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Immobilized cell reactors in mineralization of dicarboxylic acid solid waste

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Abstract

Dicarboxylic acid solid waste containing phthalic acid, malic acid, quinone, saturated and unsaturated dicarboxylic esters etc., are discharged in huge quantities during the crackdown of benzene over the catalyst vanadium at temperatures greater than 500 °C in a dicarboxylic acid manufacturing industry. Concern over the biological effects of these compounds underlines the necessity to treat this solid waste. The role of yeast *Saccharomyces cerevisiae* and anaerobic mixed bacterial cultures immobilized in activated carbon, in sequential two stage anoxic reactors, were investigated for the degradation of dicarboxylic acid solid waste (DASW). In the first stage, DASW was dissolved in water to yield a concentration of 0.5% w/v and was treated in yeast *Saccharomyces cerevisiae* immobilized reactor at an optimum residence time of 24 h. The yeast fermented samples were further treated in an upflow anaerobic reactor containing mixed culture immobilized in activated carbon at an Hydraulic Retention Time (HRT) of 0.2076 days at an hydraulic flow rate of 14.6×10^{-3} m^3/day and Chemical Oxygen Demand (COD) loading rate of 4.3 kg/m^3/day. The intermediates that were formed during the yeast fermentation and the anaerobic degradation of DASW were characterized by HPLC, proton NMR, C^{13} NMR and mass spectrometry.

Abbreviations: ^{13}C NMR – carbon 13 nuclear magnetic resonance spectrometry; ^{1}H NMR – proton nuclear magnetic resonance spectrometry; DASW – dicarboxylic acid solid waste; HPLC – high performance liquid chromatography; MS – mass spectrometry; RHAC – rice husk activated carbon

Introduction

In recent years, greater knowledge has been gained on the ability of yeast and moulds to degrade aromatic pollutants present in wastewater (Giovanni et al. 2001; Kwon et al. 2002; Martins et al. 1999). Dicarboxylic acid derivatives are refractory organic compounds that are widely used in the manufacture of industrial chemicals (Staples et al. 1997) and these derivatives have become widespread in the environment (Atlas & Giam 1981; Fatoki & Ogunfowokan 1993; Hartmanen & Abring 2002; Shelton et al. 1984). The widespread distribution of the dicarboxylic acid wastes in the environment raises concern about their toxicological effects on the living organisms (Alatriste-Mondragon et al. 2003; Sharpe 1998; USEPA 2000). Dicarboxylic acid solid waste (DASW) containing phthalic acid, malic acid, quinone, saturated and unsaturated dicarboxylic esters etc., are discharged in huge quantities during the crackdown of benzene over the catalyst vanadium at temperatures greater than 500 °C in a dicarboxylic acid manufacturing industry.

Both aerobic and anaerobic treatment processes have been developed for the treatment of various toxic effluents discharged from the industrial sources. However, aerobic processes are not always feasible because they tend to form free radicals in the presence of oxygen, which may react with exocellular polymers affecting the viability of the cells (Ulrich & Bernhard 1989). A number of anaerobic processes have also been intensively developed over the past decades for the treatment of wastewater containing complex anthropogenic compounds and/or compounds recalcitrant to biodegradation (Kleerebezem et al. 1999; Macarie et al. 1992). Yeast cells grow anaerobically by the secretion of dihydro oratate dehydrogenase using energy solely generated during the fermentation process (Nagy et al. 1992) and have the ability to utilize dicarboxylic acid derivatives as energy source (Begum et al. 2003). Researchers suggest the usage of biomass either as a fixed film or as aggregates in anaerobic wastewater treatment system (Lettinga et al. 1980; Switzenbaum 1983). Under many conditions, immobilized cells have many advantages over the use of free cells: (1) it increases the biodegradation rate through a higher cell loading effect (2) bioprocesses can be controlled more easily (3) continuous biological process can take place at a high dilution rate without washout and (4) catalytic stability of the biocatalysts as well as their tolerance towards toxic compounds can be improved. Rice husk based mesoporous activated carbon (RHAC) had been considered as a suitable carrier matrix for the immobilization of microorganisms (Kennedy et al, 2004).

The activated carbon serves as a good carrier matrix to facilitate selective solute transfer, enhanced biofilm attachment and restrict microorganism's access to the down stream permeates. Activated carbon offers a rough and fissured surface on which microorganisms can settle and colonize easily. Activated carbon acts as a modulator by adsorbing relatively high concentration of toxic compounds in the aqueous system and thereby regulates the concentration of the free material. Degradation of organic compounds by bacterial cultures immobilized on activated carbon occurs as a combination of physical adsorption and biological degradation (Ehrhardt and Rehm 1989). Due to low solubility and high hydrophobic nature of phthalates, activated carbon would be an effective adsorbent for their removal from wastewater. Hence, RHAC has been selected as the carrier matrix for the immobilization of *Saccharomyces cerevisiae* and anaerobic cultures for the degradation of DASW in aqueous phase. The objective of the present investigation was to treat DASW by two stage anaerobic processes i.e., stage I consisting of fermentation in yeast immobilized RHAC reactor and stage II consisting of the treatment in the upflow anaerobic hybrid reactor. The present work highlights the importance of *Saccharomyces cerevisiae* immobilized reactor for the breakdown of the dicarboxylic acid wastes into volatile fatty acids (VFA), thus preventing the anaerobic bacterial populations from the toxic effects of VFA during treatment in the upflow anaerobic hybrid reactor.

Materials and methods

Yeast and anaerobic culture acclimatization

A yeast strain showing high tolerance towards dicarboxylic acid was isolated, screened from distillery plant and acclimatized through successive streak transfers on nutrient agar plates containing dicarboxylic wastes, the adapted strain was maintained in deep freezer and used throughout the experimental studies. The anaerobic bacterial consortium was obtained from Upflow Anaerobic Sludge Blanket reactor treating tannery wastewater. These strains were acclimatized for the treatment of yeast fermented DASW, by the addition of yeast treated dicarboxylic acid residues at weekly intervals to anaerobic sludge.

Characteristics of activated carbon

The rice husk activated carbon (RHAC) prepared (Kennedy et al. 2004) in our laboratory was used for the immobilization of yeast and anaerobic cultures. Activated carbon was sieved to the size of 600 μ for this study. The chemical characteristics of the RHAC are given in Table 1. Surface morphology of RHAC was studied using Leo-Jeol scanning electron microscope after it was coated with gold by a gold sputtering device.

Table 1. Characteristics of rice husk activated carbon (RHAC)

S.No.	Parameters	Values
1	Carbon (%)	48.45
2	Hydrogen (%)	0.7
3	Nitrogen (%)	0.1
4	Ash content (%)	51.8
5	Bulk density (g/ml)	0.405
6	Moisture content (%)	3.8
7	Ash content (% by mass)	40
8	Matter soluble in water (%)	0.428
9	Matter soluble in acid (%)	3.908
10	Point of zero charge	6.66
11	Decolourising power (mg/g)	22
12	Phenol number (mg/g)	3.18
13	Ion exchange capacity (meq/g)	0.015
14	Surface area (BET) (m^2/g)	220
15	Pore size (Å)	39.36
16	V_{meso}/V_{tot} (%)	68.41

Batch adsorption of components of DASW by RHAC

This was carried out to determine the adsorption capacity of RHAC for COD, BOD, TOC and VFA under non-flow condition. In each of the two dry well stoppered bottles, 50 ml of 0.5% DASW solutions were taken. About 1 g of accurately weighed RHAC (dried over H_2SO_4) was added to one bottle (sample) while the other (reference/blank) containing DASW sample without RHAC served as control. The bottles were placed in a thermostatic bath and they were gently agitated at low rpm. In the batch adsorption studies, the temperature of the thermostatic bath was set at 30 °C. The bottles were then removed from the constant temperature bath after 24 h (this detention period was used in fermentation experiment) and the solutions were filtered through stainless steel wire gauze to separate the activated carbon from the solution. The filtrate was analyzed for COD, BOD, TOC and VFA. From the characteristics of DASW solution before and after adsorption onto RHAC, the adsorption capacity of RHAC was calculated.

$$x/m\text{mg/g} = [C_0 - C_t] \cdot V/m$$

where C_0 is initial characteristics (COD, BOD, TOC and VFA) of DASW solution in mg/l; C_t is the characteristics(COD, BOD, TOC and VFA) of DASW solution in mg/l at time t, V is the volume of solution in cm^3; m is the mass of RHAC in g and x/m is the adsorption capacity of the RHAC.

Table 2. Adsorption capacity of RHAC for COD, BOD, TOC and VFA in DASW solution

Parameter	Adsorption capacity (mg/g)
COD	7.14
BOD	0.6175
TOC	2.415
VFA	0.1475

Continuous mode adsorption of components of DASW by RHAC

This was performed to determine the adsorption capacity of RHAC for COD, BOD, TOC and VFA under continuous mode of operation as the upflow anaerobic immobilized reactor was operated under continuous flow condition. Upflow fixed bed adsorption experiment was conducted in a glass column of internal diameter 2.5 cm and of height 25 cm. A known quantity (25 g) of RHAC was packed in the column to yield a bed height of 12.7 cm. A peristaltic pump (Watson Marlow) was used to pump the fermented DASW sample at pH 7.0 (characteristics of which are given in Table 4) through the RHAC column in the upflow direction at a flow rate of 12.48 ml/h. The aliquots of the sample were collected to determine COD, BOD, TOC and VFA. Break through curve was drawn by plotting the outlet concentration of DASW solution on the ordinate and time on the abscissa. The area above the break through curve multiplied by the flow rate represents the quantity of DASW retained in the column (m_{ad}), which was expressed in terms of COD, BOD, TOC and VFA. The adsorption capacity of RHAC (x/m) can be determined from the ratio of m_{ad} to the mass of RHAC.

Immobilization of Saccharomyces cerevisiae

The *S. cerevisiae* cells acclimatized to 0.5% DASW sample were centrifuged and suspended in 1 M phosphate buffer (K_2HPO_4 and KH_2PO_4) to measure a concentration of 2% w/v. The *S. cerevisiae* in phosphate buffer was immobilized in RHAC by passing the yeast suspension through a reactor packed with 400 g (occupies a volume of 9.92×10^{-4} m^3) of RHAC under down flow mode.

The process was continued until the out let of the reactor showed consistent optical density at λ_{max} 550 nm. The mesoporous range of RHAC that were the active sites of immobilization and colonization were shown in Figure 1.

Batch fermentation in yeast immobilized reactor

The fermentor of volume 2260 ml with dimension of internal diameter 6 cm and height 80 cm was packed with *S. cerevisiae* immobilized RHAC of mass 400 g (occupies a volume 9.92×10^{-4} m^3 which forms about 44% of the reactor). A solution of DASW was added to the fermentor at a concentration of 5 g/l at pH (6.5). A 0.5% DASW concentration was selected by optimizing its concentration in which the yeast strain showed substantial growth. The retention time for the yeast fermentation was optimized and the fermented samples were withdrawn after 24 h retention time and were further treated in upflow anaerobic hybrid reactor.

Upflow anaerobic hybrid reactor

A laboratory scale upflow anaerobic hybrid reactor, made of glass, with dimensions of height 90 cm and diameter 6 cm was packed with RHAC of mass 200 g (occupies a volume of 4.98×10^{-4} m^3). The RHAC packed bed reactor was seeded with the acclimatized anaerobic consortium (the sludge volume was 4.8×10^{-4} m^3) obtained from the upflow anaerobic sludge blanket reactor. The anaerobic consortium was acclimatized to the yeast fermented aqueous solution of solid residue at pH 7.0 ± 0.2. The anaerobic consortium formed bottom layer and RHAC assumed the top layer. The yeast-fermented sample, after adjusting its pH to 7.0 ± 0.2, was fed on to the anaerobic digester at a flow rate 14.6×10^{-3} m^3/day which conforms to HRT of 0.2076 days and COD loading rate of 4.3 kg/m^3/day.

Biodegradation assay

The treated and untreated DASW samples were characterized for Chemical Oxygen Demand (COD), (closed reflux, titrimetric method), Biochemical oxygen demand (BOD) (Winkler method) and Volatile Fatty Acids (VFA) (distillation method) in accordance with the standard methods for analysis of water and wastewater (Lenore et al. 1989). Total organic carbon was analyzed using TOC analyzer (OI Corporation – model No. 700).

High performance liquid chromatography (HPLC)

The VFA of yeast fermented DASW, separated through distillation, and was used for the quantitative HPLC analysis using Shimadzhu VP SERIES with SpD10A detector and WINCHROM software. The column used was C18 Hypersil

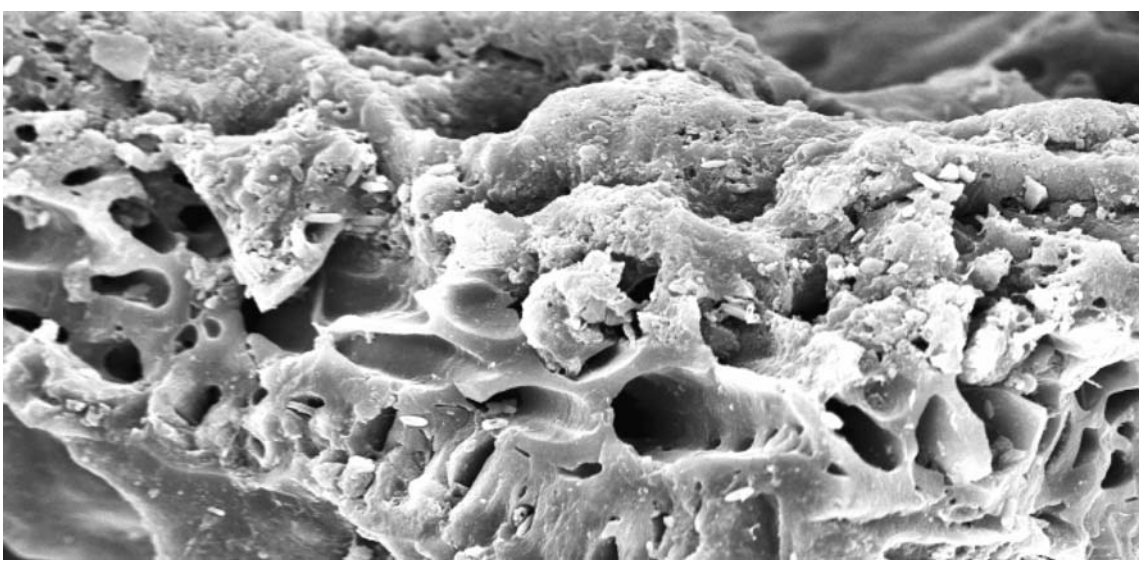

Figure 1. Scanning electron micrograph of rice husk activated carbon (RHAC).

column using methanol: acetonitrile as mobile phase. The UV detector (at 210 nm) was employed for quantification. UV–Visible spectra were recorded at the peak maxima and were corrected for solvent background. The results were calculated using the standard volatile acids (Merck, India) as control.

Mass spectroscopy (MS)

Mass spectra of the treated and the untreated aqueous samples of DASW were recorded with FINNIGAN MAT 8230, GC-MS Electron impact low resonance mass spectrometer. One milligram of lyophilized sample was used for quantification.

Proton and ^{13}C NMR

Proton and ^{13}C-NMR spectra were recorded using JEOL ECA 500 MHz spectrometer. Deuterated water was used as the solvent for recording proton NMR, and ^{13}C NMR was recorded using $CDCl_3$ as the solvent for the lyophilized samples of aqueous DASW. Proton NMR was recorded at 500 MHz and ^{13}C-NMR at 125 MHz after solvent peak suppression.

Results and discussion

Yeast immobilized RHAC reactor

The schematic flow diagram of two-phase anaerobic treatment process shown in Figure 2 consists of two reactors for the degradation of DASW prepared in water. In this treatment process *S. cerevisiae* was used in the first reactor and anaerobic mixed bacterial population was used in the second reactor. In the phase I reactor the DASW solution was fermented at a retention time of 24 h. Characterizations of the fermented sample for BOD, COD, TOC and VFA showed a reduction of 50.7 ± 3.5% in BOD, 78.8 ± 3.6% and 73.6 ± 5.4 % reduction in the COD and TOC, respectively. However, VFA has been increased by 250.8 ± 7.9%. The residual COD in the yeast treated sample was attributed to the presence of unconverted/partially transformed dicarboxylic acid, aryl alcohols and volatile fatty acids. Fermentation in the absence of aeration can transform one type of organic compound into another with the release of gaseous products and thus the TOC and COD of the fermented sample declined substantially.

The removal of pollution parameters of DASW during yeast fermentation may be partly due to adsorption by RHAC as it was used as the carrier matrix for the immobilization of *S. cerevisiae*. The adsorption capacity of the activated carbon for the constituents of DASW was determined under batch mode and the Table 2 shows that the adsorption capacity of RHAC for COD, BOD, TOC and VFA of DASW aqueous sample were, 7.14, 0.6175, 2.415 and 0.1475 mg/g, respectively. These results indicate that RHAC has good interaction capacity with the water soluble polar organic compounds of DASW, however, it may not be ruled out that adsorbed organics desorb

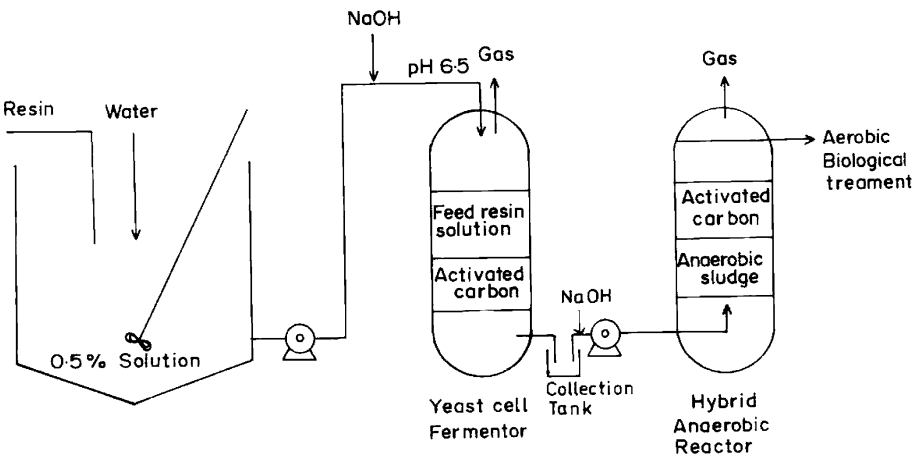

Figure 2. The schematic flow diagram of two-phase anaerobic treatment process.

and diffuse out of the activated carbon which would then be metabolized by the immobilized yeast cells. The presence of oxy functional groups (Kennedy et al. 2004) in RHAC may be attributed for its high interaction capacity with DASW. The strong bonding of the functional groups with the cell constituents prevents from getting dislodged from the RHAC. The high interaction of RHAC with DASW and yeast cells reduces mean free path for the secreted enzymes to access the substrate. Moreover, the hydroxyl groups present in RHAC contribute to the buffering action to the microbial systems to survive at very low pH (4.5–4.8) and thus accounts for the higher percentage removal of COD and BOD (Fan et al. 1990) during yeast fermentation. The fermentation of DASW constitutes a substantial increase in volatile fatty acids up to 250.8 ± 7.9% for the retention time of 24 h (Table 4). In any anaerobic degradation pathway, the complex organic compounds are initially hydrolyzed and are then fermented rapidly which in turn results in the production of VFA (Li & Noike 1992; Siegrist et al. 1993). The decrease in pH from 6.5 to 4.5 is due to the increase in VFA for a retention time of 24 h (Wang et al. 1999). In growth phase variety of acid components (acetic acid, propionic acid, butyric acid and other unknown acids) are produced by the intracellular enzyme of the yeast cells, resulting in a significant drop in pH and this corroborates with the observations made by Verstrepen et al. (2003). These results confirm the degradation of dicarboxylic acids by the enzymatic function of *Saccharomyces cerevisiae*. The drop in pH below 4.5–4.8 could be the possible reason for the non-production of methane (Weber et al. 1984) during yeast fermentation of DASW.

Upflow anaerobic hybrid reactor

The hydrolysis reactions carried out in the yeast immobilized reactor were spatially separated and further degradation processes were carried out in the methanogenic stage after adjusting the pH of the yeast fermented DASW to 7.0 ± 0.2. The pH of the yeast fermented DASW was adjusted, before feeding the upflow anaerobic reactor, to avoid the danger of acidification of the methanogenic stage. The upflow anaerobic hybrid reactor used in the present investigation consists of RHAC and anaerobic sludge acclimatized to DASW. The dry weight of the anaerobic bacterial biomass used was in the range of 3.0182 ± 0.05 mg/g of RHAC in the immobilized state. The adsorption capacity of RHAC for the constituents of yeast fermented DASW solution was determined under continuous mode of application and the results are presented in Table 3. The adsorption capacity of RHAC under continuous mode application for COD, BOD, TOC and VFA were 2.22, 0.666, 0.8538 and 0.1322 mg/g, respectively. The adsorption of the constituents of the fermented DASW might be through weak bonding with active sites of RHAC and, therefore, they desorb easily to be metabolized by the methanogens in the upflow anaerobic hybrid reactor.

The breakdown of complex molecules and their conversion into methane resulted in COD reduction by 85.9 ± 0.2%, TOC reduction by 71.1 ± 0.8% and BOD reduction by 91.8 ± 0.4%. The VFA concentration was reduced up to 95.2 ± 1.3% (Table 4). The pH of the anaerobic sample was increased to 7.8 from the influent pH of 7.0 indicating that the methanogenic bacteria were more active (Nurdan & Ayse 2003). Acetogenic and hydrogenotrophic bacteria in anaerobic reactor further fragment the volatile fatty acids produced during fermentation process. Under methanogenic conditions, dicarboxylic acid degradation is proceeded by syntrophic association between different trophic groups of anaerobes (i) hydrogenotrophic methanogens, which scavenge hydrogen, and (ii) aceticlastic methanogens which consume acetate. The intermediate compounds after fermentation are the suitable substrates for the growth of methanogenic consortium (Denac et al. 1988; Pavlostathis & Giraldo-Gomez 1991). The sludge collected from anaerobic reactor contained *Methanothrix* sp., and *Methanosarcina* sp. Identification of these acetotrophic and hydrogenotrophic methanogens in the anaerobic sludge sample was based on their characteristic morphology.

Table 3. Adsorption capacity of RHAC for COD, BOD, TOC and VFA in fermented DASW solution

Parameter	Adsorption capacity (mg/g)
COD	2.22
BOD	0.666
TOC	0.8537
VFA	0.1322

Table 4. Characteristics of DASW at different stages of treatment

Parameters	DASW (0.5% w/v)	After yeast fermentation	Percentage reduction		After upflow anaerobic reactor	Percentage reduction	
pH	6.57 ± 0.093	4.6 ± 0.11	a	b	7.8 ± 0.05	c	d
Acidity/ alkalinity	474.4 ± 37.2	1772.4 ± 7.9			198.6 ± 19.2		
COD	13556 ± 349	2242 ± 246	21.07 ± 0.56	78.8 ± 3.6	314 ± 28	19.88 ± 1.46	85.9 ± 0.2
TOC	3276 ± 229	595 ± 30	29.59 ± 1.9	73.6 ± 5.4	172 ± 6	28.82 ± 1.74	71.1 ± 0.8
VFA	541 ± 21	1690 ± 32	10.89 ± 0.34	250.8 ± 7.9	81 ± 5	1.56 ± 0.028	95.2 ± 1.3
BOD	1816 ± 22	772 ± 11	13.58 ± 0.14	50.7 ± 3.5	63 ± 5	17.24 ± 0.24	91.8 ± 0.4
CH_4 (l/day)	–	–			5.26 ± 0.243		

All the values excep pH, percentage reduction and CH_4, are expressed in mg/l.
Note: (a) Refers to percentage removal by RHAC. (b) Refers total percentage removal by RHAC and yeast cells in yeast fermentation. (c) Refers to percentage removal of fermented DASW by RHAC. (d) Refers total percentage removal by RHAC and anaerobic bacterial cultures in anaerobic digester.

Considerable attention has been paid on maintaining VFA's concentration by controlling the loading rate as it has direct impact on the anaerobic reactor performance (Wang et al. 1999). The startup of the reactor was indicated by the high conversion of VFA. In the yeast immobilized batch reactor VFA concentration reached the maximum concentration around 250.8 ± 7.9% while the TOC content was reduced by the maximum of 73.6 ± 5.4%. These results confirm the existence of the relationship between TOC and VFA i.e., the organic carbon source is utilized for the production of acids. Moreover, the VFA attained the peak value in 24 h and their increase caused a drop in pH from 6.5 to 4.6 (Wang et al. 1999). In the upflow anaerobic hybrid reactor the VFA was decreased by 95.2 ± 1.3% and the TOC was reduced by 71.1 ± 0.8% at the residence time of 0.2076 days (Table 4). Elimination of VFA and TOC resulted in the generation of methane gas in the range 5.26 ± 0.243 l/day.

HPLC analysis of VFA

The HPLC analysis of the volatile fatty acids formed in yeast immobilized reactor shown in Figure 3 reveal that the major constituent at retention time (R_t) of 3.52 corresponds to propionic acid and other major constituent at (R_t) of 2.54 corresponding to acetic acid. The percentage of propionic acid was about 23.94 and that of acetic acid was 24.49. The retention time for butyric acid (R_t) of 3.24 was 1.83%. The HPLC profile of VFA also confirms the presence of other fatty acid metabolites at (R_t) of 2.12, 3.96 and 4.07, however, the chemical nature of the metabolites could not be identified.

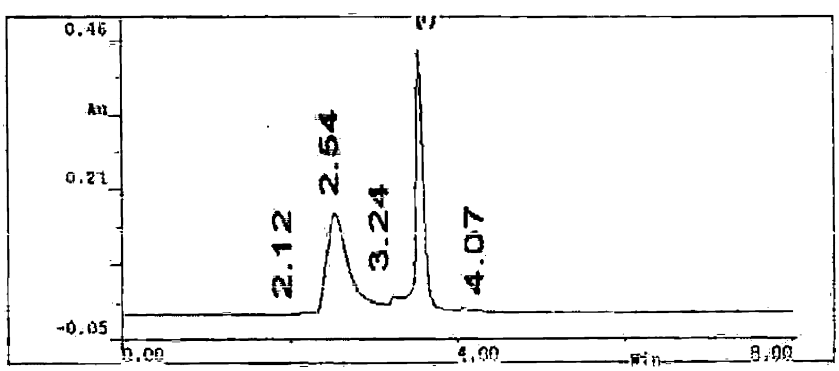

Figure 3. HPLC spectrum of volatile fatty acids.

Confirmatory analysis for biodegradation of DASW

Mass spectrum

The mass spectrum of DASW is given in Figure 4a. The molecular ion peak of phthalic anhydride was observed at m/z 148 and also has fragment peaks at $C_4H_2^+$ (50), $C_6H_4^+$ (76), $C_6H_5CO^+$ (104). The presence of maleic anhydride was also noticed which has molecular ion peak at m/z 98. The yeast treated sample showed the presence of benzoyl cation, which was formed via benzoic acid, giving the molecular ion peak at m/z 105. Benzoyl cation on fragmentation of CO group yielded phenyl cation corresponding to peak at m/z 77 and on elimination of C_2H_2 group gave a peak corresponding to m/z 51. The fragments with molecular ions confirm the presence of benzoic acid as the product of decarboxylation from phthalic anhydride during yeast fermentation. The linear chain compounds were also observed at m/z 60 and 74. The intermediate products of maleic anhydride like acrylic acid, propenal (acrolein), allyl alcohol and acetic acid were characterized from their molecular ion peaks at m/z values of 71, 56, 58 and 60, respectively (Figure 4b). The anaerobically treated waste confirms the complete degradation of phthalic acid and phthalic anhydride. The molecular ion peak at m/z value 97 and its fragment peak at m/z 69 in anaerobic treated waste may correspond to 2-hydroxy cyclohexane carboxyl CoA (Figure 4c).

^1H NMR spectroscopy

The ^1H NMR spectrum of DASW was scanned between 0 and 12 ppm. The ^1H NMR spectrum showed signals in the aromatic region (7–8 ppm) with a 2:1 ratio which indicates a 1,2-di-symmetrically substituted benzene skeleton. The chemical shift around 6.26 ppm can be assigned to the olefinic bonded protons (Figure 5a). The yeast

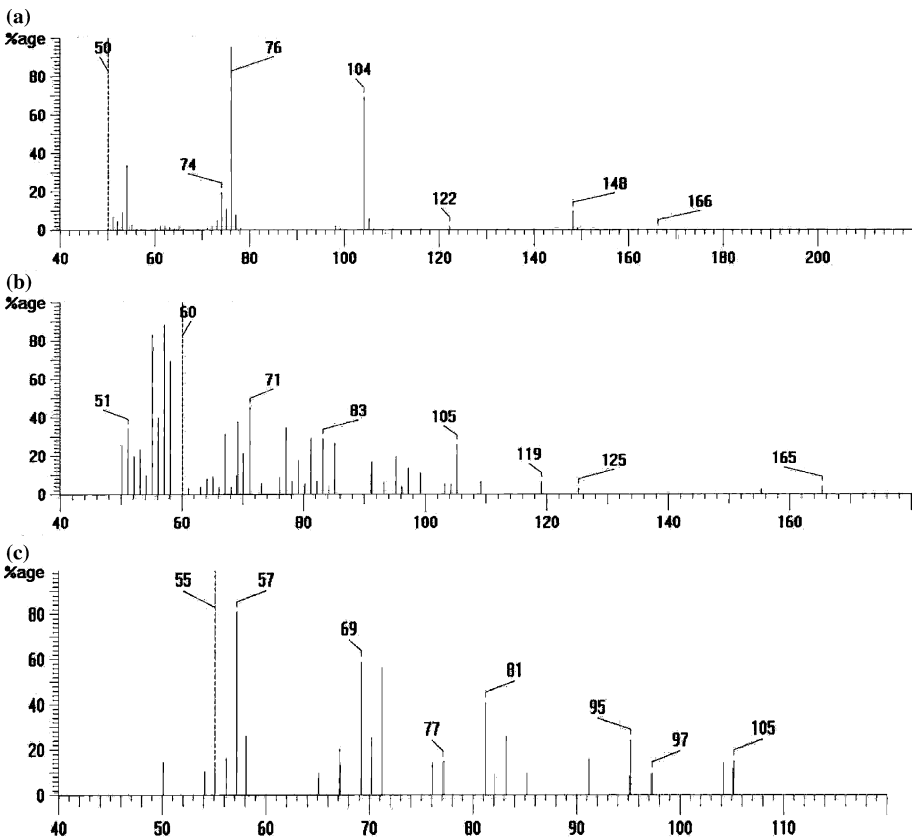

Figure 4. (a) Mass spectrum of DASW. (b) Mass spectrum of yeast treated of DASW. (c) Mass spectrum of anaerobically treated DASW.

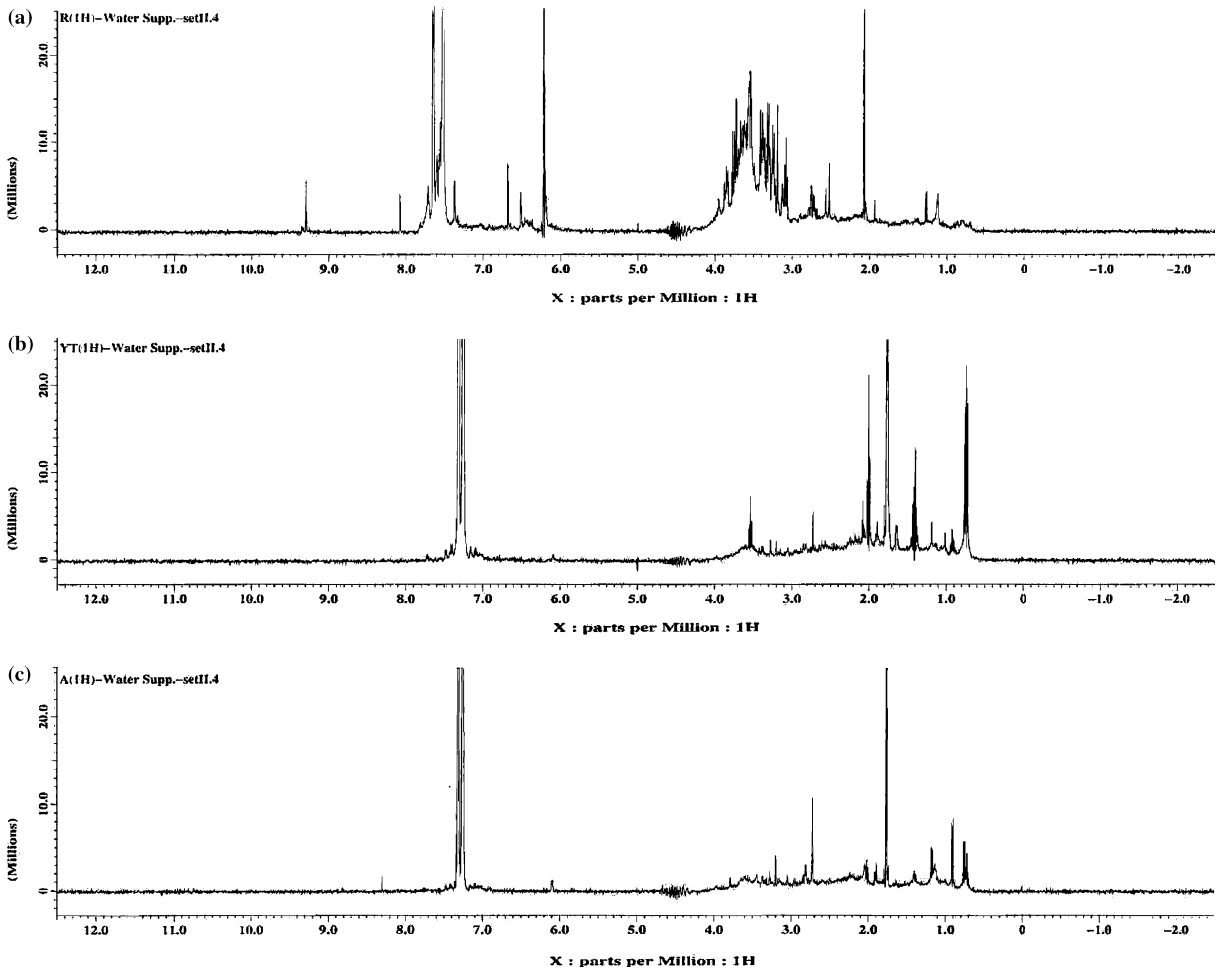

Figure 5. (a) Proton NMR of DASW. (b) Proton NMR of yeast treated DASW. (c) Proton NMR of anaerobically treated DASW.

fermented DASW sample shows the presence of aromatic protons at around 7–8 ppm. The protons in linear chain methylene groups showed chemical shift around 1.8 ppm. The protons in the alcohols give a chemical shift around 2–2.1 ppm. The peaks for the methylene protons attached to –OH group were observed at 3.5 ppm. The yeast treated sample showed many aromatic peaks (Figure 5b). The anaerobically treated sample reveals the presence of aromatic protons around 7–8 ppm in the spectrum. The linear chain methylene protons showed a peak around 1.8 ppm and the chemical shift of the linear methyl protons were, around 0.7–1 ppm that correspond to the spectrum of butyric acid. The 1.8-ppm peak was increased, showing significant change in intensity. At 8.3 ppm many small peaks were also present. The intensity of the peak at 2.8 ppm got changed and the peak at 2 ppm was completely disappeared (Figure 5c).

^{13}C NMR spectroscopy

The ^{13}C NMR spectrum of DASW was scanned between −20.0 and +220 ppm. The chemical shift around 130–140 ppm indicates the evidence of carbon in the aromatic ring (Figure 6a). The occurrence of chemical shift around 176–180 ppm confirms the presence of carboxyl carbon atoms in the yeast fermented DASW sample. The methylene group carbon showed a chemical shift around 25 ppm and methyl carbon around 0–10 ppm. The chemical shift around 160 ppm can be attributed

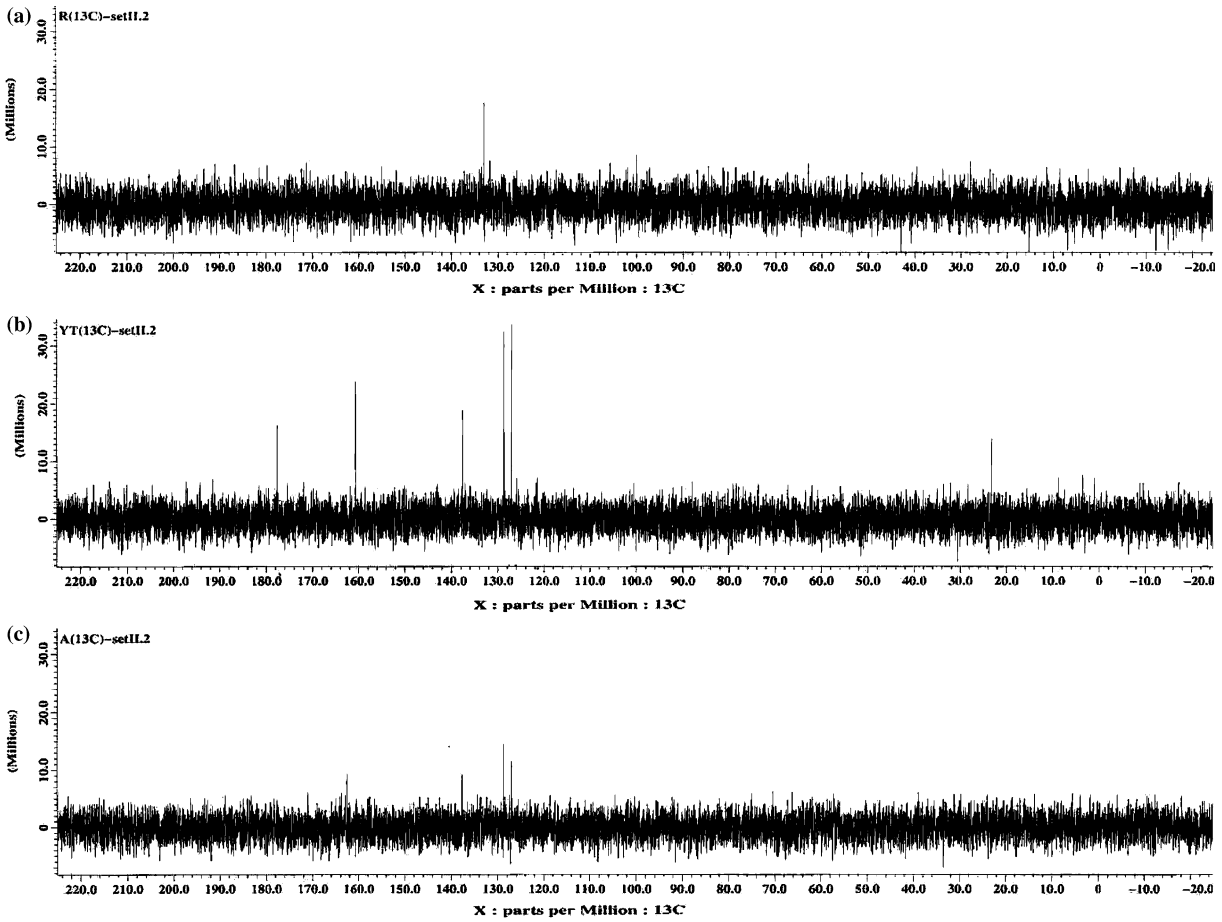

Figure 6. (a) C^{13} NMR of DASW. (b) C^{13} NMR of yeast treated DASW. (c) C^{13} NMR of anaerobically treated DASW.

to the carbon of the olefenic group (Figure 6b). The peaks at 100 and 178 ppm in the DASW has been completely disappeared after yeast fermentation. The peak at 134 ppm has disappeared from the sample collected after anaerobic treatment (Figure 6c).

The ability of the yeast strain to degrade extracellular dicarboxylic acid is dependent on the efficient transport of these acids, as well as on the efficacy of the intracellular enzymes (Ansanay et al. 1996; Delcourt et al. 1995; Salmon 1987). The yeast, *Saccharomyces cerevisiae*, import dicarboxylic acids through simple diffusion and converts them into benzoic acids by decarboxylation during fermentation. Many researchers indicate that carboxylation and decarboxylation reactions play an important role in the anaerobic breakdown of many aromatic compounds (Berry et al. 1987; Evans & Fuchs 1988; Liu & Chi 2003). Despite the intrinsic resonance stability of the aromatic ring, microbes can exploit chemicals of aromatic character as carbon and energy sources. Under anaerobic conditions, reductive processes relieve the resonance energy of the aromatic compounds leading to lowering or elimination of aromaticity of the compounds. In recent years, research work has established that structurally diverse aromatic compounds are converted into benzoate or benzoyl co enzyme A (co A) as a starting intermediate for a central anaerobic pathway of aromatic ring reduction culminating in ring cleavage (Harwood et al. 1999; Heider and Fuchs 1997). This kind of anaerobic conversion depends fully on the presence of hydrogenotrophic and acetotrophic methanogens (Matthies & Schink 1993). Based on our results and literature information (Nozawa and Maruyama 1988), both benzoate and residual dicarboxylic acid residues are to converge at

benzoyl co enzyme A (co A), a key intermediate in the anaerobic degradation of aromatic compounds.

Conclusion

Dicarboxylic acid solid waste was degraded sequentially in yeast cell immobilized reactor and in anaerobic culture – activated carbon hybrid reactor. The 0.5% (w/v) DASW concentration was selected by optimizing its concentration in which the degrading capacities of yeast strains were higher. The carrier matrix activated carbon plays a major role in immobilizing the organisms and protects them from toxicity. The first stage fermentation reaction reduced COD by 78.8 ± 3.6%, TOC by 73.6 ± 5.4%, and BOD by 50.7 ± 3.5% while VFA was increased by 250.8 ± 7.9%. The second stage anaerobic reaction caused reduction in COD by 85.9 ± 0.2%, TOC by 71.1 ± 0.8%, VFA by 95.2 ± 1.3% and BOD by 91.8 ± 0.4%. The HPLC analysis of the VFA resulted from first stage of fermentation reaction confirmed propionic acid and acetic acids as the major constituents. Mass spectrometry of fermented and anaerobically treated DASW revealed the possibility of break down of phthalic acid residues through the formation of benzoyl co A. Proton and ^{13}C NMR spectroscopy confirmed the breakdown of DASW into aliphatic carboxylic acid compounds. Hence, this study concludes the combination of yeast fermentation and anaerobic – activated carbon hybrid reactors are highly efficient for the disposal of solid waste generated from dicarboxylic acid manufacturing industries.

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