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"Gheorghe Asachi" Technical University of Iasi, Romania

EFFECT OF MOLASSES ADDITION AS BIODEGRADABLE MATERIAL ON PHENOL REMOVAL UNDER ANAEROBIC CONDITIONS

Ali Almasi[1], Abdollah Dargahi[1], Abdeltif Amrane[2], Mehdi Fazlzadeh[3,4*], Mohammad Soltanian[1], Amirhosein Hashemian[5]

[1]*Department of Environmental Health Engineering, School of Public Health, Kermanshah University of Medical Sciences, Kermanshah, Iran*
[2]*Ecole Nationale Supérieure de Chimie de Rennes, Université de Rennes 1, CNRS, UMR 6226, Rennes, Avenue du Général Leclerc, CS 50837, 35708 Rennes Cedex 7, France.*
[3]*Department of Environmental Health Engineering, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
[4]*Social Determinants of Health Research center, Ardabil University of Medical Sciences, Ardabil, Iran*
[5]*Research Center for Environnemental Déterminants of Heath (RCEDH), Départment of Biostatistics and Epidemiology, School of Public Health, Kermanshah University of Medical Sciences, Kermanshah, Iran*

Abstract

The link between the concentration of biodegradable organic matter and the rate of phenol removal in anaerobic conditions was examined in this work. This study was undertaken using a laboratory scale anaerobic stabilization pond and five closed reactors at two retention times, 2 and 5 days. The initial concentration of phenol and soluble COD decreased in the anaerobic pond effluent and the reactors with increasing hydraulic retention time; 98.6 % phenol removal was observed in reactors after 50 days and in the presence of 1000 mg/L COD, while 98.8 % removal was obtained after 5 days in the anaerobic stabilization pond system in the presence of 500 mg/L COD. Two-way ANOVA test with Scheffe post-hoc confirmed that phenol removal was optimal for 1000-2000 mg/L biodegradable COD and 50 days retention time in the reactors and with 500 mg/L biodegradable COD and 5 days retention time in the anaerobic pond.

Key words: anaerobic reactor, anaerobic stabilization pond, biodegradable organic matter, phenol removal

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1. Introduction

Phenols are among the most common organic pollutants because of their toxicity even at low concentrations (Busca et al., 2008; Almasi et al., 2014; Senturka et al., 2009; Zhonga et al., 2012). Phenol and its derivatives are common organic pollutants existing in wastewater of many chemical plants such as paper and pulp, pesticides, dyes, and chemical manufacturing industries. Besides, wastewater originating from many other industries contains various types of phenols (Bu et al., 2011; Caetano et al., 2009; Reis et al., 2011; Senturka et al., 2009; Vergara-Fernandez et al., 2017). Phenols are also present in domestic effluents and vegetation decay (Caetano et al., 2009). Therefore, wastewaters containing phenolic compounds pose a serious disposal problem due to their poor biodegradability, high toxicity and other ecological aspects (Sarac et al., 2017; Senturka et al., 2009; Zhonga et al., 2012).

On the other hand, phenol has relevant health effects on humans. Indeed, phenol is rapidly absorbed through the skin and can cause skin and eye burns upon contact. Therefore, they are considered as priority pollutants since they are harmful to organisms at low concentrations and many of them

* Author to whom all correspondence should be addressed: E-mail: m.fazlzadeh@gmail.com; Tel: +989127035079; Fax: +98 4516612004

have been classified as hazardous pollutants because of their potential harm to human health (Vimal et al., 2006; Zhonga et al., 2012). According to the World Health Organization regulation, 0.002 mg/L is the permissible limit for phenol concentration in potable water and the regulations by the Environmental Protection Agency (EPA) call for lowering phenol content in wastewaters to less than 1 mg/L (Senturka et al., 2009). Consequently, wastewaters containing phenols and other toxic compounds must be treated before discharge into the aquatic environment to avoid legal problems (Cozma et al., 2012; Reis et al., 2007). Biological processes, physicochemical processes, adsorption processes, solvent extraction, chemical oxidation, membrane processes, reverse osmosis, ion exchange and electrochemical methods are the most widely used methods to remove phenol and phenolic compounds from wastewaters (Busca et al., 2008; Saitoh et al., 2009; Senturka et al., 2009; Reis et al., 2011; Zhonga et al., 2012). Some problems, such as high cost, low efficiency, formation of toxic by-products and applicability to a limited concentration range are associated with the above methods.

Biological methods have little or no harmful effects on the environment, because these techniques do not involve the use of harmful reagents (Jarboui et al., 2010). Moreover, biological processes are less expensive if compared to other processes that can also lead to a complete mineralization of target compounds. Thus, biological removal and especially stabilization ponds have turned out to be a favorable alternative due to the absence of toxic end products and its low cost (Thavasi and Jayalakshmi, 2003).

Among the available natural biological treatment systems, stabilization ponds are among the simplest ones (Pirsaheb et al., 2014; Corbitt, 1999). Stabilization ponds provide a cheap and attractive alternative to conventional processes, when adequate land is available (Mara and Pearson, 1986; Almasi et al., 2015). Anaerobic ponds are the smallest units in the series. They are sized according to their volumetric organic loading, and may receive organic loads in the range of 100 to 350 g BOD_5 m^{-3} day^{-1}, depending on the temperature (Varon and Mara, 2004).The depth of anaerobic ponds is in the range 2−5 m and Hydraulic Retention Time are usually between 2 and 5 days (Varon and Mara, 2004; Steen, 2003). Anaerobic ponds are especially efficient in warm climates (Ghazy et al., 2008; Mara and Pearson, 1986; Varon and Mara, 2004; Naddafi et al., 2009; Steen, 2003).

In view of drawing a strategy for wastewater treatment industry, examination of anaerobic processes in order to enhance the degradation of materials in a mixed medium and then investigation of the mutual influence of recalcitrant and non-recalcitrant materials facing biological degradation was the purpose of this work, since this kind of effluents are constantly exposed to a mixture of degradable and non-degradable materials. In this aim, phenol removal in anaerobic pond system, as well as the impact of the presence of a co-substrate with high biodegradation ability, molasses, were investigated. Furthermore and to improve the knowledge regarding the biological process inside the anaerobic pond, closed lab-scale reactors (Erlenmeyer flasks) were also considered.

2. Material and methods

This study was undertaken using a laboratory scale anaerobic stabilization pond (0.2×1×1 m) made of 6 mm fiberglass plate. The temperature of the ambient air was in the range of 25 to 42 ºC. The average temperature of the pond was kept to 21±2 ºC. Hydraulic retention times of the anaerobic pond were 2 and 5 days and hydraulic loads of the system were 95 and 40 L/day, respectively. Inlet of the anaerobic pond was placed 30 cm below the level of the liquid in the pond. The full characteristics of the pilot are shown in Fig. 1. The pond was daily loaded by the wastewater output of oil and grease separator unit of Kermanshah Oil Refinery; but before the launch of the system it underwent seeding and inoculation operations. After 3 months of seeding, the anaerobic pond system was ready for launching. After system startup and ascertainment of biological stabilization, biopsy of system input and output was performed. Phenol was added to the input of the pilot at an initial concentration of 100 mg/L (oil refinery wastewater containing phenol).

To determine biological process inside the anaerobic pond, five closed reactors (five Erlenmeyer flasks equipped with air and gas permeation control system) were used in laboratory-scale. The reactors volume was set at 550 mL; characteristics and materials used in reactors are listed in Table 1. Seed containing anaerobic microorganisms was provided from pilot anaerobic lagoon. After enrichment of bacteria with medium containing essential micro-nutrients and molasses as indicated in Table 2, the seed containing enriched microorganisms was added to the reactors. Constant phenol concentration, 100 mg/L (COD 344 mg/L), was tested in the presence of sugar beet molasses (biodegradable organic matter as co-substrate) at COD values of 500, 1000, 2000, 5000 and 10000 mg/L under anaerobic conditions. For each concentration of biodegradable organic matter and constant phenol amount, five retention times (10, 20, 30, 40 and 50 days) were allocated to the reactors. Descriptive statistics used to display data and analytical statistics (e.g. ANOVA with Scheffe post-hoc) were applied to indicate variance between phenol removal in the presence of various biodegradable organic matter concentrations and retention times in the reactors using SPSS 12 software.

The measurement method for the COD of phenol was Closed Reflex (Standard methods 5220 C) and was done by the thermal COD reactor, HACH type. Phenol was also measured by Shimadzo UV-visible Spectrophotometer at a wavelength of 500 nm (APHA, 2005).

Initially, to each reactor and according to process progress and the need for nutrient addition until the time of gas production, two drops of nutrient solution were added as specified in Table 2. Reactors were magnetically stirred at a constant stirring rate and temperature was adjusted to laboratory temperature (30±5 ºC). Reactors' pH were kept constant in the range 6.5-7 and when needed, namely at time of severe pH decrease, alkalinization was provided by means of sodium carbonate addition. The assessment of reactors performance was based on the yields of COD and phenol removal. Soluble COD samples were prepared via centrifugation for 15 minutes at 6000 rpm speed. The total number of samples in this study consisted of 500 and 300 samples for reactors and anaerobic pond system, respectively.

Phenol used in this study was of analytical grade and was obtained from Merck (Darmstadt, Germany). All sampling procedures and parameters analysis were done according to standard methods for the examination of water and wastewater (APHA, 2005). After the selection of parameters, their removal percentage (*R*%) was calculated for each run as follows (Eq.1):

$$R\% = \left[\frac{Ci - Ce}{Ci}\right] \times 100 \qquad (1)$$

where C_i and C_e are the initial and final phenol concentrations, respectively.

3. Results and discussion

Quantitative results are displayed in Tables 3, 4 and 5. As seen in Table 3, the final concentrations of phenol and soluble COD decreased with the retention time in the anaerobic pond system. Maximum yields of phenol and COD removal were observed for the lowest COD amount (500 mg/L) at the highest retention time (5 days), 95.8% for phenol and 87.51% for COD; while the minimum yields of phenol and COD removal were shown for the highest COD amount (10000 mg/L) at the lowest retention time (2 days), 51.4% for phenol and 33.58% for COD. Initial launch of reactors was easy and rapid, due to using pre-prepared seed (anaerobic pond pilot seed) and acclimated micro-organisms; it is linked, on the one hand, to the presence of sufficient amount of materials that could be the subject of rapid biological degradation, and on the other hand, to the presence of selected anaerobic biomass and its adaptability to the degradation of the targeted material. Since the prepared seed was adapted to the targeted material (the seed has been about 3 months in the anaerobic pond system), after initial launch the reactors performance for molasses and phenol degradation were determined at 5 different retention times. As shown in Table 4, increasing the retention time led to increasing removals of phenol and soluble COD. Increasing the retention time and decreasing the amount of biodegradable COD led to an improvement of soluble COD and phenol removals in the anaerobic pond system (Figs. 2 and 3). Similarly, in all reactors increasing the retention time increased the removal of soluble COD and phenol (Tables 4, 5). As seen in Table 4, increasing the retention time led to more removal of oxygen-consuming organic materials; however, for each specific retention time, COD removal yield showed first a decrease followed by an increase over a certain initial COD amount, linked to an enhancement of oxygen-consuming organic materials removal which dropped slightly above a given amount.

Moreover and according to Table 4, maximum phenol removal was achieved for COD within concentration range 1000 – 2000 mg/L biodegradable COD (co-substrate), leading to 98.6 and 95.3% removal for 1000 and 2000 mg/L biodegradable COD for 50 days retention time, respectively. Effects of day and concentration separately and the effects of both on COD removal were statistically meaningful (p-value<0.001). COD removal percentage obeyed a rising trend until day 30 and declined after that.

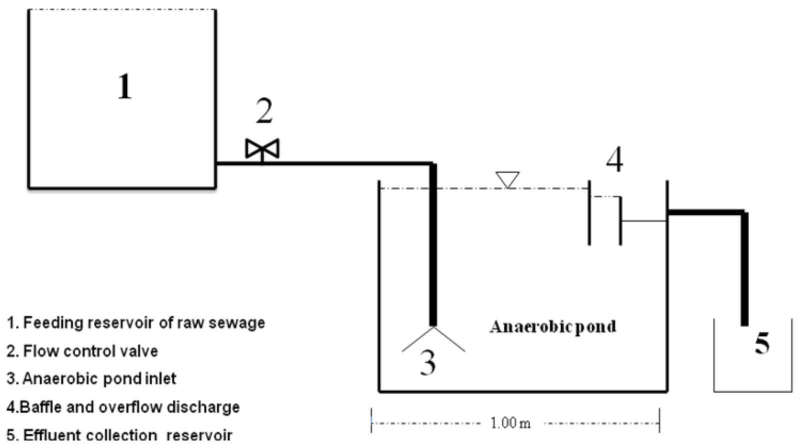

Fig. 1. Schematic diagram of the anaerobic stabilization pond pilot

Table 1. Characteristics and quantities of material used in the reactors (100 mg/L phenol concentration and 120 mg/L seed concentration; 5.5 and 1 mL of phenol solution and seed, respectively)

Reactor number	*Phenol COD (mg/L)*	*Molasses COD (mg/L)*	*Total COD (mg/L)*	*Molasses volume (mL)*	*Distilled water volume (mL)*	*Total volume (mL)*
1	344	500	964	5.5	538.0	550
2	344	1000	1464	11	532.5	550
3	344	2000	2464	22	521.5	550
4	344	5000	5464	55	488.5	550
5	344	10000	10464	110	433.5	550

Table 2. Micronutrients solution used in the preparation of seed and initial launching of reactors

Chemical compound	*Concentration (mg/L)*
Zn Cl_2	0.05
$MnSO_4.H_2O$	0.05
$(NH_4)MO_7\ O_{24}.\ {}_4H_2\ O$	0.05
$CoCl_2\ .H_2\ O$	0.05
$CuCl_2$	0.03
Cysteine hydrochloride	0.01

Table 3. Performance of the anaerobic pond system for simultaneous removal of phenol and biodegradable COD

Concentration of biodegradable matter (molasses) (mg/L COD)	*COD of phenol (mg/L)*	*Retention time (day)*	*Initial COD concentration (mg/L)*	*Average residual COD concentration (mg/L)*	*Average COD removal* (%)	*Initial phenol concentration (mg/L)*	*Average residual phenol concentration* (*mg/L*)	*Average Phenol removal* (%)
500	344	2 5	1466	353.3 183.1	75.9 87.5	169.6	17.09 7.12	89.92 95.8
1000	344	2 5	1966	621.06 355.84	68.4 81.9	169.6	40.19 19.69	76.3 88.39
2000	344	2 5	2966	1131.53 794.77	61.8 73.2	169.6	52.93 32.73	68.79 80.7
5000	344	2 5	5966	3484.14 2293.13	41.6 61.6	169.6	69.36 43.21	59.1 74.52
10000	344	2 5	10966	7283.61 5742.89	33.6 47.6	169.6	82.42 52.4	51.4 69.1

The results obtained at days 10and 20 were the same (p-value=0.999) and for the remaining days, it has been meaningful. The trend shows a meaningful increase in all levels of concentrations. Table 5 indicates the statistical results of COD and phenol removal yields related to the retention time and the amount of biodegradable material.On the other hand, two-way ANOVA along with Scheffe pot-hoc confirmed that the maximum yield of phenol removal was achieved for 1000 – 2000 mg/L biodegradable COD and 50 days retention time in reactors and for 500 mg/L biodegradable COD and 5 days retention time in the anaerobic pond.

The effects of day and concentration separately and also the effects of both on phenol removal have been statistically meaningful (p-value<0.001). The percent of phenol removal had fluctuations in different days. The percent of phenol removal was maximum at

day 20, after that it decreased until day 40, but a slight increase was seen at day 50 which was not statistically meaningful. The trend shows a meaningful increase in all levels of concentrations. Since wastewaters are constantly exposed to a mixture of degradable and non-degradable materials, examination of anaerobic processes in order to enhance the degradation of materials in a mixed medium and then investigation of the mutual influence of recalcitrant and non- recalcitrant materials facing biological degradation was the purpose of this work, to draw a strategy for their treatment. Accordingly, Fang et al. (2006) carried out an investigation under thermophilic conditions to examine the treatment of a relatively high phenol concentration (630 mg/L) in a wastewater containing sucrose as a co-substrate and a total amount of 1500 mg/L COD, showing 99% of phenol removal in the Stabilized UASB reactor during 177 days running at 40 hours retention time (Fang et al., 2006).

This short retention time after a long-term experiment and a temperature of 55 °C did not seem surprising owing to the biodegradability of the considered co-substrate, sucrose (Fang et al., 2006). The temperature also plays an important role in anaerobic degradation, and maximum efficiency for anaerobic processes was obtained in a relatively long time and under psychrophilic conditions.

Table 4. Performance of the reactors (Erlenmeyer flasks) for simultaneous removal of phenol and biodegradable COD

Reactor number	*Concentration of molasses (mg/L COD)*	*Retention time (day)*	*Initial COD concentration (mg/L)*	*Average residual COD concentration (mg/L)*	*Average COD removal (%)*	*Initial phenol concentra-tion (mg/L)*	*Average residual phenol concentration (mg/L)*	*Average phenol removal (%)*
1	500	10	964	648.58	32.72	100	74.83	25.17
		20		583.83	39.44		67.79	32.21
		30		462.05	52.07		58.61	41.39
		40		362.75	62.37		52.44	47.56
		50		315.10	67.31		47.70	52.30
2	1000	10	1464	1133.72	22.56	100	79.82	20.18
		20		919.59	37.19		64.69	35.31
		30		882.06	39.75		55.88	44.12
		40		429.10	70.69		7.27	92.73
		50		226.82	84.51		1.38	98.62
3	2000	10	2464	1777.04	27.88	100	84.19	15.81
		20		1227.73	50.17		59.24	40.67
		30		1099.85	55.36		54.90	45.10
		40		597.52	75.75		7.20	92.80
		50		541.26	78.03		4.71	95.29
4	5000	10	5464	4019.32	26.44	100	85.84	14.16
		20		3241.07	40.68		78.50	21.15
		30		2505.24	54.15		69.82	30.18
		40		1531.92	71.96		52.60	47.40
		50		1380.75	74.73		49.01	50.99
5	10000	10	10464	8491.54	18.85	100	88.13	11.87
		20		7344.33	29.81		81.08	18.92
		30		5390.36	48.49		66.65	33.35
		40		4418.25	57.78		54.68	45.32
		50		3028.70	71.15		42.72	57.28

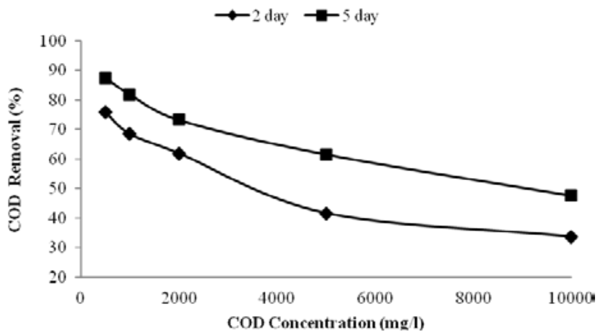

Fig. 2. COD Removal based on biodegradeble COD concentration and retention time in anearobic pond system

Indeed, Scully et al. indicated that in a temperature range of 9.5 to 15 ºC, the removal of phenol reached its highest value after 415 days (99%) (Scully et al., 2006). The results obtained in the present study are consistent with those of Almasi and Dargahi who used an anaerobic stabilization pond for phenol removal from oil refinery wastewater (Almasi and Dargahi, 2010). They are also in agreement with the findings of Shui-Zho et al. (2004) who showed that increasing the COD load decreased phenol removal, from 88.8% to 32.5 % for increasing organic loads from 6000 to 18000 mg/L COD (Shui-Zhou et al., 2004); as well as with Streekanth et al. (2008) using a laboratory-scale UASB system to remove phenolic compounds from wastewater, which also showed that increasing the retention time decreased phenolic compounds removal. The present findings were also confirmed by Zhouyang et al. showing 99% and 100 % phenol removal obtained within 37 and 50 days retention time in an IALR (Internal Air Lift Reactor) reactor containing 210 mg/L phenol and glucose as a co-substrate (Zhouyang et al., 2009).

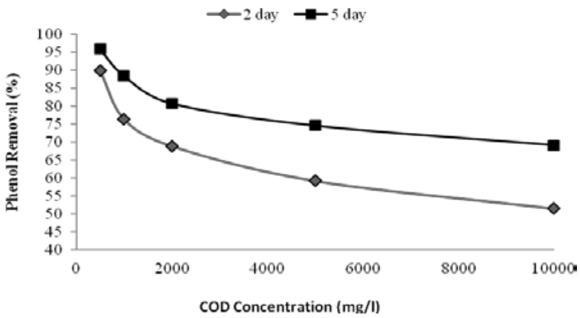

Fig. 3. Phenol removal based on biodegradable COD concentration and retention time in anearobic pond system

Charest et al. (1999) studied the removal of phenolic compounds of petrochemical wastewater using closed biological system under anaerobic conditions and observed that the highest yield for phenol removal was achieved after 13 days (97%), namely in line with the present findings. Increasing the co-substrate concentration showed negative effects, on the yield of phenol removal on the one hand, and on the time needed to achieve a stability of reactors performance on the other hand.

Table 5. Means and standard deviations of COD and Phenol removal in reactors for various retention times and biodegradable organic matter concentrations

Reactor	*Initial concentration of molasses (COD mg/L)*	*Retention time (10 days)*		*Retention time (20 days)*		*Retention time (30 days)*		*Retention time (40 days)*		*Retention time (50 days)*	
		Phenol removal (%)	*COD removal (%)*	*Phenol removal (%)*	*COD removal (%)*	*Phenol removal (%)*	*COD removal (%)*	*Phenol removal (%)*	*COD removal (%)*	*Phenol removal (%)*	*COD removal (%)*
1	500	25.17±3.58	32.72±0.56	32.21±2.97	39.44±1.51	41.39±0.77	52.07±3.06	47.56±1.81	62.37±1.86	52.30±0.66	67.31±2.96
2	1000	20.18±1.56	22.56±2.81	35.31±1.67	37.19±2.73	44.12±1.31	39.75±0.87	92.73±0.53	70.69±2.54	98.62±0.56	84.51±1.31
3	2000	15.81±3.22	27.88±1.72	40.67±2.00	50.17±2.52	45.10±1.80	55.36±2.33	92.80±1.46	75.75±1.46	95.29±0.65	78.03±2.31
4	5000	14.16±1.88	26.44±1.64	21.15±1.73	40.68±0.92	30.18±1.10	54.15±2.42	47.40±1.27	71.96±3.09	50.99±0.95	74.73±2.36
5	10000	11.87±2.25	18.85±2.16	18.92±1.00	29.81±3.30	33.35±1.23	48.49±1.69	45.32±1.42	57.78±1.49	57.28±1.25	71.15±1.77

Bajaj et al. (2009) reported that the maximum of phenol removal observed before a phenol shock load was 39.47 mmol/L or 3.7 g phenol/L for a hydraulic retention time (HRT) of 2.5 days and an organic loading rate (OLR) of 5.3 g L^{-1} day^{-1}. After a shock load induced by increasing the phenol concentration from 40 to 50 mmol/L in the influent, maximum phenol removal decreased to 18 mmol L^{-1} day^{-1} for 5.7 g COD L^{-1} day^{-1} (Bajaj et al., 2009). Furthermore, Tay et al. also reported that phenol removal efficiency dropped from 88% to 14% within 30 days after increasing feeding phenol amount from 13.3 to 17.8 mmol L^{-1} (Tay et al., 2001). Elias et al. examined the degradability of phenolic compounds under anaerobic conditions in batch and continuous systems and indicated that for an organic load of 7 kg COD m^{-3} day^{-1}, an UASB system showed more than 90% COD removal, while phenolic compounds could be also simultaneously removed during anaerobic treatment of petrochemical effluents (Elias et al., 2003).

Regarding the performances of biological processes in the case of continuous or batch feeding of reactors, the results should be related to the mode of culture, which may have an impact on the results. In the present study, the reactor feeding occurred in one stage, and hence the obtained findings are in accordance with the related studies. Azbar et al. (2009) showed that anaerobic hybrid reactor could tolerate high influent COD concentrations and obtained removal efficiencies in the range 50–94% and 39–80% for COD and total phenol (Azbar et al., 2009). Carbajo et al. (2010) also examined phenol removal in the presence of various volatile fatty acid concentrations as co-substrates and indicated that 95% phenol was removed after 212 days (Carbajo et al., 2010); in addition, when feeding reactor discontinuously, 95% of phenol was removed after 32 days, in agreement with the present findings.

4. Conclusions

The biodegradation of recalcitrant compounds was enhanced by the presence of biodegradable co-substrates. From the present investigation, other issues appear which may have a severe impact on the degradation of the refractory materials and also and may have an inhibitory effect.

Regarding these results and before considering a mixture of substrates, it is therefore recommended to beforehand determine at laboratory-scale the most appropriate ratio of auxiliary biodegradable material and biologically recalcitrant material.

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