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Application of Box–Wilson experimental design method for 2,4-dinitrotoluene treatment in a sequential anaerobic migrating blanket reactor (AMBR)/aerobic completely stirred tank reactor (CSTR) system

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ABSTRACT

A sequential aerobic completely stirred tank reactor (CSTR) following the anaerobic migrating blanket reactor (AMBR) was used to treat a synthetic wastewater containing 2,4-dinitrotoluene (2,4-DNT). A Box–Wilson statistical experiment design was used to determine the effects of 2,4-DNT and the hydraulic retention times (HRTs) on 2,4-DNT and COD removal efficiencies in the AMBR reactor. The 2,4-DNT concentrations in the feed (0–280 mg/L) and the HRT (0.5–10 days) were considered as the independent variables while the 2,4-DNT and chemical oxygen demand (COD) removal efficiencies, total and methane gas productions, methane gas percentage, pH, total volatile fatty acid (TVFA) and total volatile fatty acid/bicarbonate alkalinity (TVFA/Bic.Alk.) ratio were considered as the objective functions in the Box–Wilson statistical experiment design in the AMBR. The predicted data for the parameters given above were determined from the response functions by regression analysis of the experimental data and exhibited excellent agreement with the experimental results. The optimum HRT which gave the maximum COD (97.00%) and 2,4-DNT removal (99.90%) efficiencies was between 5 and 10 days at influent 2,4-DNT concentrations 1–280 mg/L in the AMBR. The aerobic CSTR was used for removals of residual COD remaining from the AMBR, and for metabolites of 2,4-DNT. The maximum COD removal efficiency was 99% at an HRT of 1.89 days at a 2,4-DNT concentration of 239 mg/L in the aerobic CSTR. It was found that 280 mg/L 2,4-DNT transformed to 2,4-diaminotoluene (2,4-DAT) via 2-amino-4-nitrotoluene (2-A-4-NT) and 4-amino-2-nitrotoluene (4-A-2-NT) in the AMBR. The maximum 2,4-DAT removal was 82% at an HRT of 8.61 days in the aerobic CSTR. The maximum total COD and 2,4-DNT removal efficiencies were 99.00% and 99.99%, respectively, at an influent 2,4-DNT concentration of 239 mg/L and at 1.89 days of HRT in the sequential AMBR/CSTR.

1. Introduction

2,4-Dinitrotoluene (2,4-DNT) (CAS No. 121-14-2) is one of the six dinitrotoluene isomers and is used commercially as an intermediate in the production of dyes and as a precursor to toluene diisocyanate, which is used to manufacture polyurethane foams. Besides, DNT is used as a waterproofing, plasticizing and gelatinizing agent in explosives and as a modifier for smokeless powders [1–4]. The environmental impact of DNT exposure is also a major public concern. 2,4-DNT is highly toxic to aquatic organisms including fish, invertebrates, algae, protozoa and bacteria with the lowest observed effect concentration of 0.05 mg/L [5]. Oral LD_{50} (the LD_{50} is the dose that kills half (50%) of the animals tested; LD = lethal dose) values for 2,4-DNT range from 268 to 650 mg/kg for rats and from 1250 to 1954 mg/kg for mice [6,7]. US waste water treatment standard for 2,4-DNT was determined as 0.32 mg/L for discharge to the streams [7]. There have been several reports about DNT removal using various physico/chemical technologies such as adsorption [1,8,9], air stripping [1] and advanced oxidation processes [10–13]. One major drawback of adsorption is that it does not actually treat the DNT but simply removes it from the aqueous phase. Other physical/chemical methods, such as ultrafiltration, reverse osmosis, liquid–liquid extraction or resin adsorption all suffer from similar drawbacks [14].

Biological methods have been proposed as a more affordable and complete solution for degrading DNT [14–17]. DNT is generally resistant to aerobic biotransformation due to the strong electron attracting capacity of the nitro group. Therefore, with industrial applications of the aerobic biodegradation of 2,4-DNT it is very difficult to achieve compliance with EPA discharge limits using aerobic treatment systems [6]. In the anaerobic system, DNT was transformed to nitroso, hydroxylamino, and eventually amine groups

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[6] which are easily degraded aerobically. Cheng et al. [6] reported complete biotransformation of 2,4-DNT to 2,4-DAT via 4-A-2-NT or 2-A-4-NT with methanol, acetic acid or hydrogen as the primary substrate under anaerobic conditions. However, the accumulation of nitroso and hydroxyl amino intermediates such as 2-amino-4-nitrotoluene (2-A-4-NT), 4-amino-2-nitrotoluene (4-A-2-NT) and 2,4-diaminotoluene (2,4-DAT) are more toxic than the parent compounds. Therefore, sequential anaerobic–aerobic treatment was necessary for the complete mineralization of 2,4-DNT. Maloney et al. [15] reported a sequential anaerobic/aerobic treatment process to treat the DNT. In this reactor system, DNT was converted to DAT via anaerobic mineralization process and then DAT was oxidized to ammonia in aerobic activated sludge reactor [15]. In another study performed by Sponza and Atalay [16] a sequential anaerobic upflow anaerobic sludge blanket (UASB)/completely stirred tank reactor (CSTR) system was used to treat the 2,4-DNT using molasses as primary substrate. Over 85% and 90% COD and DNT removal efficiencies were obtained, respectively, at a DNT loading rate of 250 mg/L day and at an HRT of 0.5 day in the UASB reactor. More than 95% COD and 2,4-DNT removal efficiencies were achieved in the combined UASB/CSTR system at an initial DNT loading rate of 250 mg/L day.

Combined anaerobic/aerobic processes are a viable alternative for the treatment of xenobiotic compounds such as 2,4-DNT that are difficult to treat by traditional processes [17]. The mineralization of some recalcitrant pollutants has been possible by using sequential anaerobic/aerobic treatments [17]. Although several studies have been performed concerning the treatment of DNT and its intermediate products, little work has been done to determine the sequential anaerobic and anaerobic/aerobic treatment of 2,4-DNT. Anaerobic treatment of 2,4-DNT by the AMBR has also not been reported in the literature.

The AMBR was developed as a high rate anaerobic treatment system that combines compartmentalization, continuous flow, short hydraulic retention time, simple design, no gas–liquid separation, with no feed distribution system and no recycling [18–20]. AMBR staging was beneficial for more favorable conditions for volatile fatty acid degradation, high level of biomass in the first compartment, and removal of recalcitrant substances since it provides sufficient contact between microorganisms and toxic substances [21]. The studies related to AMBR are limited to the treatment of synthetic wastewaters containing sucrose and non-fat dry milk as substrate, p-nitrophenol and nitrobenzene [18–23]. Therefore, this study was designed to investigate the treatment of 2,4-DNT in a sequential AMBR/CSTR system.

The Box–Wilson design is a response surface methodology, an empirical modeling technique, devoted to the evaluation of the relationship of a set of controlled experimental factors and observed results. This optimization process involves three major steps: performing the statistical design experiments, estimating the coefficients in a mathematical model, and predicting the response and checking the adequacy of the model [24]. In this study, a Box–Wilson statistical experimental design method was employed to determine the effect of operation conditions such as HRT and influent 2,4-DNT concentration on COD and 2,4-DNT removal efficiencies, total gas, methane gas and methane gas percentage, pH, TVFA and TVFA/Bic.Alk. ratio in the effluent of the AMBR.

The major objectives of this study were to investigate the effects of HRT and influent 2,4-DNT concentrations on the removal efficiencies of COD, 2,4-DNT; total, methane gas productions, methane percentages, the pH levels, TVFA and TVFA/Bic.Alk. ratios in effluent of the AMBR using a Box–Wilson statistical experiment design method and to determine the optimal conditions maximizing the percentage of COD and 2,4-DNT removal in the AMBR. Furthermore, the effect of the aerobic CSTR reactor on the yields of COD remaining from the AMBR and 2,4-DNT metabolites produced in the AMBR reactor was investigated.

2. Material and methods

2.1. Experimental set-up

A continuously fed stainless steel AMBR and CSTR were used in sequence for the experiment. A schematic diagram of the lab-scale sequential AMBR/CSTR used in this study is presented in Fig. 1. The effluent of the AMBR was used as the influent of the CSTR. The AMBR was constructed from a stainless steel rectangular container with inner dimensions of 45 cm length, 20 cm height and 15 cm width with an active volume of 13.5 L.

The AMBR was divided into three equal compartments with two vertical stainless steel sheets and was operated by reversing the flow periodically. Round openings with a diameter of 2.5 cm located in the back of the stainless steel sheets were placed at a height of 0.5 cm from the bottom to create sufficient contact between biomass and substrate. The AMBR was provided with six equidistant sampling ports along its length at heights of 5 cm and 20 cm from the bottom. The three compartments were mixed equally every 15 min at 60 rpm with a mixer (Heidolph) to ensure gentle mixing. The flow over the horizontal plane of the reactor was reversed once a week. A weekly change in flow direction was chosen to prevent a pH drop due to VFA buildup in the initial compartment and to prevent unequal biomass levels due to anticipated biomass migration between compartments. The influent feed was pumped to the middle compartment for a certain amount of time before the flow was reversed. In this way, a breakthrough of substrate was prevented. The samples were withdrawn from the AMBR after stopping the mixing process for 15 min. Four automatic ball valves, with an internal diameter of 2.54 cm, were used to open and close the influent and effluent ports. The gas produced was collected via a porthole in the top of reactor. The operating temperature of the AMBR was maintained constant at $37 \pm 1\,^{\circ}C$ through continuous operation using an electronic heater located in the lower part of the reactor. This provided a homogenous temperature in all the compartments of the AMBR. A digital temperature probe located in the middle part of the second compartment showed the constant operating temperature. The CSTR reactor consisted of an aerobic (effective volume = 9 L) and a settling compartment (effective volume = 1.32 L) and was operated in continuous mode with AMBR.

2.2. Wastewater composition

Synthetic wastewater contained glucose, $NaHCO_3$, sodium thioglycollate, Vanderbilt mineral medium and 2,4-DNT. 5000 mg/L of $NaHCO_3$ was added in the feed to prevent the accumulation of total volatile fatty acid (TVFA) and to provide a neutral pH (7.0–7.8) in AMBR. Additionally, 667 mg/L of sodium thioglycollate was added to provide anaerobic conditions in the feed wastewater. Vanderbilt mineral medium was used as nutrient and trace minerals for the microorganism. This medium was prepared in distilled water by dissolving 0.4 g $MgSO_4$, 0.4 g NH_4Cl, 0.4 g KCl, 0.3 g Na_2S, 0.08 g $(NH_4)_2HPO_4$, 0.05 g $CaCl_2$, 0.04 $FeCl_2$, 0.01 g $CoCl_2$, 0.01 g KI, 0.01 g $Na(PO_3)$, 0.5 mg $AlCl_3$, 0.5 mg $MnCl_2$, 0.5 mg $CuCl_2$, 0.5 mg $ZnCl_2$, 0.5 mg NH_4VO_3, 0.5 mg $NaMoO_4$, 0.5 mg H_3BO_3, 0.5 mg $NiCl_2$, 0.5 mg $NaWO_4$, 0.5 mg Na_2SeO, and 0.01 g cystein per liter [17]. The influent COD concentrations and the COD loading rates were kept constant at approximately 3000 mg/L through anaerobic operation in the AMBR reactor. Glucose, giving a COD concentration of 3000 mg/L was used as co-substrate to provide electrons for the reduction of 2,4-DNT. Although the influent COD concentration was

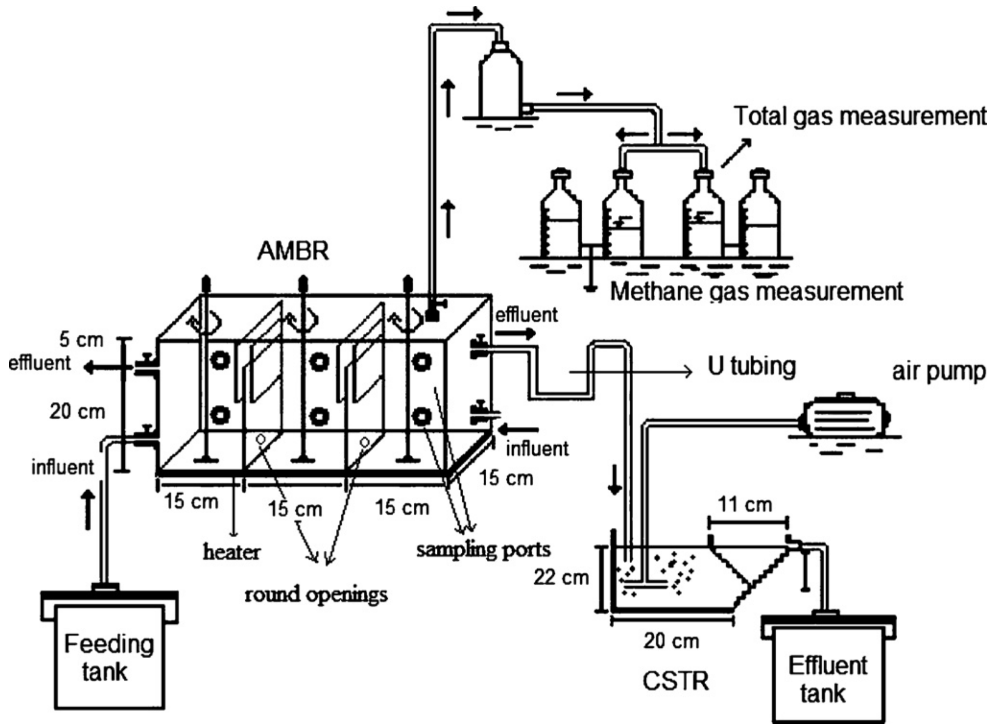

Fig. 1. Schematic configuration of lab-scale AMBR/CSTR sequential reactor system.

kept constant at 3000 mg/L with glucose, the influent COD concentrations increased from 3000 to 3350 mg/L when the 2,4-DNT concentration was increased from 0 to 280 mg/L since 2,4-DNT gave additional COD to the synthetic wastewater.

2.3. Seed of the reactors

Partially granulated anaerobic sludge was used as seed in the AMBR and was obtained from an UASB reactor containing partially granulated biomass (containing *acidogenic* and *methanogenic Archae* bacteria) from the Pakmaya Yeast Beaker Factory in Izmir, Turkey. The volatile suspended solid (VSS) concentration of anaerobic sludge used as seed in the AMBR was 18 g/L. The sludge in the CSTR was taken from the aeration tank of Pakmaya Yeast Beaker Factory in Izmir, Turkey. The suspended solid (SS) in the CSTR was 3 g/L.

2.4. Analytical methods

Total suspended solid (TSS) and mixed liquor suspended solid (MLSS) in granulated and activated sludge were measured by the filtration technique using membrane filters with pores sized 0.45 μm [25]. The soluble COD concentrations in samples were detected using the closed reflux colorimetric method following standard methods [25]. Biogas production was measured with liquid displacement method. Total gas was measured by passing the gas through distilled water containing 2% (v/v) H_2SO_4 and 10% (w/v) NaCl [26]. Methane gas was detected by using distilled water containing 3% NaOH (w/v) [27]. Methane percentage in biogas was determined by Dräger (Stuttgart, Germany) Pac-Ex methane gas analyzer.

Bicarbonate alkalinity (Bic.Alk.) and TVFA concentrations were determined by titrating the sample with 0.1N of standard sulphuric acid solution to first pH 5.1, then from pH 5.1–3.5 [18]. Then the TVFA and bicarbonate alkalinity concentrations were calculated with a computer program by solving the following equations:

$$A_1 = \frac{[HCO_3^-]([H]_2 - [H]_1)}{[H]_1 + K_C} + \frac{[VA]([H]_2 - [H]_1)}{[H]_2 + K_{VA}} \quad (1)$$

$$A_2 = \frac{[HCO_3^-]([H]_3 - [H]_1)}{[H]_3 + K_C} + \frac{[VA]([H]_3 - [H]_1)}{[H]_3 + K_{VA}} \quad (2)$$

where A_1 and A_2 are the molar equivalent of the standard acid consumed to the first and second end points; $[HCO_3^-]$ the bicarbonate concentration; [VA] or [TVFA] the volatile fatty acid ion concentration; $[H]_{1,2,3}$ the hydrogen ion concentrations of the original sample and at the first and the second end points; K_C the conditional dissociation constant of carbonic acid; K_{VA} is the combined dissociation constant of the volatile fatty acids (C2–C6), this pair of constants was assumed to be 6.6×10^{-7} for bicarbonate and 2.4×10^{-5} for volatile acids.

2,4-DNT, 2,4-DAT, 2A4NT and 4A2NT were determined by high-pressure liquid chromatography (HPLC) [28] using an Agilent (1100) instrument with a Packing Ace 5-C18 reversed phase column (25 cm × 4.6 mm ID). All the samples were initially centrifuged (SED 5X model) to remove any particulate matter and then filtered through a 0.45 μm teflon filter using a disposable syringe (Agilent 5185-5835) prior to HPLC analysis. The auto sampler was set for an injection volume of 10 μL. The quantification was accomplished with a wavelength of 210 nm for 2,4-DNT. Elution was prepared with isocratic solvent system consisting of 50% methanol and 50% HPLC water at a flow-rate of 1.4 mL/min [28]. Quantification was carried out by the integration of the peak area.

2.5. Experimental and theoretical procedures

The AMBR was operated through 30 days only with glucose under steady-state conditions for acclimation of partially granulated anaerobic biomass in the AMBR. The steady state was arbitrarily considered as variation of COD in the effluent and the variations of the methane gas production and methane percentage less than 5%. COD removal efficiency and methane gas percentage

Table 1
Operational conditions for AMBR and sequential AMBR/CSTR during continuous operation.

Runs	Periods (days)	HRT	OLR	DNT conc.	DNT LR	SRT	HRT	OLR	DNT conc.	DNT LR	SRT
		AMBR					Sequential AMBR/CSTR				
1	25	10.00	0.32	140	0.14	360	16.60	0.32	140	0.14	380
2	30	8.61	0.35	41	0.05	108	14.34	0.35	41	0.05	128
3	25	8.61	0.37	239	0.28	322	14.34	0.37	239	0.28	342
4	20	5.25	0.57	0.00	0.00	183	8.75	0.57	0.00	0.00	203
5	25	5.25	0.6	140	0.27	194	8.75	0.60	140	0.27	214
6	20	5.25	0.61	280	0.53	147	8.75	0.61	280	0.53	167
7	25	1.89	1.59	41	0.22	57	3.15	1.59	41	0.22	77
8	25	1.89	1.69	239	1.26	55	3.15	1.69	239	1.26	75
9	25	0.50	6.30	140	2.80	15	0.83	6.30	140	2.80	35

OLR = organic loading rate (kg COD/L day), HRT = hydraulic retention time (day), SRT = solid retention time (day), DNT LR = 2,4-DNT loading rate (g DNT/L day), DNT conc. = 2,4-DNT concentration (mg/L), and SRT = 20 days in aerobic CSTR.

Table 2
The coded values for the operating levels of the variables in Box–Wilson statistical experiment design in AMBR.

Variable	Symbol	Coded variable level				
		Maximum (+*k*)	Minimum (−*k*)	Central (0)	Intermediate-a (+1)	Intermediate-b (−1)
2,4-DNT dose (mg/L)	X_1	280	0	140	239	41
HRT (day)	X_2	10	0.5	5.25	8.65	1.89

were measured as 92% and 56%, respectively, after 30 days of operation period in the AMBR. After this operation time, the COD removal efficiency and methane gas percentage were constant for 7 consecutive days, showing that steady-state conditions were reached in the AMBR without 2,4-DNT addition. Continuous operation with 2,4-DNT was started after the start-up period.

Table 1 shows the hydraulic retention time (HRT), organic loading rate (OLR), 2,4-DNT loading rates (DNT LR), 2,4-DNT concentrations (DNT conc.) and solid retention time (SRT) applied to the AMBR and to the sequential AMBR/CSTR system through continuous operations, which was dependent on Box–Wilson experimental procedure. The AMBR and CSTR were operated at steady-state conditions for approximately 20–30 days in every HRT and 2,4-DNT concentration in continuous mode. The HRTs were not decreased before reaching steady-state conditions. Steady state throughout continuous operation was defined by the constant daily biogas production, effluent COD and DNT concentrations until a maximum variation of 5% for 4 consecutive days in the AMBR. All the datasets were collected under steady-state conditions. The results given in the figures and tables are the average values of the triplicate samplings.

The experimental conditions through continuous operation of AMBR were determined by the Box–Wilson statistical experiment design. This method was used to investigate the effects of the two independent variables (HRT and 2,4-DNT concentration in the influent) on the response functions (COD removal efficiency, 2,4-DNT removal efficiency, biogas production, effluent pH, TVFA and TVFA/Bic.Alk. ratio) and to determine the optimal conditions maximizing the percent COD and 2,4-DNT removals in the AMBR.

In the experimental procedure, 2,4-DNT concentration (X_1) and HRT (X_2) were chosen as independent variables. 2,4-DNT concentration (X_1) was changed between 0 and 280 mg/L and HRT (X_2) varied between 0.5 and 10 days. The feed 2,4-DNT concentration did not increase >280 mg/L due to it having a solubility in water of 270 mg/L at 22 °C [1].

In this study, COD and 2,4-DNT removal efficiencies, biogas production, effluent pH, TVFA concentration and TVFA/Bic.Alk. ratios were considered as dependent variables in the Box–Wilson statistical design method.

The design principle includes three type of combinations: the axial (*A*), factorial (*F*), and center (*C*) points. The axial points include each variable at its extreme levels coded as $-k$ and $+k$ with the others at their center point level. The factorial points, with two levels of each of the factors coded as −1 and +1, include all combinations of intermediate levels. The center point, coded as 0, is a single test at the average level of each variable. The coded values for the operating levels of the variables are used for convenience. Table 2 shows the coded values for the operating levels of the variables in Box–Wilson statistical experiment design in AMBR. Where k is defined as minimum ($-k$) and maximum ($+k$) values for each variable, central (0) = range/2 is defined for each variable, and intermediate values are defined as central ± range/2t, where t

Table 3
Experimental conditions according to Box–Wilson statistical design in AMBR.

		Coded values		Real values	
		X_1	X_2	2,4-DNT conc. (mg/L)	HRT (days)
Axial point	A1	0	$+k$	140	10.00
	A2	0	$-k$	140	0.50
	A3	$+k$	0	280	5.25
	A4	$-k$	0	0	5.25
Factorial point	F1	1	−1	239	1.89
	F2	−1	1	41	8.61
	F3	1	1	239	8.61
	F4	−1	−1	41	1.89
Control point	C1	0	0	140	5.25
	C2	0	0	140	5.25
	C3	0	0	140	5.25

Table 4
Coefficients of the response functions for COD and 2,4-DNT removal efficiencies, total gas, methane gas productions, methane percentages, pH, TVFA concentrations and TVFA/Bic.Alk. ratios in AMBR.

	B_0	B_1	B_2	B_{12}	B_{11}	B_{22}
2,4-DNT (mg/L)	100.06	−0.0013	−0.0153	3.29×10^{-5}	3.87×10^{-6}	0.001
COD (mg/L)	90.71	−0.0396	1.8333	4.6×10^{-4}	6.62×10^{-5}	−0.119
Total gas (L/day)	12.23	−0.0115	−2.1054	1.4×10^{-3}	-2.5×10^{-5}	0.108
Methane gas (L/day)	4.45	−0.0047	−0.6377	5.87×10^{-4}	-1.1×10^{-5}	0.029
% Methane	41.55	−0.188	4.580	-3.7×10^{-3}	3.76×10^{-4}	−0.249
pH	6.95	0.0023	0.1995	-1.3×10^{-4}	-9.8×10^{-6}	−0.010
TVFA (mg/L)	407.19	3.7846	−144.894	−0.145	-7.4×10^{-3}	9.653
TVFA/Bic.Alk.	0.198	0.0013	−0.0692	-5.6×10^{-5}	-2.4×10^{-6}	0.005

Table 5
Experimental and predicted COD and 2,4-DNT removal efficiencies in AMBR.

	COD removal efficiency (%)		2,4-DNT removal efficiency (%)	
	Experimental	Predicted	Experimental	Predicted
A1	93.60	93.51	99.64	99.65
A2	86.33	87.38	99.15	99.12
A3	91.94	91.82	99.65	99.67
A4	96.04	97.04	*	*
F1	88.75	88.27	99.59	99.59
F2	96.70	96.30	99.88	99.85
F3	92.53	92.91	99.54	99.61
F4	93.54	92.27	99.46	99.36
C1	93.14	93.14	99.42	99.42

* No removal, without 2,4-DNT.

is equal to $\sqrt{p}$ (p = number of variables). The experimental conditions determined by the Box–Wilson statistical design method for the AMBR are presented in Table 3. The experiments consist of four axial (A), four factorial (F), and three central points (C) totaling 11 experiments. Computation was carried out by using the multiple regression analysis with the least squares method.

The predicted COD removal efficiency (Y_{COD}), 2,4-DNT removal efficiency (Y_{DNT}), biogas production efficiency (Y_B), effluent pH (Y_{pH}), effluent TVFA (Y_{TVFA}) and effluent TVFA/Bic.Alk. ($Y_{TVFA/B.A}$) were correlated with the other independent parameters 2,4-DNT concentration (X_1) and HRT (X_2) using Eq. (3).

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_{12} + b_{22}X_2^2 \quad (3)$$

where Y = the predicted response function (COD and 2,4-DNT removal efficiencies, biogas production, pH, TVFA concentration and TVFA/Bic.Alk. ratio), b_0 = constant, b_1–b_2 = the linear coefficients, b_{12} = cross product coefficient and b_{11}–b_{22} = quadratic coefficients. These coefficients were determined by using a STATISTICA 5.0 computer program.

3. Results and discussion

Experimental results found in this study were used to determine the coefficients of the response function (Eq. (1)) using a statistical regression analysis program "STATISTICA". The calculated coefficients are listed in Table 4 and they were used to calculate the predicted values of COD and 2,4-DNT removal efficiencies, total gas and methane gas productions, methane percentage, pH levels, TVFA concentrations and TVFA/Bic.Alk. ratios in the effluent of the AMBR. Response functions for COD (Y_{COD}) and 2,4-DNT (Y_{DNT}) removals are presented in Eqs. (4) and (5) with the determined coefficients.

$$Y_{COD} = 90.71 + (-0.0396)X_1 + (1.83)X_2 + (0.00046)X_1X_2 + (6.62 \times 10^{-5})X_1{}^2 + (-0.119)X_2^2 \quad (4)$$

$$Y_{DNT} = 100.06 + (-0.0013)X_1 + (-0.0153)X_2 + (3.29 \times 10^{-5})X_1X_2 + (3.87 \times 10^{-6})X_1{}^2 + (0.001)X_2^2 \quad (5)$$

The coefficients determined from the response function (Y_{COD}) were used to estimate the COD removal efficiency with the independent variables such as different HRTs and 2,4-DNT concentrations in the feed. The experimental and predicted COD removal efficiencies are depicted in Table 5 for the AMBR. A significant correlation between the experimental and the predicted COD removal efficiencies (R^2 = 0.95; F = 12.56, p = 0.01) (data not shown) was found in the AMBR. This result indicated that the predicted COD yields exhibited excellent agreement with the experimental ones. As shown in Tables 2 and 5, the experimental and predicted COD removal efficiencies were 93.60% and 93.51%, respectively, at a 2,4-DNT concentration of 140 mg/L in the feed at an HRT of 10 days. The maximum experimental and predicted COD removal efficiencies were found as 96.70% and 96.30%, respectively, at a 2,4-DNT concentration of 41 mg/L in the feed at an HRT of 8.61 days. The minimum experimental and predicted COD removal efficiencies were 86.30% and 87.38%, respectively, at a 2,4-DNT concentration of 140 mg/L in the feed at an HRT of 0.50 day.

A significant correlation between the experimental and the predicted 2,4-DNT removal efficiencies was found in the AMBR (R^2 = 0.95; F = 13.67, p = 0.01) (data not shown). This result indicated that the predicted 2,4-DNT yields exhibited excellent agreement with the experimental ones (see Table 5). The experimental and predicted 2,4-DNT removal efficiencies were 99.65% and 99.65%, respectively, at a 2,4-DNT concentration of 140 mg/L in the feed at an HRT of 10 days (see Tables 2 and 5). The maximum experimental and predicted 2,4-DNT removal efficiencies were 99.85% and 99.85%, respectively, at a 2,4-DNT concentration of 41 mg/L in the feed at an HRT of 8.61 days. The minimum experimental and predicted 2,4-DNT removal efficiencies were found as 99.15% and 99.12%, respectively, at a 2,4-DNT concentration of 140 mg/L in the feed at an HRT of 0.5 day.

Table 6 shows the experimental and the predicted total and methane gas productions and methane percentages. The experimental total gas, methane gas productions and the methane percentage values showed good agreement with high correlation coefficients in the AMBR (R^2 = 0.99, F = 12.56, p = 0.01; R^2 = 0.97, F = 13.67, p = 0.01; R^2 = 0.95, F = 14.76, p = 1, respectively) (data not

Table 6
Experimental and predicted total gas, methane gas and methane percentage in the AMBR reactor.

	Total gas (L/day)		Methane gas (L/day)		Methane percentage (%)	
	Experimental	Predicted	Experimental	Predicted	Experimental	Predicted
A1	1.90	1.93	0.85	0.81	38	38
A2	9.00	9.20	3.35	3.39	24	25
A3	0.95	1.08	0.55	0.60	29	30
A4	4.00	4.17	2.15	1.86	59	59
F1	5.20	5.10	1.92	1.97	25	24
F2	2.15	2.14	0.85	1.03	54	51
F3	0.95	0.91	0.54	0.47	32	31
F4	8.40	8.24	2.85	3.19	42	42
C1	3.10	3.12	1.56	1.56	37	37

shown). The maximum experimental and predicted total and methane gas productions were 9.00, 9.20 L/day and 3.36, 3.39 L/day, respectively, at a 2,4-DNT concentration of 140 mg/L in the feed at an HRT of 0.5 day (see Tables 2 and 6). The minimum experimental and predicted total gas and methane gas productions were 0.91, 0.96 L/day and 0.47, 0.54 L/day, respectively, at a 2,4-DNT concentration of 239 mg/L in the feed at an HRT of 8.61 days. The maximum methane percentage (49%) was found in the influent wastewater samples without 2,4-DNT at an HRT of 5.25 days.

Table 7 shows the experimental and the predicted pH and TVFA concentrations and TVFA/Bic.Alk. ratios in the effluent of the AMBR. The experimental and the predicted pH, TVFA and TVFA/Bic.Alk. ratios showed a good agreement with the correlation coefficients (R^2) of 0.98 and 0.92 (R^2 = 0.98, F = 14.97, p = 0.01; R^2 = 0.92, F = 14.65, p = 0.01; R^2 = 0.96, F = 14.77, p = 0.01; respectively) (data not shown). The maximum experimental and predicted TVFA concentrations were measured as 850 and 715 mg/L at a 2,4-DNT concentration of 140 mg/L, respectively, in the feed at an HRT of 0.5 day (see Tables 2 and 7). The minimum experimental and predicted TVFA concentrations were obtained at an HRT of 5.25 days without 2,4-DNT. The pH and TVFA/Bic.Alk. ratios were between 7.17 and 7.20 and between 0.35 and 0.31, respectively, at the HRT mentioned above.

3.1. Variation of predicted COD removal efficiencies depending on 2,4-DNT concentration as functions of HRTs in an AMBR reactor

Fig. 2 shows the variations of predicted COD removal efficiencies with increasing 2,4-DNT concentrations in the feed at different HRTs calculated from the Box–Wilson model used. As shown in Fig. 2, the predicted maximum COD removal efficiencies decreased from 96.70% to 86.00% as the HRTs decreased from 10 days to 0.5 day at a 2,4-DNT concentration of 280 mg/L in the feed. The lowest COD removals (85.70–87.30%) were obtained at an HRT of 0.5 day for >140 mg/L 2,4-DNT concentrations (140–280 mg/L). The predicted effluent COD concentrations were 270 mg/L and 495 mg/L at HRTs of 0.5 day and 10 days, respectively, at the highest 2,4-DNT

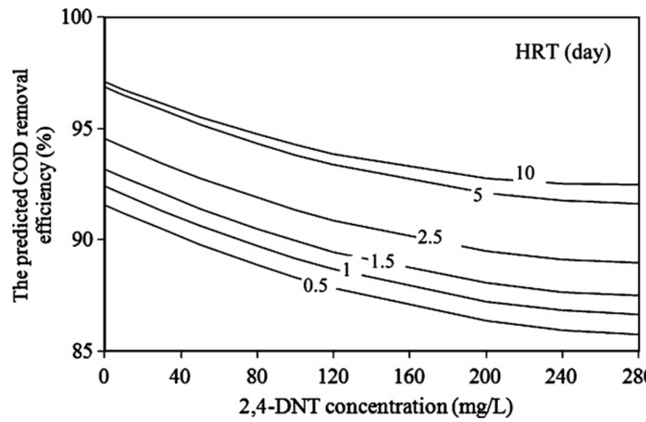

Fig. 2. Variations of predicted COD removal efficiency (%) with 2,4-DNT concentration in the feed as a function of HRT in AMBR.

concentrations of 280 mg/L (data not shown). In order to obtain high COD removals varying between 93.00% and 96.00%, the HRT should be between 2.5 and 10 days for 2,4-DNT concentrations <40 mg/L.

A decrease in the predicted COD removal efficiencies of the AMBR in the effluent of the AMBR with reduction of HRTs and an increase of 2,4-DNT concentrations are expected phenomena (see Fig. 2). The non-metabolized and non-mineralized intermediate products such as 2-amino-4-nitrotoluene (2-A-4-NT), 4-amino-2-nitrotoluene (4-A-2-NT) and 2,4-diaminotoluene (2,4-DAT) of 2,4-DNT were measured as COD and increased the COD concentrations in the effluent of the AMBR resulting in lower COD removal efficiencies (see Section 3.6). The toxicities of intermediate products such as the 2-A-4-NT, 4-A-2-NT and 2,4-DAT could also be the reason for low COD removals at low HRTs and high 2,4-DNT concentrations. It was reported that the accumulation of nitroso and hydroxyl amino intermediates such as 2-A-4-NT, 4-A-2-NT and 2,4-DAT could be more toxic than the parent compound (2,4-DNT) [6]. Oral LD_{50} values (the LD_{50} is the dose that kills half of the animals tested; LD = lethal dose) involving the acute exposure of rats,

Table 7
Experimental and predicted pH, TVFA concentrations and TVFA/Bic.Alk. ratios in the effluent of the AMBR reactor.

	pH		TVFA (mg/L)		TVFA/Bic.Alk. ratio	
	Experimental	Predicted	Experimental	Predicted	Experimental	Predicted
A1	7.82	7.83	210	223	0.06	0.07
A2	7.20	7.17	850	715	0.35	0.31
A3	7.50	7.39	125	89	0.03	0.03
A4	7.71	7.71	0.00	0.00	0.00	0.00
F1	7.20	7.23	405	499	0.21	0.23
F2	7.93	7.92	0.00	26.66	0.00	0.00
F3	7.60	7.60	105.00	95.67	0.03	0.01
F4	7.34	7.36	189	319	0.10	0.14
C1	7.74	7.74	686	186	0.06	0.07

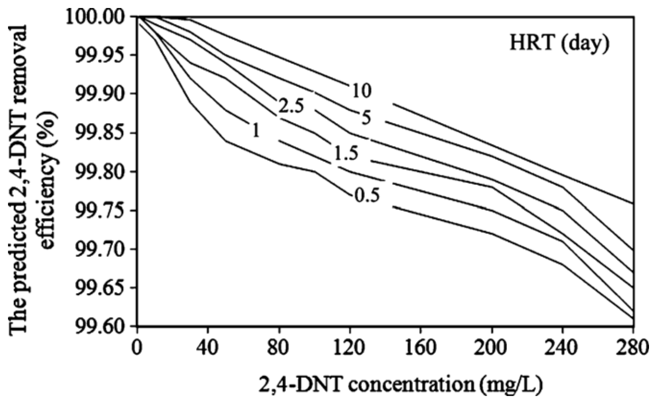

Fig. 3. Variations of predicted 2,4-DNT removal efficiency (%) with feed 2,4-DNT concentration as a function of HRT in AMBR.

mice, and rabbits to the 2,4-DAT have shown that they have moderate toxicities from low (500–5000 mg/kg) to high (50–500 mg/kg) toxicities [29].

3.2. Variation of predicted 2,4-DNT removal efficiencies depending on 2,4-DNT concentrations as functions of HRT in the AMBR

Variations of predicted 2,4-DNT removal efficiencies versus increasing 2,4-DNT concentrations are illustrated in Fig. 3 as functions of the HRT. The predicted 2,4-DNT removal efficiencies decreased from 99.85% to 99.63% as the HRTs decreased from 10 days to 0.5 day at a 2,4-DNT concentration of 280 mg/L in the feed. Nearly 100% 2,4-DNT removals were obtained at <40 mg/L DNT concentrations for HRTs between 2.5 and 10 days while the predicted 2,4-DNT was removed with a yield around 99.85% in the AMBR even at an HRT as low as 0.5 day for 2,4-DNT concentration <40 mg/L. In some recent studies the AMBRs were used to treat the nitrobenzene (NB) which is another toxic nitroorganic compound [22,30]. In the first study, the NB and COD removals were found to be 85% and 100% at an influent NB concentration of 400 mg/L and at an HRT of 10 days in the AMBR [22]. The COD and NB removal efficiencies were 79% and 100% at an HRT of 1 day and at an influent NB concentration of 60 mg/L [30].

In the present study the optimum HRT was considered as the HRT which gives the highest COD and 2,4-DNT removal efficiencies in the AMBR. Thus, the optimum HRTs and 2,4-DNT concentration in the influent resulting in maximum COD (96.70%) and 2,4-DNT removal efficiencies (around 100%) were determined between 2.5 and 10 days and <40 mg/L in the AMBR using the Box–Wilson design method (see Figs. 2 and 3). The COD removal efficiencies were nearly 97%, 95%, 94%, 93% and 92% for 2,4-DNT concentrations varying between 0 and 10 mg/L, 10 and 30 mg/L, 30 and 50 mg/L, 50 and 100 mg/L, 100 and 120 mg/L and between 120 and 280 mg/L at an HRT of 5 days. The 2,4-DNT removal efficiencies were approximately 99.92%, 99.87%, 99.80% and 99.76% for 2,4-DNT concentrations of 80, 120, 200 and 280 mg/L at a 5-day HRT.

The results obtained in this study were higher that those reported by Sponza and Atalay [16]. 85% COD and 90% DNT removal efficiencies were obtained at a maximum DNT concentration of 125 mg/L corresponding to a loading rate of 250 mg DNT/L day in an UASB reactor at an HRT of 0.5 day [16]. Vanderloop et al. [31] reported 100% removal for 2,4-DNT in anaerobic/anoxic fluidized-bed granular activated carbon (GAC) bioreactor. The 2,4-DNT yields obtained in this study agree with the data mentioned above with a 2,4-DNT removal efficiency of 99.90% in the AMBR.

If the AMBRs were to be operated at lower HRTs giving higher COD and 2,4-DNT removal efficiencies, the recommended optimum HRTs would change depending on influent 2,4-DNT concentrations. Table 8 shows the recommended optimum HRTs depending on influent 2,4-DNT concentration, COD and 2,4-DNT removal efficiencies, total gas, methane gas productions, methane gas percentages, pH, TVFA levels and TVFA/Bic.Alk. ratios in the AMBR. As shown in Table 8, the optimum HRT which gives the maximum COD (97.00%) and 2,4-DNT removal (99.90%) efficiencies would be between 5 and 10 days at influent 2,4-DNT concentrations between 1 and 280 mg/L. If the AMBR were to be operated at low HRTs such as <5 days the COD and the 2,4-DNT removal efficiencies would change depending on the influent 2,4-DNT concentrations (see Table 8): As the influent 2,4-DNT concentration is between 1 and 50 mg/L, the recommended HRT was 0.5 day to obtain 90–92% COD and 99.90% 2,4-DNT removal efficiencies. If the influent 2,4-DNT concentrations were between 1–80, 1–100 and 1–200 mg/L for maximum 2,4-DNT yields the recommended HRTs would be 1 day, 1.5 days and 2.5 days, respectively (Table 8). For COD removal efficiencies varying between 92–97%, 90–92% and 90–95% the recommended HRTs would be 1 day, 1.5 days and 2.5 days, respectively, while the 2,4-DNT removal efficiencies were 99.85% at all HRTs (Table 8). Table 8 also shows the recommended HRTs for total and methane gases, methane gas percentages, pH, TVFA and TVFA/Bic.Alk. ratios in the effluent of the AMBR depending on the influent 2,4-DNT concentrations. For maximum total and methane gas productions and methane percentages varying between 10.6 and 11.2 L/day, 3.88 and 4.13 L/day and 35–44%, respectively, the recommended HRT would be 0.5 day for 1–50 mg/L 2,4-DNT concentrations (Table 8). For the pH and TVFA concentrations in the effluent of the AMBR varying between 7.05–7.14 and 341–504 mg/L, respectively, the recommended HRT should be 0.5 day at 2,4-DNT concentrations of 1–50 mg/L (see Table 8). For low TVFA concentrations (0.00–240 mg/L) the HRT would be between 5 and 10 days for influent 2,4-DNT concentrations of 1–280 mg/L.

3.3. Variations of predicted total, methane gas productions and methane percentages depending on 2,4-DNT concentrations as functions of HRTs in AMBR

Figs. 4 and 5 show the variations of predicted total gas and methane gas productions depending on 2,4-DNT concentrations as

Table 8
Recommended optimum HRTs depending on 2,4-DNT and COD concentrations in the feed, 2,4-DNT removal efficiencies, total gas, methane gas productions, methane percentages, pH, TVFA concentrations and TVFA/Bic.Alk. in the effluent of AMBR.

Inf. 2,4-DNT conc. (mg/L)	Recommended HRT (day)	COD removal eff. (%)	2,4-DNT removal eff. (%)	Total gas (L/day)	Methane gas (L/day)	Methane (%)	pH	TVFA (mg/L)	TVFA/Bic.Alk. ratio
1–50	0.5	90.00–92.00	99.00–99.90	10.6–11.2	3.88–4.13	35–44	7.05–7.14	341–504	0.17–0.22
1–80	1	90.00–92.00	99.00–99.90	9.27–10.23	3.43–3.83	33–46	7.14–7.25	276–516	0.14–0.22
1–100	1.5	90.00–93.00	99.00–99.90	8.13–9.31	3.06–3.55	32–48	7.23–7.34	216–495	0.11–0.20
1–200	2.5	90.00–95.00	99.00–99.90	5.06–7.64	1.93–3.02	27–51	7.38–7.49	108–495	0.06–0.19
1–280	5	92.00–97.00	99.00–99.90	1.25–4.41	0.58–1.97	29–58	7.39–7.69	0.00–241	0.00–0.08
1–280	10	92.00–97.00	99.00–99.90	0.87–2.02	0.36–0.93	29–62	7.40–7.90	0.00–102	0.00–0.05

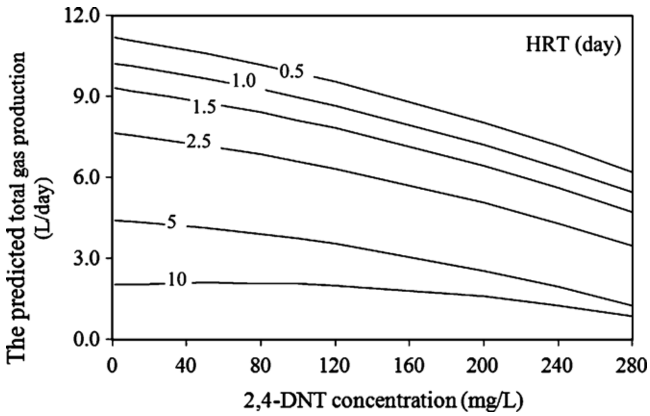

Fig. 4. Variations of predicted total gas production with feed 2,4-DNT concentration as a function of HRT in AMBR.

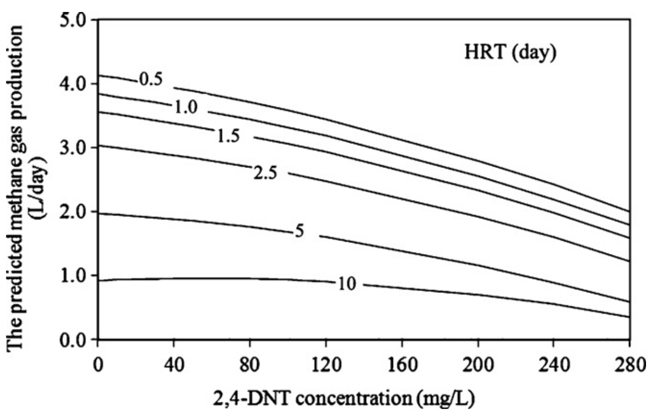

Fig. 5. Variations of predicted methane gas production with feed 2,4-DNT concentration as a function of HRT in AMBR.

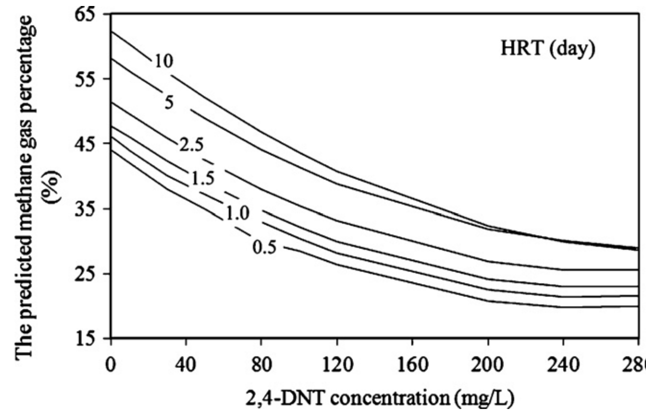

Fig. 6. Variations of predicted percent methane gas with feed 2,4-DNT concentration as a function of HRT in AMBR.

functions of HRTs. The total gas productions were very high (10 and 11 L/day) at short HRTs (0.5 and 1 day) at influent 2,4-DNT concentration of <40 mg/L. These yields were also the highest maximum total gas productions found in this study (Fig. 4). As the 2,4-DNT concentrations increased to 280 mg/L the total gas productions decreased at all HRTs due to toxic effect of 2,4-DNT and intermediate products to the methanogens since it was reported that high 2,4-DNT concentrations decreased the methanogenic activity in anaerobic processes. For example total gas production decreased to 2.00 L/day at an HRT of 10 days and at a 2,4-DNT concentration of 280 mg/L. In the study performed by Razo-Flores et al. [27], the 2,4-DNT concentration inhibiting the half of the methanogenic activity in granular anaerobic sludge (IC_{50}) was 4.91 mg/L indicating the inhibition of the methanogens.

The maximum methane gas productions were found around 4.0 L/day at 0.5 day HRT for 2,4-DNT concentration <40 mg/L in the influent (Fig. 5). Total and methane gas productions increased with decreasing HRTs and decreasing 2,4-DNT concentrations. The minimum methane gas production was 0.36 L/day, respectively, at 280 mg/L of 2,4-DNT concentration at 10 days HRT.

As shown in Figs. 4 and 5, when the HRT decreased from 10 days to 0.5 day, the total and methane gas productions increased from 0.87 to 6.22 L/day and from 0.36 to 2.0 L/day, respectively, at the highest 2,4-DNT concentration of 280 mg/L in the feed. Kuscu and Sponza [22,23] reported that the total and methane gas productions increased with decreasing HRT in the AMBR treating para-nitrophenol (p-NP) and NB. In this study, the total and methane gas productions increased from 2.7 to 11.7 L/day and from 1.3 to 3.3 L/day, respectively, at 40 mg/L of p-NP concentration in the feed when the HRT was decreased from 10.38 days to 1 day in the AMBR [22]. In another study performed by Kuscu and Sponza [23], the total and methane gas productions increased from 2.16 to 12.25 L/day and from 1.1 to 3.8 L/day by decreasing the HRT from 10.38 days to 1 day in the AMBR at a NB concentration of 60 mg/L in the feed [23].

The studies performed previously using the same AMBR [22] were compared with the data obtained in this study: In this study, the methane and total gas productions were found as 0.96–2.09 L/day (HRT = 10 days) and 3.94–10.74 L/day (HRT = 0.5 day) respectively, at an influent 2,4-DNT concentration of 40 mg/L from Figs. 4 and 5 using Box–Wilson statistical experiment design. These results were lower than the total and methane gas productions in the AMBR treating 40 mg/L p-NP at 0.5 and 10 days HRTs. This was due to high toxicity of 2,4-DNT (IC_{50} = 4.91 mg/L) compared to p-NP (IC_{50} = 8.45 mg/L) [27]. These results were higher than those reported by Sponza and Atalay [16]. The total gas, methane gas productions and methane yields were recorded as 2500 mL/day, 1800 mL/day and 73–76%, respectively, at a DNT loading rate of 6 mg/L day at an HRT of 0.5 day in an UASB [16]. Therefore, these results indicated that the gas productions in the AMBR were higher than those in the UASB reactor.

The predicted methane gas percentages with increasing 2,4-DNT concentrations in the AMBR are depicted in Fig. 6 as functions of HRTs. The methane gas percentage decreased by increasing the 2,4-DNT concentration and decreasing the HRT. The methane gas percentage decreased from 62% to 29% as the 2,4-DNT concentrations increased from 0 to 280 mg/L at an HRT of 10 days. The methane percentage decreased from 29% to 20% on decreasing HRTs from 10 days to 0.5 day at a 2,4-DNT concentration of 280 mg/L in the AMBR. The maximum methane percentage (49%) was found at 2,4-DNT concentrations of <40 mg/L at an HRT of 10 days.

The methane gas percentage decreased with decreasing of HRTs due to high TVFA accumulation in the AMBR at low HRTs and at high 2,4-DNT concentrations (Fig. 8). This can be explained by the partial dominancy of *acidogens* compared to *methanogens* at high organic loading rates or at low HRTs in the AMBR. In a study performed by our team the number of *acidogen* bacteria reached a value of 4×10^5 colony found unit/100 mL while the number of methanogens remained between 2×10^1 and 4×10^2 colony found unit (data not shown). The accumulation of VFA at low HRTs will be explained in the next subheading (Section 3.4). If the rate of acid formation exceeds the rate of breakdown to methane, a process unbalance results with decreases in methane content of biogas [32]. On the other hand, the volatile fatty acids converted to the intermediates and end products such as N_2 and H_2 instead of methane at short HRTs and high VFA concentrations [32]. Chaisri et al. [33]

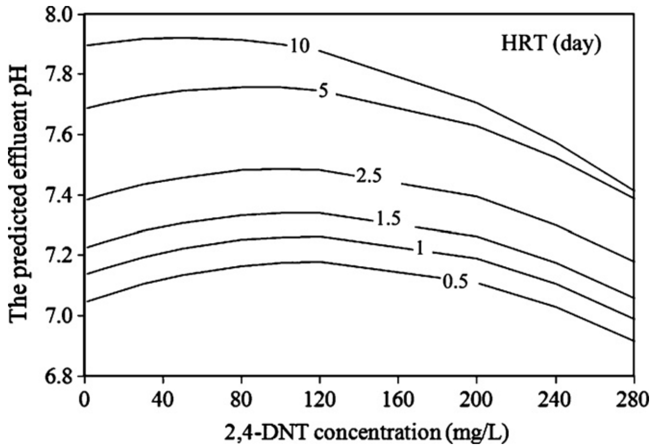

Fig. 7. Variations of predicted effluent pH with feed 2,4-DNT concentration as a function of HRT in AMBR.

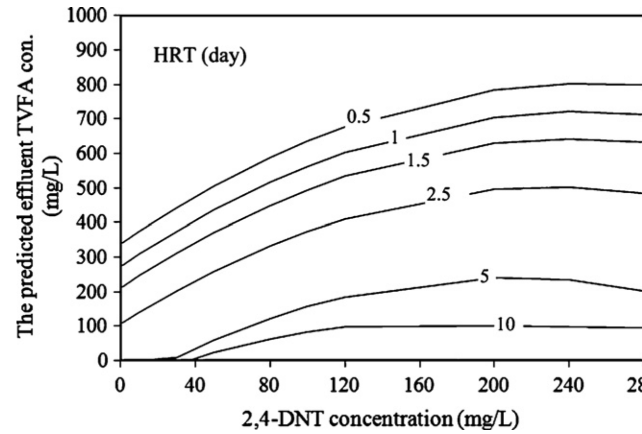

Fig. 8. Variations of predicted effluent TVFA with feed 2,4-DNT concentration as a function of HRT in AMBR.

reported that the conversion of refractory organic substances in wastewater to VFA was different and the conversion of VFA to CH_4 decreased with decreasing HRT under anaerobic conditions. In this study it was reported that when the HRT was decreased from 20 to 2.86 days, the *acidogenesis* increased from 24% to 33% while the *methanogenesis* decreased from 33% to 22% in an UASB reactor.

3.4. Variations of predicted pH and TVFA with 2,4-DNT concentration as a function of HRT in the AMBR

pH, alkalinity additionally volatile fatty acids are integral expressions of the acid–base conditions of anaerobic microbial treatment process, as well as an intrinsic index of the balance between two of the most important microbial groups, the obligate methanogens and the aceticlastic methanogens [34]. Methanogens prefer nearly neutral pH conditions with a generally accepted optimum range of about 6.5–8.2. Conditions above or below this range decrease the rate of methane production. Figs. 7 and 8 also show the variations of pH and TVFA concentrations with 2,4-DNT concentrations in the AMBR as functions of HRT. As shown in Fig. 7, the effluent pH values were optimum range between 6.5 and 8.5 at all HRTs and 2,4-DNT concentrations in the AMBR. The pH values decreased from 7.05 to 6.90 and from 7.9 to 7.45 at 0.5 and 10 days HRTs on increasing 2,4-DNT concentrations from 0 to 280 mg/L. This can be explained by the accumulation of TVFA in the AMBR with increasing 2,4-DNT concentrations (see Fig. 8). The TVFA concentrations increased from 95 to 798 mg/L, respectively, at the 2,4-DNT concentrations of 280 mg/L as the HRT decreased from 10 days to 0.5 day. The maximum TVFA concentration was 798 mg/L at 280 mg/L 2,4-DNT at 0.5 day HRT. The minimum TVFA concentration in the effluent of AMBR also was found as 0 mg/L at 10 days HRT at 2,4-DNT concentrations of <40 mg/L.

TVFA production is an important step for the metabolism of organisms under anaerobic conditions. Low VFA concentration indicates a stable reactor performance. In anaerobic compartmentalized reactor, the first compartment is referred as “acid fermentation” and involves the production of volatile fatty acids (VFA), while the second phase is referred as “methane fermentation” because the VFAs are converted to methane and carbon dioxide production [34,35]. The produced volatile fatty acids, mainly acetic acid, lower the pH in the first compartments of the AMBR (data not show). Despite the decrease of the pH values in the first compartments of the AMBR, the effluent pH value was optimum range of 6.5–8.2 at all HRTs and all 2,4-DNT concentrations.

Alkalinity is one of the most central concepts because it controls the pH and this is a measure to buffer the pH in the presence of acids in the anaerobic reactors [17]. Therefore, a sufficient bicarbonate alkalinity must be present to neutralize the wastewater under anaerobic conditions. If the acid concentrations (H_2CO_3 and TVFA) exceed the available alkalinity, the reactor will sour; this will severely inhibit the methanogens [17]. The alkalinity requirement is 1.2–1.6 g alkalinity as $CaCO_3$/g COD influent which is sufficient to maintain the pH above 6.6 under anaerobic conditions [17]. Therefore, in this study a required alkalinity was added to the feed wastewater to provide an optimum pH in the AMBR at the beginning of the study.

TVFA/Bic.Alk. ratio gives necessary information to determine the stability of the anaerobic reactor. If the TVFA/Bic.Alk. ratio is lower than 0.4, the reactor is stable. When the TVFA/Bic.Alk. ratio is lower than 0.8, the reactor system is moderately stable or unstable, as reported by Behling et al. [36]. Fig. 9 shows the variations of TVFA/Bic.Alk. ratios depending on increasing 2,4-DNT concentration in the feed as functions of HRTs. TVFA/Bic.Alk. ratios were lower than 0.4 at all HRTs and all 2,4-DNT concentrations. This showed that AMBR was stable when it was operated at HRTs varying between 0.5 and 10 days at 2,4-DNT concentrations increasing from 1 to 280 mg/L.

Table 9
COD removal efficiencies depending on HRTs in the aerobic CSTR reactor.

2,4-DNT conc. (mg/L)	HRT (day)	Anaerobic inf. COD (mg/L)	Anaerobic eff. COD (mg/L)	Aerobic eff. COD (mg/L)	Aerobic removal eff. COD (%)	Anaerobic/aerobic total COD removal (%)
0	5.25	3043	121	50	59	98
41	8.60	2930	93	45	52	98
41	1.89	2925	183	92	50	97
140	10.00	3270	207	95	54	97
140	5.25	3271	225	110	51	97
140	0.50	3230	395	180	54	94
239	8.60	3200	239	120	50	96
239	1.89	3298	371	202	46	99
280	5.25	3350	270	140	48	96

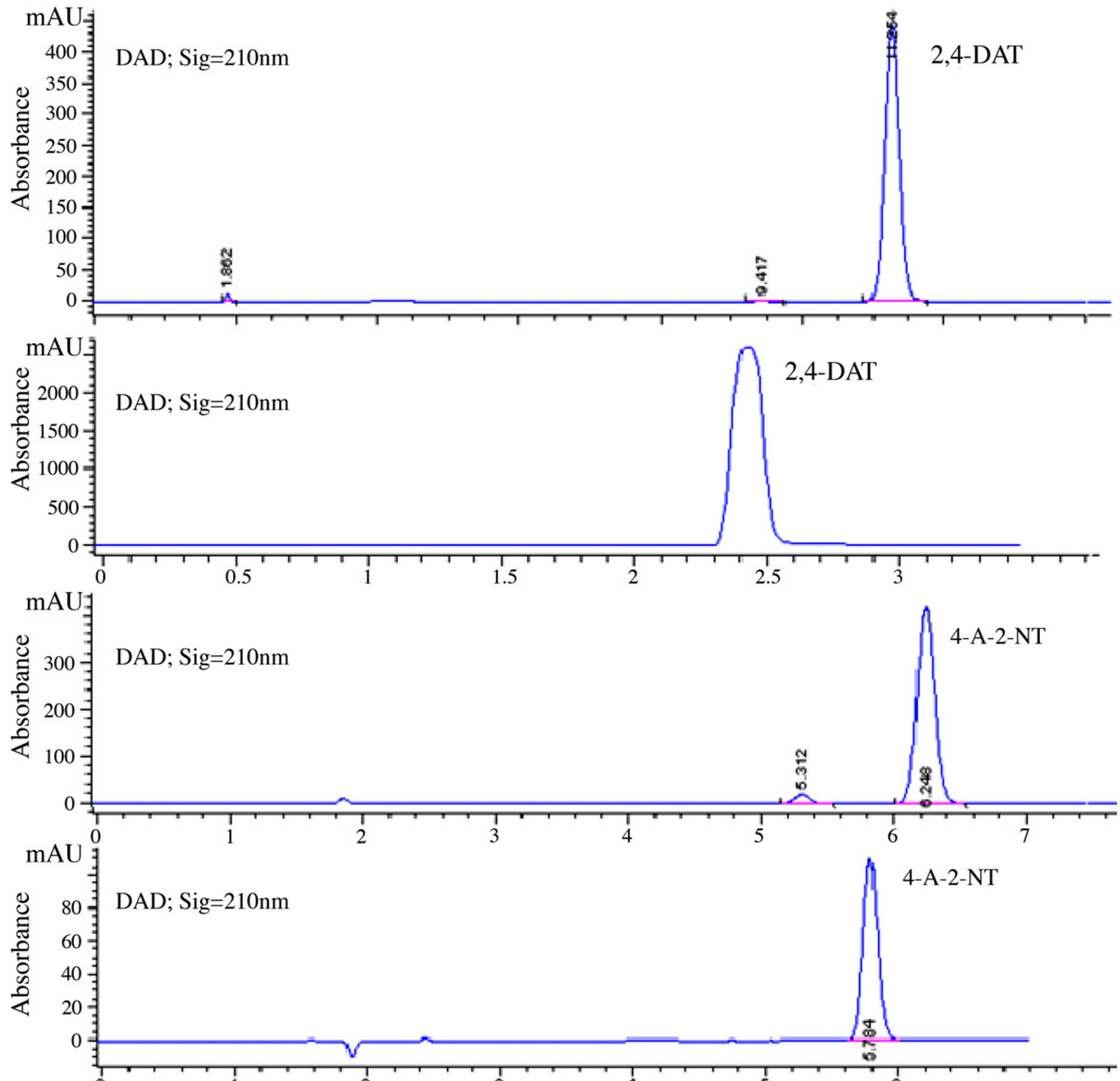

Fig. 10. The HPLC chromatogram of 2,4-DNT, 2,4-DAT, 2-A-4-NT and 4-A-2-NT standard solutions (100 mg/L) (Sig = 210 nm).

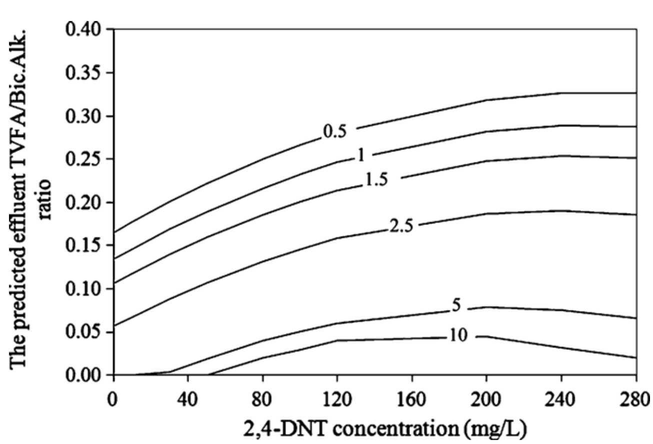

Fig. 9. Variations of predicted effluent TVFA/Bic.Alk. ratio with feed 2,4-DNT concentration as a function of HRT in AMBR.

3.5. The effect of aerobic stage on the COD and 2,4-DNT removals

A CSTR following the AMBR was used for the residual COD removal and mineralization of 2,4-DNT metabolites. The predicted or experimental effluent COD concentrations and the COD removal efficiencies in the aerobic reactor are shown in Table 9. The COD removal efficiencies varied between 46% and 59% depending on the influent 2,4-DNT concentrations and HRTs in the aerobic CSTR. High COD removal efficiencies were found (54–59%) at HRTs varying between 5.25 and 10 days HRT at 2,4-DNT concentrations varying between 1 and 140 mg/L in the CSTR. Lower COD removal efficiency (46%) was found at 1.89 days HRT at 239 mg/L 2,4-DNT in the CSTR. COD removal efficiency decreased from 54% to 47% on decreasing HRT from 10 days to 0.5 day at a 2,4-DNT concentration of 140 mg/L in this reactor. The maximum total COD removal efficiency was 99% at 239 mg/L 2,4-DNT concentration in the influent at 1.89 days HRT in the sequential AMBR/CSTR system.

3.6. Metabolic pathway of 2,4-DNT through sequential anaerobic (AMBR) and aerobic (CSTR)

It is reported that 2,4-DNT was transformed to aminonitrotoluenes such as 2,4-diaminotoluene (2,4-DAT), 2-amino-4-nitrotoluene (2Am4NT), and 4-amino-2-nitrotoluene (4Am2NT) in the AMBR [6,15,37,38]. Fig. 10 shows the HPLC chromatograms of 2,4-DNT, 2,4-DAT, 2-A-4-NT and 4-A-2-NT standard solutions. The peaks were obtained at retention times of 11.26, 2.45, 6.25 and 5.78 min for 2,4-DNT, 2,4-DAT, 2-A-4-NT and 4-A-2-NT, respec-

Table 10
Experimental transformation products and removal efficiencies of 2,4-DNT throughout sequential anaerobic/aerobic degradation in AMBR and CSTR.

Runs	HRT	AMBR reactor								
		2,4-DNT			2A4NT		4A2NT		2,4-DAT	
		Inf. (mg/L)	Eff. (mg/L)	Eff. (%)	Inf. (mg/L)	Eff. (mg/L)	Inf. (mg/L)	Eff. (mg/L)	Inf. (mg/L)	Eff. (mg/L)
1	5.25	0.00	0.00	99.99	0.00	0.00	0.00	0.00	0.00	0.00
2	8.60	41	0.00	99.99	0.00	0.52	0.00	0.50	0.00	25.17
3	1.89	41	0.00	99.99	0.00	0.40	0.00	0.50	0.00	23.90
4	10.00	140	0.00	99.99	0.00	0.60	0.00	1.91	0.00	91.76
5	5.25	140	0.00	99.99	0.00	2.96	0.00	0.37	0.00	87.70
6	0.50	140	0.00	99.99	0.00	1.27	0.00	4.18	0.00	89.60
7	8.61	239	0.00	99.99	0.00	1.89	0.00	0.20	0.00	158.00
8	1.89	239	0.00	99.99	0.00	2.05	0.00	0.70	0.00	144.00
9	5.25	280	0.00	99.99	0.00	2.44	0.00	0.40	0.00	182.50

Runs	HRT	CSTR reactor										
		2,4-DNT		2A4NT			4A2NT			2,4-DAT		
		Inf. (mg/L)	Eff. (mg/L)	Inf. (mg/L)	Eff. (mg/L)	Eff. (%)	Inf. (mg/L)	Eff. (mg/L)	Eff. (%)	Inf. (mg/L)	Eff. (mg/L)	Eff. (%)
1	5.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	8.60	0.00	0.00	0.52	0.20	62	0.50	0.00	100	25.17	4.50	82
3	1.89	0.00	0.00	0.40	0.10	75	0.50	0.00	100	23.90	5.70	76
4	10.00	0.00	0.00	0.60	0.10	83	1.91	0.00	100	91.76	23.75	74
5	5.25	0.00	0.00	2.96	1.31	56	0.37	0.05	86	87.70	32.70	63
6	0.50	0.00	0.00	1.27	0.50	60	4.18	0.80	81	89.60	39.20	56
7	8.61	0.00	0.00	1.89	0.70	89	0.20	0.00	100	158.00	32.00	80
8	1.89	0.00	0.00	2.05	0.50	76	0.70	0.00	100	144.00	41.00	72
9	5.25	0.00	0.00	2.44	0.63	74	0.40	0.00	100	182.50	76.90	58

tively. Table 10 shows the transformation products and removal efficiencies of 2,4-DNT through anaerobic/aerobic degradation. It was found that the 2,4-DAT is the main degradation product of the 2,4-DNT while 30–40% of the 2,4-DNT transformed to 2-A-4-NT and 4-A-2-NT metabolites in AMBR. Low 2,4-DAT production was obtained (23.9 mg/L) at an influent 2,4-DNT concentration of 41 mg/L at 1.89 days HRT in the AMBR. 2-A-4-NT and 4-A-2-NT were found as trace metabolites in which the concentrations were as low as 1.89 and 0.20 mg/L, respectively, in the effluent of the AMBR at an HRT of 8.61 days. This showed that 2,4-DNT transformed to 2,4-DAT via 2-A-4-NT and 4-A-2-NT under anaerobic conditions as reported by Cheng et al. [6] (see Table 10). From 280 mg/L 2,4-DNT in the influent of AMBR 182.50 mg/L 2,4-DAT, 2.44 mg/L 2A4NT and 0.40 mg/L 4A2NT were produced under anaerobic conditions at an HRT of 5.25 days. The 2,4-DAT, 2A4NT and 4A2NT were removed with yields of 58%, 74% and 100% at an HRT of 5.25 days in the effluent of aerobic CSTR (Table 10). Fig. 11 shows the metabolic pathway of 2,4-DNT through anaerobic degradation. 2,4-DAT, 2-A-4-NT and

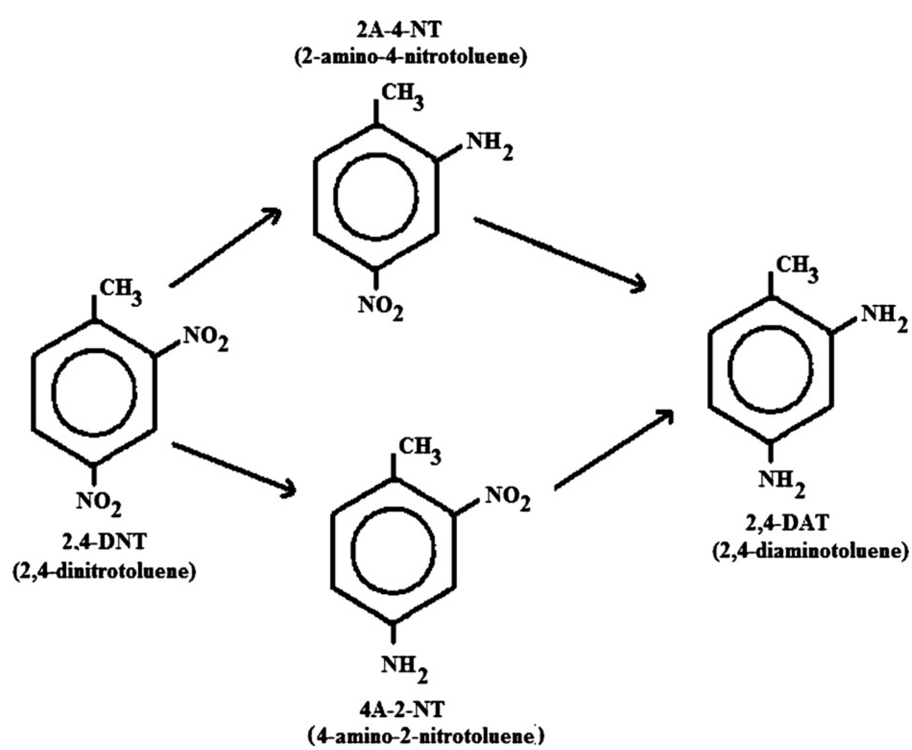

Fig. 11. The anaerobic pathway of 2,4-DNT.

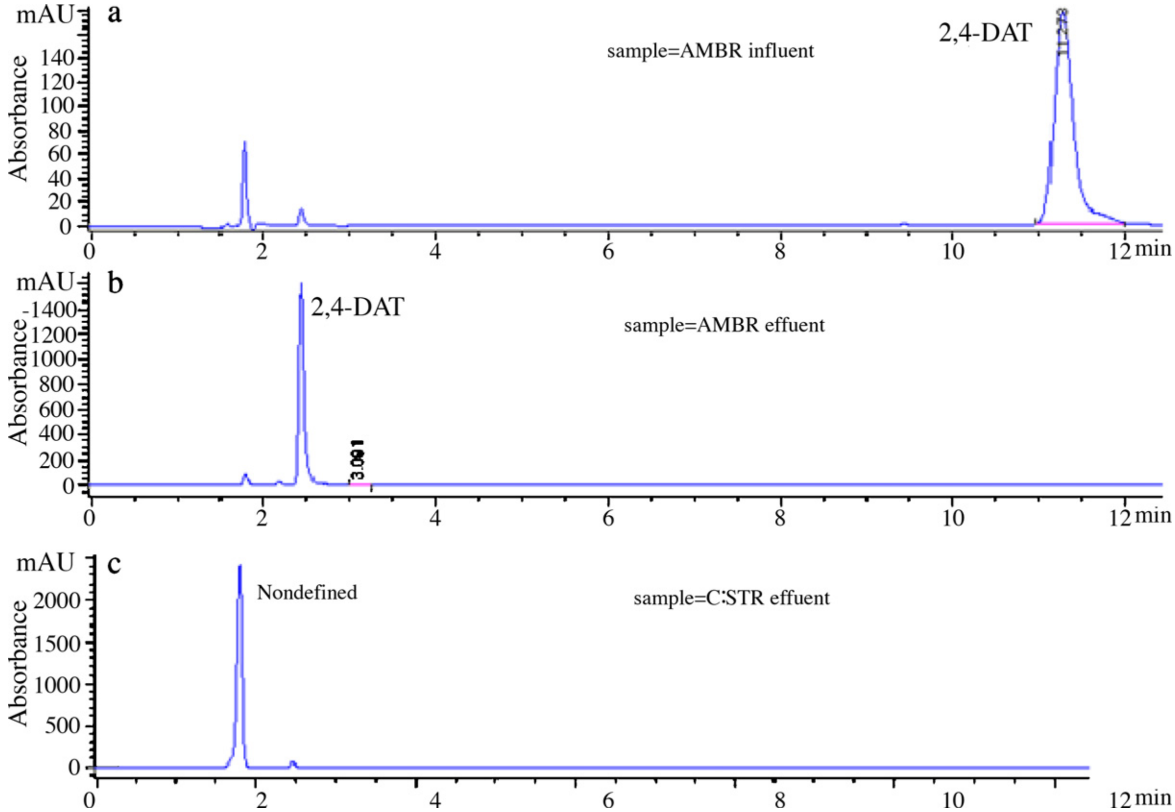

Fig. 12. The HPLC chromatogram of the influent (a) and effluent (b) of AMBR and the effluent of CSTR (c) (Sig = 210 nm).

4-A-2-NT concentrations were 76.9, 0.63 and 0.4 mg/L, respectively, at 280 mg/L 2,4-DNT concentration at 5.25 days HRT in the effluent of the CSTR. The maximum 2,4-DNT removal was 99.99% for HRTs varying between 0.50 and 10 days in AMBR at all 2,4-DNT concentrations. 2A4NT and 4A2NT were degraded with 89% and 100% yields at HRTs of 8.61 and 1.89 days, respectively, in the aerobic CSTR reactor at an initial 2,4-DNT concentration of 239 mg/L in the influent of the AMBR. 2,4-DAT was removed with yields varying between 80% and 82% at HRTs between 8.60 and 8.61 days in the aerobic reactor at 41–239 mg/L initial 2,4-DNT concentrations in the influent of the AMBR.

Fig. 12a, b shows the HPLC chromatograms of the metabolites in the influent and effluent of the AMBR while Fig. 12c shows metabolites in the effluent of the CSTR at an HRT of 10 days. In the first two chromatograms, a peak was obtained at retention time of 1.8 min which are the decomposition products of 2,4-DAT, 2-A-4-NT and 4-A-2-NT. This peak could not be identified in the aerobic reactor as shown in Fig. 12c. Maloney et al. [15] reported that all the DAT present in the activated sludge reactor was removed and oxidized to ammonia. As a result, 2,4-DNT was degraded to 2,4-DAT via 2-A-4-NT and 4-A-2-NT under anaerobic conditions, and then 2,4-DAT was mineralized under aerobic conditions in the sequential AMBR/CSTR.

4. Conclusions

A Box–Wilson statistical experiment design was used to determine the effects of operating parameters such as HRT and 2,4-DNT concentrations in the feed on COD and 2,4-DNT removal efficiencies, total gas and methane gas productions, methane percentage; pH, TVFA and TVFA/Bic.Alk. ratio in the effluent of the AMBR. In a Box–Wilson statistical experiment design, response function coefficients were determined by regression analysis of the experimental data and predicted results obtained from the response functions were in good agreement with the experimental results indicating the reliability of the methodology used.

The COD remaining from the anaerobic reactor and mineralization of 2,4-DNT metabolites were performed in the aerobic stage in the CSTR. In AMBR, 2,4-DNT was converted to 2,4-DAT via 2-A-4-NT and 4-A-2-NT, which are easily degraded in the CSTR. The maximum total COD and 2,4-DNT removal efficiencies were 99.00% and 99.99%, respectively, at an influent 2,4-DNT concentration of 239 mg/L at an HRT of 1.89 days in the sequential AMBR/CSTR. This shows that sequential AMBR/CSTR reactor system was successful in treating the toxic nitro organics such as 2,4-DNT.

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