysis. The obtained tags were aligned and subjected to Blast against the known 16-18S rRNA gene tag database. The maximum E-value cut-off of 0.005 based on the entire available source databases was used for the analysis through genomic CLC software. Dissimilarity cut-off of 0.03 and 0.20 was used to cluster the cleaned reads into operational taxonomic units (OTUs). Taxonomic classification into domain, phylum, order, class, families and genus was performed with a set confidence threshold based on OTU diversity and reads (OTU abundance). The relative taxonomic abundance (%) of individual within the community was calculated by comparing the number of sequences assigned to a specific taxon against the number of total sequences obtained for the sample. The FASTQ file has been uploaded to the NCBI sequence Read Archive.

3. Results and discussion

3.1. Production of 3-biofuels (H_2, ethanol and CH_4) and VFAs at different OLRs

Table 2 summarizes the effect of organic loading rate (OLR) on the pathway of H_2 and CH_4 production from petrochemical industry wastewater containing MEG. Equations (Eqs. (8)–(13)) show the degradation pathway and metabolite products from anaerobic degradation of MEG [17,46]. H_2 production rate (HPR) and specific H_2 production rate (SHPR) significantly ($P < 0.05$) increased from 1.070 ± 0.10 L/d and 71.35 ± 6.66 ml H_2/L/d to 6.571 ± 0.65 L/d and 438.07 ± 43.02 ml H_2/L/d at increasing OLR from 0.67 to 4 gCOD/L/d, respectively. The CH_4 production rate (MPR) and specific CH_4 production rate (SMPR) simultaneously increased from 1.702 ± 0.14 L/d and 113.47 ± 9.16 ml CH_4/L/d to 3.567 ± 0.65 L/d and 237.80 ± 21.67 ml CH_4/L/d, respectively. These results are attributed to the increase in substrate availability to be converted into H_2 and subsequently to CH_4, as described in Eqs. (11)–(13), particularly if the organic removal rate (ORR) was steadily increased, as shown later in Fig. 4b. These results are comparable to those obtained by Marin et al. [12], who found that increasing OLR from 0.3 to 6 gCOD/L/d positively affected on the biogas production rate, from anaerobic degradation of ethylene glycol based-aircraft de-icing fluid wastewater. This was not the case for CH_4 yield at increasing OLR from 0.67 to 4 gCOD/L/d; the yield dropped from 170.20 ± 13.74 to 59.45 ± 5.42 ml $CH_4/gCOD_{add}$; moreover, the methane content significantly ($P < 0.05$) reduced by a value of 50%. Similar trends were recorded by Liu et al. [47], who observed a certain decrease of methane content from 55% to 20% at increasing OLR from 2.01 to 6.17 gCOD/L/d. At the same time, the H_2 yield was increased from 107.03 ± 9.99 to 122.23 ± 10.33 ml $H_2/gCOD_{add}$, at increasing OLR from 0.67 to 2 gCOD/L/d. The further increase in OLR up to 4 gCOD/L/d led to a slight decrease in H_2 yield to 109.52 ± 10.75 ml $H_2/gCOD_{add}$. This finding may be attributed to the effect of VFA/Alkalinity ratio on both acidogenesis and methanogenesis, which is favorable to be between 0.40 and 0.80 to have a stable anaerobic fermentation [39,48]. Therefore, the increase in VFA/Alkalinity ratio to 1.87 at OLR of 2 gCOD/L/d indicates performance instability comprised by acidification preference (increase in H_2 yield) and methanisation drop (20%). Moreover, the further increase of VFA/Alkalinity to 4.95 caused, however, a decrease in H_2 yield. Thus, it can be concluded that for higher methane production, an excess addition of $NaHCO_3$ (>0.5 g/L) is suggested as alkalinity source. $NaHCO_3$ concentration between 1 and 1.5 g/L was reported by Liu et al. [47] to keep alkalinity and pH of the system stable, during the anaerobic treatment of industrial wastewater at OLRs between 1 to 6 gCOD/L/d. In accordance, the VFA/Alkalinity ratio can efficiently express the acids formation inhibition effect on the methanogenesis, particularly when the VFAs accumulation doesn't exceed 2000 mg/L, as a minimum value obtained from different substrates [49]. Lower H_2 yield of 54.58 ml $H_2/gCOD_{add}$ at OLR of 0.67 gCOD/L/d was obtained by Elreedy and Tawfik [50], using ABR, treating MEG

Table 2
H_2 and CH_4 production at different OLRs.

Parameter	OLR, gCOD/L/d			
	0.67	1.0	2.0	4.0
Total Biogas volume (L/d)	3.67 ± 0.28	4.96 ± 0.51	8.82 ± 0.85	15.22 ± 1.62
H_2 (%)	29.16 ± 2.72	34.51 ± 3.85	41.52 ± 3.52	43.19 ± 4.24
CH_4 (%)	46.41 ± 3.74	37.76 ± 4.23	24.99 ± 2.32	23.47 ± 2.14
CO_2 (%)	22.59 ± 2.11	26.47 ± 2.86	23.23 ± 2.29	22.71 ± 2.30
HPR (L/d)	1.070 ± 0.10	1.712 ± 0.19	3.667 ± 0.31	6.571 ± 0.65
SHPR (ml H_2/L/d)	71.35 ± 6.66	114.11 ± 12.74	244.47 ± 20.67	438.07 ± 43.02
H_2 yield (ml H_2/gCOD_{add})	107.03 ± 9.99	114.11 ± 12.74	122.23 ± 10.33	109.52 ± 10.75
MPR (L/d)	1.702 ± 0.14	1.878 ± 0.21	2.203 ± 0.24	3.567 ± 0.33
SMPR (ml CH_4/L/d)	113.47 ± 9.16	125.20 ± 142	146.87 ± 13.67	237.80 ± 21.67
CH_4 yield (ml CH_4/gCOD_{add})	170.20 ± 13.74	125.20 ± 142	73.43 ± 6.83	59.45 ± 5.42
Alkalinity (mg$CaCo_3$/L)	566.52 ± 39.6	518.77 ± 34.5	391.26 ± 29.2	356.76 ± 24.4
VFA/Alkalinity	0.43	0.79	1.87	4.95
Acetate/Ethanol	3.53	3.96	5.28	5.64
Biodegradability (%)	98	94	71	85
Methanisation (%)	45	33	20	16
COD mass balance (%)	82	87	91	80

Values represent average ± standard deviation.

contaminated wastewater under similar conditions. In addition, acetate/ethanol ratio was calculated as an indicator of better acetogenic reactions as previously depicted in Eq. (11). The higher the acetate/ethanol ratio, the higher the H_2 content was observed with an improvement from 29.16 ± 2.82 to 43.19 ± 4.24% at acetate/ethanol ratios of 3.53 and 5.64, respectively.

$$C_2H_6O_2\ (\text{MEG}) \rightarrow\ C_2H_4O(\text{Acetaldehyde}) +\ H_2O; \quad (8)$$

$$C_2H_4O\ (\text{Acetaldehyde}) + H_2O \rightarrow CH_3COO^-(\text{Acetate}) + H^+ + H_2; \quad (9)$$

$$C_2H_4O\ (\text{Acetaldehyde}) + H_2 \rightarrow C_2H_6O\ (\text{Ethanol}); \quad (10)$$

$$C_2H_6O\ (\text{Ethanol}) + H_2O \rightarrow CH_3COO^-(\text{Acetate}) + H^+ + 2H_2 \quad (11)$$

$$CH_3COO^-\ (\text{Acetate}) + H_2O \rightarrow\ HCO_3^- + CH_4; \quad (12)$$

$$4H_2 + H^+ + HCO_3^- \rightarrow CH_4 + 3H_2O \quad (13)$$

The substrate biodegradability and methanisation were calculated according to Eqs. (1) and (2) in order to investigate the methanogenesis inhibition. The highest biodegradability was observed (>94%) at low OLRs of 0.67 and 1 gCOD/L/d. Low substrate methanisation (22 and 16%) was observed at OLRs of 2 and 4 gCOD/L/d, corresponded by higher differences between their values and the biodegradability values. This finding emphasizes the phase separation effect of the AnPBBR, where methanogenesis can be improved at the later compartments, causing that difference to be reduced. Additionally, increasing the imposed OLR significantly decreased the substrate methanisation, which indicates a higher methanogenesis inhibition that caused mainly by the pH deterioration.

Fig. 2 shows the time course generation of volatile fatty acids (VFAs) with their corresponding pH changes, versus the OLR variation. The effluent VFAs concentrations significantly ($P < 0.05$) increased from 245.19 ± 14.78 to 1766.27 ± 255.84 mg/L, at increasing the OLR from 0.67 to 4.0 gCOD/L/d, respectively. The effluent pH values dropped from 6.11 ± 0.62 to 4.77 ± 0.51 as a result of increasing VFAs along with the OLR increase (Fig. 2). An increase in the $NaHCO_3$ addition to 500 mg/L prevented the alkalinity to be dropped at increasing the OLR from 2 to 4 gCOD/L/d, as shown in Table 2, especially that the generated VFAs were increased by more than 2 times, that were observed at OLR of 2 gCOD/L/d. Moreover, the pH drop to 4.77 ± 0.51, which is in the

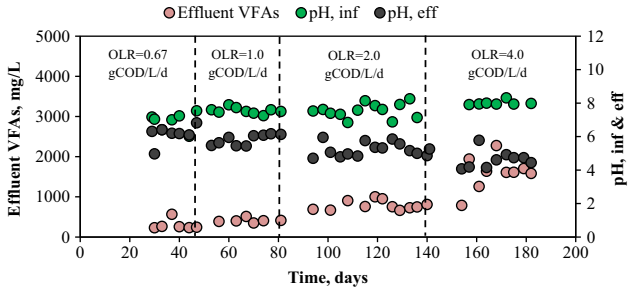

Fig. 2. Variations of total volatile fatty acids (VFAs) and pH values at different OLRs.

methanogenses inhibition range (below 5) [51] strongly deteriorated the methane content (Table 2); however, most of that methane production (≈70%) was occurred in the first two compartments (pH > 5), as shown later in Table 3. Furthermore, previous studies emphasized that minor methanogenic activity was found at a pH level of 4.5 [51,52]. This finding was also explained by the higher tolerance of the hydrogen-utilizing over acetoclastic methanogenesis, under low pH environments [49,51], which is inconsistency, in this study, with the highest acetate/ethanol ratio observed at OLR of 4 gCOD/L/d (Table 3). Similar trends of VFAs increase at increasing the OLRs, were observed [12,53–55]. Furthermore, the pathway of VFAs' production/consumption and pH drop/rise was carefully investigated along each of the AnPBBR compartments, in order to describe the intermediate variations, as shown later in Table 3. As depicted in Fig. 3a, acetate and ethanol production significantly ($P < 0.05$) increased from 225.25 ± 20.27 and 57.15 ± 2.31 mg/L to 1353.70 ± 159.85 and 240.19 ± 34.69 mg/L, at increasing the OLR from 0.67 to 4 gCOD/L/d, respectively. The results obtained revealed that the disparity between the acetate/ethanol concentrations was improved at increasing the OLR. This finding is due to the enhancement in the acetogenesis phase, which is responsible for oxidizing ethanol to acetate (Eq. (11)). In addition, the high difference between acetate and ethanol concentrations, as observed at OLRs of 2 and 4 gCOD/L/d, undoubtedly confirmed the complete acidification preference (Eq. (11)) over methanisation process. However, at low OLRs of 0.67 and 1 gCOD/L/d, a decrease in acetate concentrations was occurred, which is due to the sufficient consumption by the aceticlastic methanogenesis [56]. The acetate and ethanol generation was constantly increased with increasing the OLR, resulting good correlation coefficients ($R^2 = 0.848$ and 0.888, respectively) as shown in Fig. 3b.

Table 3
AnPBBR compartment-wise profiles at different OLRs.

Compartment no.	In	1	2	3	4
HRT (h)	0	9	18	27	36
OLR = 0.67 gCOD/L/d					
COD (mg/L)	994 ± 40	889 ± 79	784 ± 73	547 ± 49	315 ± 50
VFAs (mg/L)	–	311.55 ± 28.70	285.57 ± 20.88	275.32 ± 19.11	245.19 ± 14.78
pH	6.88 ± 0.41	5.99 ± 0.77	6.01 ± 0.30	6.06 ± 0.26	6.11 ± 0.62
Acetate (mg/L)	–	310.46 ± 30.55	275.45 ± 21.74	261.17 ± 18.84	225.51 ± 20.27
Ethanol (mg/L)	–	117.90 ± 11.29	72.26 ± 6.80	66.00 ± 5.30	57.15 ± 2.31
Cumulative HPR (L/d)	–	1.021 ± 0.82	1.071 ± 0.10	1.071 ± 0.10	1.071 ± 0.10
Cumulative MPR (L/d)	–	0.169 ± 0.01	0.489 ± 0.04	0.995 ± 0.08	1.702 ± 0.14
OLR = 1.0 gCOD/L/d					
COD (mg/L)	1488 ± 60	1293 ± 108	1345 ± 122	1058 ± 85	695 ± 74
VFAs (mg/L)	–	348.74 ± 33.53	510.47 ± 46.05	423.06 ± 40.38	411.55 ± 53.57
pH	7.55 ± 0.49	5.51 ± 0.35	5.24 ± 0.46	5.25 ± 0.29	5.82 ± 0.29
Acetate (mg/L)	–	313.25 ± 25.08	493.45 ± 39.77	417.35 ± 28.07	379.77 ± 34.10
Ethanol (mg/L)	–	181.86 ± 19.50	111.83 ± 12.30	109.94 ± 13.52	85.07 ± 19.20
Cumulative HPR (L/d)	–	0.866 ± 0.06	1.667 ± 0.14	1.712 ± 0.14	1.712 ± 0.19
Cumulative MPR (L/d)	–	0.345 ± 0.03	0.386 ± 0.03	0.865 ± 0.06	1.878 ± 0.21
OLR = 2.0 gCOD/L/d					
COD (mg/L)	2975 ± 122	2609 ± 258	2389 ± 215	2370 ± 194	1881 ± 108
VFAs (mg/L)	–	681.16 ± 59.22	729.27 ± 63.81	866.09 ± 69.33	733.53 ± 52.97
pH	7.58 ± 0.52	5.15 ± 0.44	5.06 ± 0.50	5.08 ± 0.20	5.28 ± 0.45
Acetate (mg/L)	–	590.89 ± 50.15	658.75 ± 68.09	771.64 ± 81.59	652.88 ± 99.39
Ethanol (mg/L)	–	130.66 ± 10.47	234.94 ± 21.65	166.12 ± 19.45	123.57 ± 25.72
Cumulative HPR (L/d)	–	2.965 ± 0.27	3.005 ± 0.22	3.667 ± 0.32	3.667 ± 0.31
Cumulative MPR (L/d)	–	0.542 ± 0.04	1.170 ± 0.01	1.225 ± 0.09	2.203 ± 0.24
OLR = 4.0 gCOD/L/d					
COD (mg/L)	6039 ± 446	4549 ± 442	4208 ± 173	3718 ± 205	3298 ± 159
VFAs (mg/L)	–	1099.13 ± 85.90	1140.87 ± 129.98	1501.74 ± 62.40	1766.27 ± 255.84
pH	8.04 ± 0.27	5.58 ± 0.23	5.28 ± 0.15	4.88 ± 0.42	4.77 ± 0.51
Acetate (mg/L)	–	987.22 ± 78.13	1065.89 ± 96.26	1191.99 ± 108.77	1353.70 ± 159.85
Ethanol (mg/L)	–	237.13 ± 25.86	264.42 ± 28.80	192.78 ± 9.16	240.19 ± 34.69
Cumulative HPR (L/d)	–	4.937 ± 0.41	5.298 ± 0.32	6.232 ± 0.53	6.571 ± 0.65
Cumulative MPR (L/d)	–	1.640 ± 0.19	2.458 ± 0.28	3.164 ± 0.26	3.567 ± 0.33

Values represent average ± standard deviation.

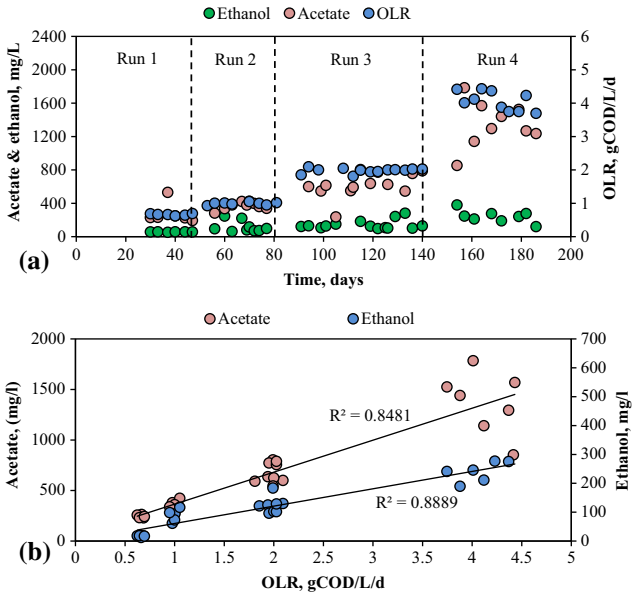

Fig. 3. (a) Time course of acetate and ethanol production versus OLRs, and (b) the relationship between acetate and ethanol generation at different OLRs.

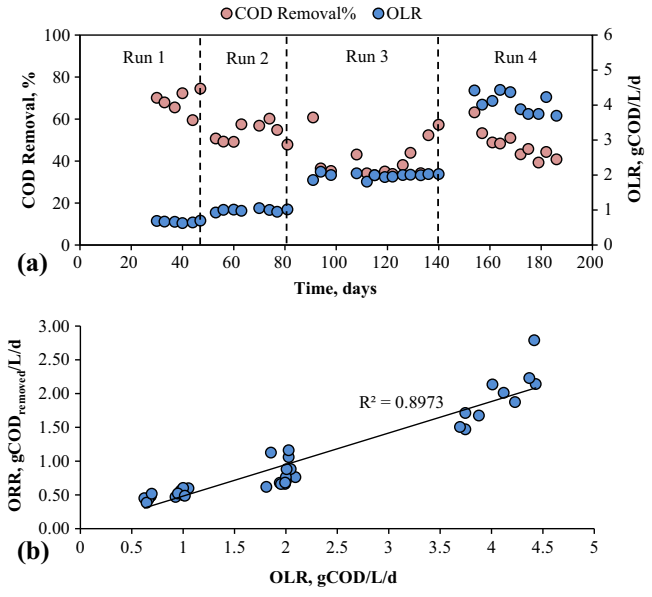

Fig. 4. (a) Time course of COD removal in the AnPBBR at different OLRs, and (b) organic removal rates (ORRs) versus OLRs.

The results presented in Fig. 4a show the time course of COD removal efficiency at different imposed organic loading rates (OLRs). The COD removal efficiency was gradually decreased from 68.29 ± 5.35% to 36.71 ± 3.84% at increasing the OLR from 0.67 to 2 gCOD/L/d, respectively. However, the COD removal efficiency was slightly increased to 45.18 ± 4.10%, at OLR of 4 gCOD/L/d. This result may be attributed to the increase in the buffer addition ($NaHCO_3$), in addition to a considerable increase in the ambient temperature (from 20 ± 5 to 25 ± 6 °C), at this scenario. This is in consistency with Donoso-Bravo et al. [57] who concluded that aci-

dogenesis, in the anaerobic process, showed the highest effect by temperature, since a change in the operational temperature, resulted in a decrease of the acidogenesis reaction rate, consequently influencing the methanogenesis. Similar findings were observed by Marin et al. [12], who reported that increasing OLR from 0.75 to 3.0 gCOD/L/d reduced the COD removal efficiency from 92% to 77%, respectively, in an anaerobic reactor treating MEG-based aircraft-de-icing fluid at 33 °C. Furthermore, Sawajneh et al. [58] found that 50% COD removal was obtained at OLR of 1.4 gCOD/L/d at 12–23 °C. Compared to our previous work [50], using ABR, the AnPBBR showed a significant enhancement in COD removal (from 38.7 ± 2.78% to 68.29 ± 5.35%), at the same OLR. The organic removal rate (ORR) was steadily increased (R^2 = 0.897) from 0.450 ± 0.045 to 1.827 ± 0.284 $gCOD_{removed}$/L/d with increasing the OLR from 0.67 to 4 gCOD/L/d, respectively, as shown in Fig. 4b. This result indicates a stable performance of the AnPBBR through the OLR rising from 0.67 to 4 gCOD/L/d, in addition to still have yet got the highest operational limit [59].

3.2. Pathways of 3-biofuels (H_2, ethanol and CH_4) generation at different HRTs (Compartment-wise profile)

The results obtained, at steady-state of each operated OLR, along the AnPBBR length were highlighted in order to investigate the compartments' separation effect (four different HRTs; 9, 18, 27 and 36 h) on the pathway of each parameter, as depicted in Table 3. It can be noticed that the higher COD removal efficiencies were started after reaching the peak of VFA production, which were observed at the 1st, 2nd and 3rd compartments at OLRs of 0.67, 1 and 2 gCOD/L/d, respectively. Nevertheless, at OLR of 4 gCOD/L/d, the VFAs production was gradually increased through the four compartments, with a final concentration of 1766.27 ± 255.84 mg/L, indicating the prevalence of acidogenesis and acetogenesis over methanogenesis. In addition, an unusual increase in the COD concentration was recorded at the 3rd compartment (from 1293 to 1345 mg/L) at OLR of 1 gCOD/L/d, which was attributed to the high VFAs generation. This observation is in agreement with Marin et al. [12], who mentioned the same behavior, particularly at lower OLRs (0.6 and 1.2 gCOD/L/d). This is the same case of pH profile results, which show concurrent drops to 5.99 ± 0.77, 5.24 ± 0.46, 5.06 ± 0.50 and 4.77 ± 0.19 at the VFAs production's peak points at OLRs of 0.67, 1, 2 and 4 gCOD/L/d, respectively. The pH final recovery (6.11 ± 0.62 and 5.82 ± 0.29), for the effluent, that was respectively recorded at OLRs of 0.67 and 1 gCOD/L/d, was associated with alkalinity production through the conversion of H_2 and acetate by methanogens to CH_4. This is in consistency with Gopala Krishna et al. [60], who reported that lower pH was associated with earlier compartments and then was increased along the reactor length due to VFAs consumption.

Furthermore, Table 3 depicts that the maximum H_2 production was observed at HRT of 9 h (1st compartment), even with increasing the OLR (0.67, 1, 2 and 4 gCOD/L/d). Moreover, this is the same finding for acetate as the resulted metabolite from the anaerobic decomposition of MEG as aforementioned in Eqs. (2) and (4). Similar trends for acetate production from ethylene glycol based-substrate were obtained by Marin et al. [12]. In addition, Zhu et al. [61] recorded that at OLRs between 0.9 and 2.6 gCOD/L/d, the acetate generation was appeared in the ABR first two compartments followed by its consumption at later compartments. Likewise, the highest ethanol production was observed at the 1st compartment (HRT of 9 h), where its values were 117.90 ± 11.29, 181.86 ± 19.50, 130.66 ± 10.47 and 237.13 ± 25.86 mg/L at OLRs of 0.67, 1, 2 and 4 gCOD/L/d, respectively. Conceptually, this is associated to the concurrent reactions (Eqs. (9) and (10)), which describe the ethanol formation by the acetaldehyde dehydrogenase activity [62]. However, the maximum ethanol production was further dropped (according to Eq. (11)) at the 2nd compartment, when the operated OLRs were 0.67 and 1 gCOD/L/d. However, the further increase in OLR to 2 and 4 gCOD/L/d caused an increase of ethanol at the 2nd compartment (HRT of 18 h), before the later consumption, to reach the maximum concentrations of 234.94 ± 21.65 and 264.42 ± 28.80 mg/L, respectively. Therefore, considerable improvement in hydrogen production was in consistency with ethanol consumption. Zhu et al. [63] found that the ethanol production was observed only at the ABR first compartment while treating soybean processing wastewater at OLR 0.9–2.6 gCOD/L/d.

On the contrary, the maximum methane production was markedly mentioned at the last two compartments (HRTs of 27 and 36 h) at OLR of 0.67 and 1 gCOD/L/d, and its values were 1.213 and 1.488 ml CH_4/d, respectively. This finding was shifted to the last compartment (0.978 ± 0.08 ml CH_4/d) at OLR of 2 gCOD/L/d. This strongly support the hypothesis of the long HRT is preferable for methanogenesis process. Afterwards, at OLR of 4 gCOD/L/d, the methane gas was constantly produced along the four compartments with final low CH_4 content of 23.44 ± 2.14%. This is mainly due to the certain pH drop (below 5) in the last two compartments, showing lower methane production. Noteworthy, Dwyer and Tiedje [17] found that after 70 h, the production of ethanol (Eq. (3)) with simultaneous CH_4 production was readily observed in the MEG consortia. Moreover, he proved that MEG degradation seemed to be completed before the CH_4 production. This finding describes the high aforementioned biodegradability (>94%) with concurrent high methane content at the lower OLRs of 0.67 and 1 gCOD/L/d. Moreover, the registered cumulative methane production (MPR) was progressively increased as 1.702 ± 0.14, 1.878 ± 0.21, 2.203 ± 0.24 and 3.567 ± 0.33 L CH_4/d at OLRs of 0.67, 1, 2 and 4 gCOD/L/d. Eventually, it can be concluded that the compartments separation with different HRTs through the AnPBBR, controlled the various microbial activities, with considerable shifting at increasing OLRs.

3.3. Stover–Kincannon and CSTR in series models

The Stover–Kincannon model was applied for the AnPBBR using different initial COD concentrations at a constant HRT of 36 h, as shown in Fig. 5. The results obtained confirmed that this model was fitted (R^2 = 0.897) to the AnPBBR, treating MEG contaminated effluents. Moreover, the model can be considered to describe and predict the system performance. The kinetic coefficients μ_{max} (maximum utilization rate) and K_B (saturation constant) were 2.02 and 2.46 g/L/d, respectively. Interestingly, the maximum obtained utilization rate (μ_{max}) was almost 3.5-fold higher than the reported value by Elreedy et al. [33] using stepped anaerobic baffled (SAB) reactor for petrochemical wastewater treatment. This can be attributed to the effect of using packing media to enhance the reactor performance. As well, lower values for μ_{max} of 1.92 g/

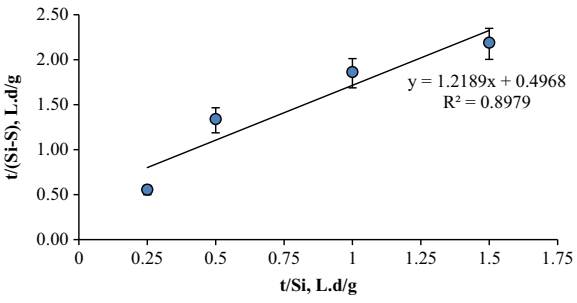

Fig. 5. Stover–Kincannon model representation for the AnPBBR performance.

L/d and K_B of 1.51 g/L/d were reported by Aydinol et al. [64], for treatment of wastewater in an UASB reactor (operated at OLR of 1.2–11.7 gCOD/L/d; at 17–21 °C). Similar results for μ_{max} and K_B of 3.36 and 4.56 g/L/d were achieved by Raja Priya et al. [65] using Up-flow anaerobic fixed film reactor (UAFB), treating formaldehyde wastewater at 30 °C and OLR of 0.18–3.16 gCOD/L/d. The low μ_{max} and K_B values could be attributed to the slowly biodegradable substrates [39]. Adding on to this, Coskun et al. [66], found that the operational temperature affected substantially on the values of μ_{max} and K_B. As a result, the effluent substrate concentration (S_e, gCOD/L) can be determined as a function of the influent substrate concentration (S_i, gCOD/L), OLR (gCOD/L/d) as well as the AnPBBR kinetic coefficients, as follows in Eq. (14):

$$S_e = S_i - [2.02S_i/(2.46 + (S_i/\mathrm{OLR}))] \quad (14)$$

The CSTR in series model was proposed in order to perform an internal kinetic study for the AnPBBR compartment-wise performance, which can be used to predict the effect of organic loading rate variations on the removal efficiency. The model was applied at a constant overall HRT of 36 h, with 9 h-HRT at each of the four compartments. The initial COD concentrations were varied from 1000 to 6000 mg/L. The value of the kinetic constant (k, h^{-1}) was mathematically determined according to Eq. (6). The kinetic constant (k) was 0.018 ± 0.002 h^{-1} (Mean ± standard deviation) which was higher than the specific substrate utilization rate coefficient for ABR with three chambers (0.012 h^{-1}), treating molasses wastewater at an OLR of 5–25 gCOD/L/d [67]. On the other hand, this model was successfully used by Nardi et al. [68], who used a horizontal flow anaerobic immobilized sludge (HAIS) reactor, treating synthetic wastewater and the obtained kinetic constant was 0.45 h^{-1}. Likewise, Ghaniyari-Benis et al. [42], used hybrid anaerobic baffled reactor (HABR) for treatment of synthetic wastewater and the retrieved kinetic constant amounted to 0.60 ± 0.07 h^{-1}. Based on these results, the CSTR in series model was validated as depicted in Fig. 6a by comparing the model theoretical graph with the experimental data of the fractional conversions for the different initial COD concentrations. Subsequently, Fig. 6b shows the model evaluation step between the experimental data of fractional conversion (*Y*-axis) and theoretical data (*X*-axis) at different initial COD concentrations. As evident, the deviations of equal to or lower than 15% between the experimental and theoretical values of the COD fractional conversion were achieved in this study. Hence, this model, based on the substrate utilization rate kinetic constant (K, h^{-1}), shows a representative prediction of MEG removal as COD fractional conversion values. Correspondingly, it confirms the substrate utilization rate dependency with the activity of the different consortia (at each compartment) involved in the anaerobic process in the AnPBBR.

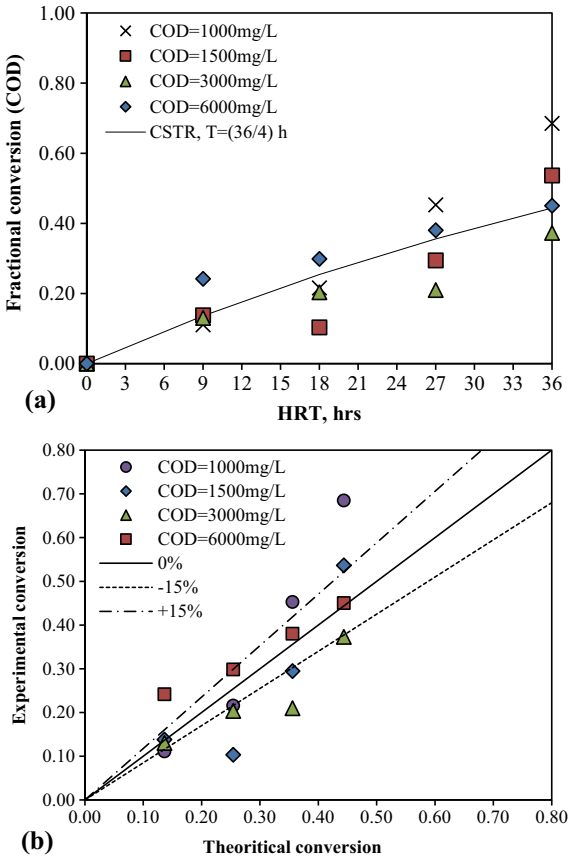

Fig. 6. (a) Experimental and theoretical fractional conversion (CSTR in series model) results' variation at each compartment with changing the initial COD, and (b) evaluation of the model versus experimental results.

3.4. Economic benefits of using AnPBBR

Comparison between the economic analysis of stepped anaerobic baffled (SAB) reactor Elreedy et al. [33] and AnPBBR for treatment of petrochemical wastewater are presented in Table 4. This comparison was conducted to evaluate the economic aspects of adding PU sheets as packing material. The related costs were calculated according to the results obtained in the two studies under same conditions, which corresponded by COD removal of 70.77 ± 2.55%. Nevertheless, the AnPBBR showed higher COD removal efficiency with higher concurrent biogas production at the further increase in OLR. A case study of polyester company producing approximately 1000 m^3/d MEG contaminated wastewater with effluent COD of 1920 ± 316 mg/L, was considered for the cost analysis. The designed working volume for SAB reactor and AnPBBR was 3300 m^3 (including 10% as head space for biogas collection). The cost estimation results, including capital, operation and maintenance costs, in addition to the possible revenues are summarized in Table 4. The construction cost (capital cost) included basins, heating, pumping and piping systems, was estimated to be 1,180,000 LE (1$ = 7.65 LE, LE refers to Egyptian pound). The packing bed PU sheets cost is 5 $/$m^2$, according to the manufacturer. The annual operation and maintenance costs have been divided into three categories; energy consumption, nutrients cost and other related expenses (staff, chemicals, waste management and maintenance). The latter cost was estimated to be 0.0944 €/m^3 (1€ = 1.139$) [69]. The pumping energy consumption for feeding was calculated according to the required horsepower (hydrostatic head = 6 m and pump efficiency of 70%). The heating energy for maintaining the temperature to be not less than 15 °C was calculated according to the assumption that the required heating periods are equal to three cold months per year (current weather in Egypt).The nutrients costs, for COD/N/P adjustment, buffer addition and trace elements supplementation, are presented in Table 4. The possible revenues were calculated based on: (a) the energy profit in term of bio-hydrogen and methane energy, which calculated based on obtaining 70% efficiency from the heating conversions (125 MJ per kg-H_2 and 50.2 MJ per kg-CH_4) [70], (b) the environmental benefits as reported by Molinos-Senante et al. [69] who estimated the pollutants discharging add-value, were stated based on the value of 0.1312 €/$kgCOD_{removed}$. Moreover, the excess sludge cost and/or profit were assumed to be neglected; this is due to the negligible influent suspended solids' concentrations and low excess sludge production, particularly in the AnPBBR. It's interesting to point out that the energy saving, which can be achieved by producing bio-hydrogen and methane, was about 3.23 and 4.09 times the desired energy for the process operation

Table 4
Economic analysis for AnPBBR versus stepped anaerobic baffled (SAB) reactor.

Costs/profits	Quantity		Unit	Unit price		Total cost/profit	
	SAB	AnPBBR				SAB	AnPBBR
Capital cost ($)							
1. Construction	3300		m^3	44.07	$/$m^3$	−145434[a]	−145434
2. Polyurethane (PU) sheets	–	306	m^3	5.00	$/$m^2$	–	−61200
Operation and maintenance cost ($/year)							
1. Energy consumption							
a. Pumping	−6533		kW h/year	0.106	$/kW h	−692	
b. Heating (maintaining 22 ± 7 °C)	−229,950		kW h/year	0.106	$/kW h	−24375	
2. Nutrients supplementation cost	−54,000		Kg/year	1.468	$/Kg	−79272	
3. Other expenses	−365,000		m^3/year	0.108	$/$m^3$	−39238	
Profits ($/year)							
1. Bio-hydrogen and methane, energy profit	736580	966269	kW h/year	0.106	$/kW h	78,078	102,424
2. Environmental benefits	535090	498079	$kgCOD_{removed}$/year	0.149	$/$kgCOD_{removed}$	79,728	74,214
Net profit ($/year)						**14,229**	**33,062**
Payback period (years)						**10.22**	**6.25**

[a] Negative values represent costs; Positive values for profits.

(pumping and heating). As well, their revenues represent approximately 54.38% and 71.34% of the total annual operation and maintenance cost for the AnPBBR and SAB reactor, respectively (Table 4). Meanwhile, the net annual profit and payback period (the required period for the investment value to cover the capital cost) values showed that adding PU sheets of about 10% of the reactor working volume (only 0.3% based on the media gross volume) reduced the payback period by 40% of that was achieved by the SAB reactor.

3.5. Microbial community

From the sequence obtained after analysis using IlluminaMiseq Platform, a total of 20,119 sequence reads met the quality criteria ($Q < 30$) after filtering without any mismatch. The phylogenetic analysis of the sequence reads revealed the dominance of bacterial community in the reactor (97.88%). Archaea was detected in very low numbers (0.61%) and along with other kingdom such as protozoa and Animalia (Fig. 8a). Tags with 97% similarity were grouped into single OTUs and the results indicated presence of diverse group of bio-hydrogen, ethanol, methane-producing and methane oxidizing bacteria based on the OTU clustering analysis. Different types of hydrolytic, acidogenic and acetogenic bacteria that have the ability to produce ethanol and hydrogen as a metabolite during dark fermentation process were identified in the reactor (Fig. 7b). The taxonomic affiliation of the bacterial community at the different structural level was analyzed for better understanding and in total, 26 phyla were identified apart from the unknown sequences (Fig. 7b). *Proteobacteria* (36.62%), *Firmicutes* (20.85%) and *Bacteroidetes* (3.44%) were the most dominant phyla within the reads; *Actinobacteria* (0.85%), *Acidobacteria* (0.71%) and *Chloroflexi* (0.51%) were detected but in small percentage (Fig. 7b) and the remaining sequences were within the minor phyla. Similar phyla have been reported in anaerobic digesters fed with different feed stocks [71,72].

Further classification into lower taxonomic levels showed up to 36 classes, 66 orders (Fig. 8) and 99 families. Dark fermentation bacteria that have the capacity to degrade and metabolize carbohydrates into H_2, CO_2 and ethanol were found abundant in these groups [71,73]. Majority of the enriched dominant populations in the system were affiliated with low-G + C-content gram-positive bacteria, Alpha, Gamma, Epsilon, and *Betaproteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Clostridia*, *Enterobacter, Spirochaetes* and other group of H_2 producing microorganisms as shown in Fig. 8. The highest reads in order and family level classification belonged to *Clostridiales/aceae* (~20%) (Fig. 8), *Enterobacteriales/aceae* (~20.91%) which are known, spore forming, hydrogen producers. Similarly, Proteobacteria and class *Betaproteobacteria* of the orders *Nitrosomonadales*, *Rhodocyclales* and *Burkholderiales* as detected in this study were also observed by Silva et al. [74] in a bioreactor treating petroleum refinery wastewater as the main group in the selected DNA-bands using amplified 16S rRNA gene fragments of DGGE-PCR (Denaturing gradient gel electrophoresis of Polymerase Chain Reaction). Few other studies have also reported the func-

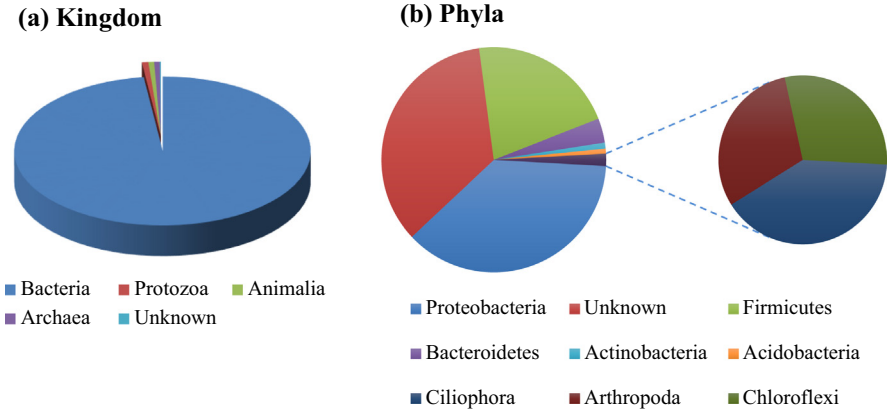

Fig. 7. Relative abundance of major (a) domain and (b) phyla in the AnPBBR sludge sample based on IlluminaMiSeq sequencing data.

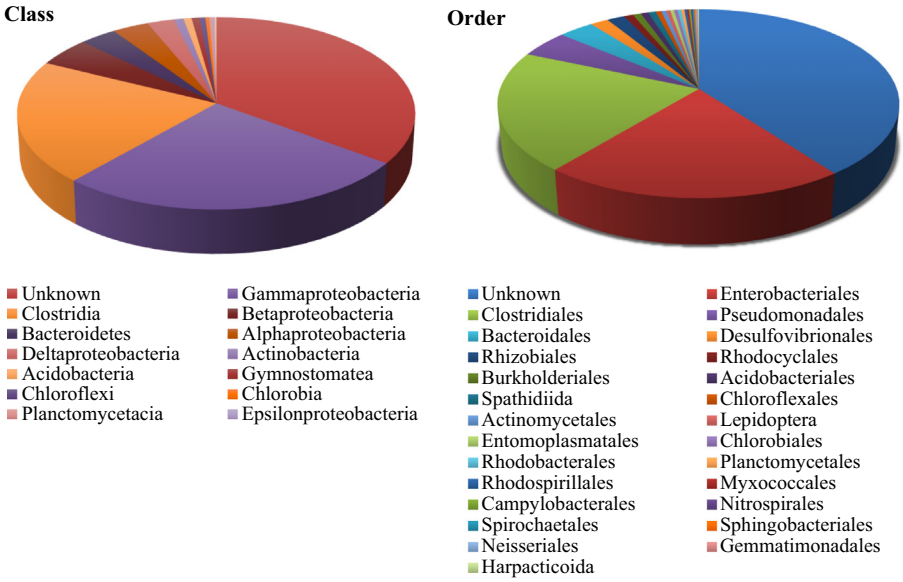

Fig. 8. Pie chart showing taxonomic distribution of different bacterial groups in the sludge samplebased on our Illumina dataset. In total 36 classes and 66 orders were identified in the sample taken during simultaneous H_2/CH_4 production in the reactor.

tional role of these bacterial communities in removing petroleum contaminant and production of biofuels [75,76].

Furthermore, the taxonomic richness and abundance shown in rank abundance plots (see supplementary, Fig. S1) revealed the dominant and presence of diverse strains of H_2 producing microorganisms including *Bacillus*, *Clostridium*, *Desulfovibrionales*, *Ethanoligenens*, *Enterobacter*, *Rhodobacter*, *Thermoanaerobacterium* and *Thermotogales* spp. in the reactor [71]. Interestingly, these strains are known to be hydrogen producers during anaerobic fermentation process and maximum H_2 and ethanol production showed that these groups were active in the 1st compartment. Eighteen different phylo-types of *Clostridium* strains as well as *Ethanoligenensharbinense* were identified as the key dominant hydrogen producers in the 1st compartments [77,78]. Characterization results of microbial communities in this study from phylum to genus level suggest their uniqueness and main role in the production of the three studied biofuels (hydrogen, ethanol and methane) as the by-products of anaerobic digestion of MEG using AnPBBR.

It is interesting to know that most of the 16S rRNA tag Illumina sequencing data for Proteobacteria belonging to γ-Proteobacteria (26.01%), ε-Proteobacteria (0.18%), β-Proteobacteria (4.77%) and α-Proteobacteria (3.23%) were affiliated to methanotrophic and non-methanotrophic proteobacterial population belonging to type I, II and the unclassified methanotrophs. Methanotrophs among the group of Proteobacteria have been noted for their role in reducing methane produced in natural environment through biological oxidation of methane [79]. Op Den Camp et al. [80] summarized the taxonomic framework and properties of Verrucomicrobia and Proteobacterial methanotrophs. They have methane monooxygenase (pMMO and sMMO) as their unique functional gene/enzyme (pmoA and mmoX) that are used for the oxidation of methane. As seen in this study, the detected methanotrophic group belonged to the Proteobacteria phylo-types and the highest sequence reads belonged to γ-, α- and β-Proteobacteria. The γ- and α-Proteobacteria are known for using methane as sole source of carbon and energy, while methylotrophic denitrifiers of the Methylophilaceae family in the β-Proteobacteria uses methane as hydrogen donor for in-situ denitrification [81] when NH_4Cl is added as an additional N-source [82,83]. These organisms play an important role in CH_4 and methanol oxidation as well as denitrification process [84]. Recently described type I methanotrophs of the order *Methylococcales* belonging to genus *Methylocaldum* (*Methylocaldumtepidum*) and type II in the order *Rhizobiales* (1.65%) of family *Methylobacteriaceae* and *Methylocystaceae* were detected in the sludge sample taken from the 1st compartment of the reactor [85,86]. The sequence reads were affiliated to *Methylosarcina fibrate*, *Methylophilusmethylotrophus*, *Methylobacteriumisbiliense*, *methylocaldumtepidum*, *Methylocaldumszegendiense*, *Methylocystis* spp. and uncultured *methylobacterium* strains.

Even though, Archaea community that belong to methane–producing group were detected at the kingdom level, but the methane produced are probably been used by the methanotrophic-proteobacterial population [81]. The results showed that the composition and function of archaea community was greatly affected by the reactor condition. Low pH in the 1st compartment have also been found as one of the contributing factors for low archaea population which might have led to low concentration of methane [87,88]. We further postulate that the low diversity and distribution of archaea community in the sludge obtained from the reactor could be the cause of low methane concentration at HRT of 9 h (1st compartment) and OLR of 2 gCOD/L/d.

4. Conclusions

The AnPBBR is a promising technology and efficient for separation of different consortium bacteria responsible for 3-bio (hydrogen, ethanol and methane) production from treatment of petrochemical wastewater containing MEG. However, the behavior, pathway and efficiency of the anaerobic degradation is strongly affected by the organic loading rate (OLR). The obtained substrate biodegradability was varied from 98 to 85% at increasing the OLR from 0.67 to 4.0 gCOD/L/d, respectively. Besides, the maximum H_2 and CH_4 production rates of 6.571 ± 0.65 and 3.567 ± 0.33 L/d were respectively achieved at OLR of 4 gCOD/L/d. The AnPBBR compartment-wise study demonstrated that the maximum H_2 and ethanol production (4.937 ± 0.385 L/d and 237.13 ± 25.86 mg/L, respectively) were achieved at HRT of 9 h. The Stover–Kincannon and CSTR in series models were successfully applied to the overall and compartment-to-compartment performance of the AnPBBR, respectively. A cost estimation analysis showed a significant reduction in the payback period (40%) as compared to the classical ABR,

treating MEG containing wastewater. The microbial community analysis confirmed the presence of a diverse group of bacteria in the AnPBBR with *Proteobacteria* as the highest phylum.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.enconman.2016.05.067.

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