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Chemical characteristics of the granular sludge from an UASB reactor treating binary mixture of catechol and resorcinol in an aqueous solution

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Abstract. The chemical characteristics of the granules acclimated with binary mixture of catechol and resorcinol in an aqueous solution were investigated by means of inductively coupled plasma mass spectrometer (ICP MS), scanning electron microscopy coupled with electron disperse x-ray analysis (SEM EDAX), X-ray diffractometer (XRD) and fourier transform infra-red spectrometer (FTIR) techniques. ICP MS results showed that most of the metals decreased drastically after treatment but Ca and Fe increased. EDAX analysis showed that mineral content Si, Al, S, Fe, Mg, Na increased and Ca, P, K decreased after the treatment. XRD analysis indicates minor changes occurring in the sludge. The Fourier transform infra-red spectroscopy analysis confirmed the biodegradation of amine salts and slight accumulation of volatile fatty acids, aliphatic and aromatic ethers, and polysaccharides.

Keywords: chemical oxygen demand, sludge characteristics, catechol, resorcinol

1. Introduction

Catechol and resorcinol belong to phenol family and are derivatives of benzene. These are hematotoxic and hepatotoxic, reveal peroxidative capacity and provoke mutagenesis and carcinogenesis toward humans and other living organisms [1]. Catechol and resorcinol are found in many industrial wastewaters. It is, therefore, necessary that the phenolic compounds must be removed from the wastewaters to meet the regulatory discharge standards and also to improve the removal efficiency of other compounds which contribute to the chemical oxygen demand (COD) in the effluents.

Recently, Subramanyam and Mishra [2] reviewed the literature on the co-degradation of catechol and resorcinol and reported on their degradation in an UASB reactor acclimatized to catechol concentrations. They found that resorcinol/catechol in a ratio of 1:4 in a total concentration of 1000 mg/L can be treated effectively in an UASB reactor having a hydraulic retention time (HRT) of 8 h and an organic loading rate (OLR) of 5.7 kg COD/m^3.d. They compared their results with those reported in literature [3,4]. It was found that the catechol acclimated sludge can adapt itself to resorcinol and can effectively biodegrade it if present in a ratio of 1:4. However, the reverse is not true. This is basically because of the totally different degradation pathways of catechol and resorcinol [5,6]. The characteristics of the granular sludge during treatment of phenol and its derivatives have been reported and discussed by many investigators [7,8]. Recently, Subramanyam and Mishra [8] reported the characteristics of the granular sludge during biodegradation of catechol, with and without glucose as a co-substrate, in a bench scale UASB reactor. However, there is not much information on the characteristics of the granular sludge treating a mixture of

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phenolics in wastewaters in an UASB reactor. Since the degradation of mixed pollutants exhibit their particular characteristics, the characterization of the granular sludge adapted to such mixed substrates is very important. Microbial granules have a vital role to play in the field of biological wastewater treatment. The characteristics of granules have all been identified as of a great significance [9-11]. The characteristics of granular sludge varied by reactor operating conditions as well as the wastewater composition. Whenever the composition of wastewater is changed, the UASB granule-associated bacteria have to re-organise microbial spatial distribution and structure in order to adapt to new metabolic processes required for the oxidation of present organic substrate. Such a substrate change-induced structural re-organisation would result in a partial or complete breakup of the UASB granules grown on the former substrate [2,12].

For a steady operation, it is significant to be able to determine and manipulate the granular sludge characteristics, which can be used as a basis for the design and monitoring of bioreactors. Thus, it is essential to elucidate the physicochemical characteristics of microbial granules [11]. No study has been reported by the previous researchers to examine the influence of resorcinol on catechol acclimated sludge characteristics using UASB reactor.

The present paper deals with the chemical characteristics of the granular sludge of an UASB reactor, which has shown high treatment efficiency for catechol and resorcinol in aqueous solutions.

2. Materials and Methods

2.1. The operating conditions of the reactor

A 9.75 L laboratory scale UASB reactor (100 mm diameter x 1200 mm height) initially charged with a 3 litre screened anaerobically digested sludge taken from a full-scale UASB reactor treating municipal wastewater and fed on synthetic wastewater (SWW) containing glucose as a substrate (concentration 470-4686 mg/L) for the startup and granulation over a period of 120 d. The granules were observed to be of the size between 0.5 and 2.5 mm at the end of day 120. This was acclimated and stabilized with first catechol (concentration 100-1500 mg/L) in the presence of glucose in the SWW and later on only with catechol up to day 325, at 35±2 ^{o}C.

The thoroughly acclimatized reactor biomass with catechol, was then fed with an aqueous solution of catechol and resorcinol in different concentration ratios while maintaining a total concentration of binary at 1000 mg/L. The details of acclimatization of the sludge and the treatment of catechol and resorcinol are given elsewhere [2,8,13]. The steady state performance results of the UASB reactor treating mixed feed of catechol and resorcinol are presented in Table 1.

Table 1 Operational parameters and the reactor performance results during co-degradation of catechol and resorcinol in an UASB reactor

Duration days	HRT h	Feed Concentration mg l^{-1} R	Feed Concentration mg l^{-1} C	R/C ratio	Influent* COD mg l^{-1} Avg.±S.D.	OLR kgCOD $m^{-3}d^{-1}$ Avg.±S.D.	Effluent pH	COD removal % Avg.±S.D.	Biogas production $m^3m^{-3}d^{-1}$ Avg.±S.D.
326-350	8	50	950	1/19	2036±385.33	6.01±1.14	7.00-6.65	63.7±14.17	1.20±0.62
351-364	8	100	900	1/9	1902±554.28	5.62±0.16	7.12-6.73	73.18±1.58	1.54±0.16
365-379	8	150	850	3/17	1934±77.05	5.71±0.23	7.01-6.60	87.22±2.22	1.86±0.19
380-393	8	200	800	1/4	1905±51.69	5.63±0.15	6.98-6.55	89.07±2.12	2.15±0.14
394-407	8	250	750	1/3	1895±57.1	5.60±0.17	7.05-6.68	78.07±2.24	1.83±0.06
408-422	8	300	700	3/7	1849±96.72	5.46±0.29	6.95-6.76	71.88±1.38	1.72±0.05
423-453	8	400	600	2/3	1884±86.80	5.56±0.26	7.08-6.63	59.12±6.78	1.38±0.16
454-470	8	250	750	1/3	1918±59.21	5.66±0.17	6.96-6.71	38.99±4.52	0.77±0.05

* The values have been approximated as 1889 mg l^{-1}. Note: R: Resorcinol; C: Catechol

2.2. Analysis

The metal concentration in the sludge samples was determined by an inductively coupled plasma mass spectrometer (ICP MS), (Perkin Elmer Sciex, Elan DRC-e, USA). A scanning electron microscope (SEM) coupled with electron disperse X-ray analysis (EDAX) (SEM EDAX, Philips XL-30) was used for the elemental analysis of the surface of the sampled granules. The compositional characteristics of the sludge sampled from the UASB reactor were analysed by an X-ray diffractometer (Brucker AXS, Diffraktometer D8, Germany). A Fourier transform infra-red spectrometer (FTIR-Thermo Electron Corporation, Nicolet

Avatar 370 CsI) was used to find out the surface functional groups of the sludge samples. All analytical reagent grade chemicals were used in the present investigation. The procedure for preparation of samples for ICP-MS and FTIR tests were described previously (8).

3. Results and Discussion

3.1. Metal content of the sludge

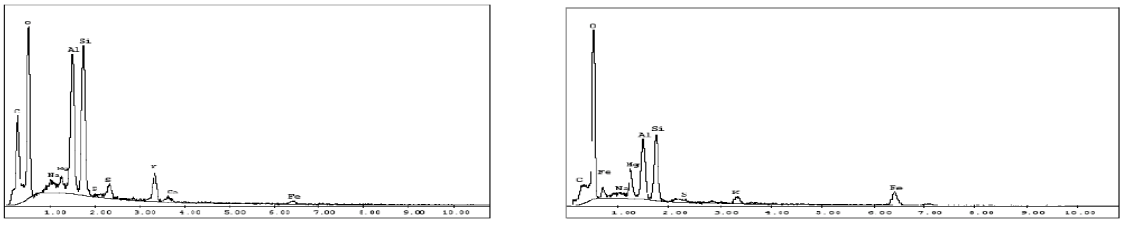
The mineral compositions of the methanogenic granular sludge grown on catechol and the binary mixture of catechol and resorcinol are reported in Table 2a. The sludge acclimated with catechol alone shows high amounts of Na, K, Mg and Ca with a very small amount of P, Co and Fe. When the reactor was switched over to the binary mixture of catechol and resorcinol, the granules exhibited high amounts of Fe and Ca. It is seen that the granules leach out Na, K and Mg substantially and accumulate calcium and iron, although phosphorous level remains intact. Divalent cations such as calcium and magnesium facilitate the adhesion of cells resulting in the formation of pellets and granular type of aggregation [14]. Calcium increased by ~51% from 12.36 to 18.66 mg (g dry sludge)$^{-1}$ and iron increased by ~2890% from 0.68 to 20.25 mg (g dry sludge)$^{-1}$. Ramakrishnan and Gupta [7] reported that relatively higher percentage of iron and calcium and a lower percentage of other inorganic components including sodium, potassium, etc., got incorporated in the granules during the treatment of phenolic wastewaters. Our results indicate that the calcium and iron at increased levels in the granules play a very important role in the treatment of the mixed feed of catechol and resorcinol in a catechol acclimated UASB reactor.

Table 2
Elemental composition of the ash for various sludges.
2a. ICP-MS results (mg/g dry sludge)

Element	Sludge after catechol treatment (day 325)	Sludge after catechol and resorcinol treatment (day 470)
Na	79.48	4.39
K	68.54	5.21
Mg	47.38	10.29
Ca	12.36	18.66
P	1.39	1.34
Co	1.15	0.26
Fe	0.68	20.25
2b. EDAX results (average atom %)		
C	66.175	49.47
O	22.17	33.08
Si	4.005	6.02
Al	3.345	4.26
K	1.165	0.60
S	0.815	1.15
Fe	0.6	2.71
Mg	0.565	1.39
Na	0.56	0.83
Ca	0.305	0.30
P	0.3	0.19

3.2. SEM-EDAX analysis

SEM-EDAX of the granules grown initially on glucose and catechol, and thereafter catechol alone and then acclimated and stabilized with the binary mixture of catechol and resorcinol in the UASB reactor was carried out at two locations on the granule surface. The average elemental composition for the two surface locations in terms of atom % is presented in Table 2b. One of the spectra that elucidates the local concentration of individual elements on the granular surface on day 325 and 470 is shown in Fig. 1.



(a) Granular sludge observed on day 325 (b) Granular sludge observed on day 470

Fig. 1

Inorganic elements that precipitated in the granules and are identified from the EDAX of the granular surface are silicon, aluminum, potassium, sulphur, ferrous, magnesium, and minor amounts of sodium, calcium and phosphorous (Table 2b). The granule surface contained more silicon and aluminum than that of the other inorganic elements. This may be the reason for high resistance of granules to toxics-catechol and resorcinol even at a high total concentration of 1000 mg L^{-1}. Similar observation was reported by Kosaric et

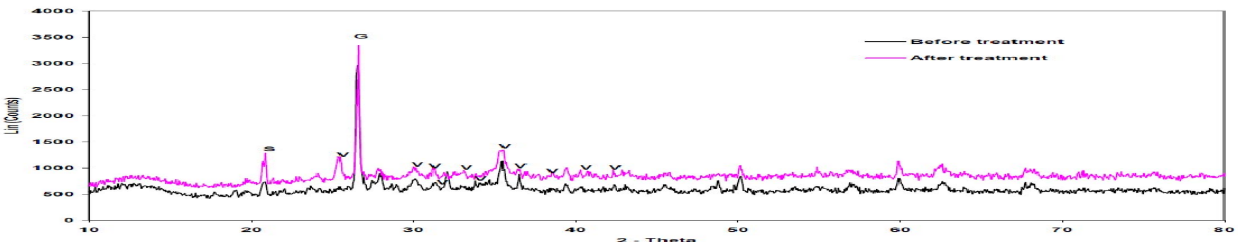
al. [15] about the granules grown on a synthetic volatile fatty acid medium. The granules sampled after the washout of a part of the biomass from the reactor, when the reactor was operated at different (R/C) ratios after the operation at the optimum R/C ratio of 1:4, show a slight decrease in the total sum of carbon and oxygen from 88.3% to 82.6%. It is seen from Table 2b that the carbon content decreased from 66.2% to 49.5% and oxygen increased from 22.2% to 33% when the reactor was switched over from catechol-bearing feed to the binary feed of catechol and resorcinol. The total inorganic mineral content on the granular surface for the granules acclimatized with catechol only was ~12% which increased to ~18% after the treatment of the binary mixture of catechol and resorcinol. An increase in the inorganic mineral content is a clear indication of the fact that the microbial mass in the granular sludge decreased due to a sudden decrease in the resorcinol concentration from 400 mg L^{-1} to 250 mg L^{-1} in a total concentration of 1000 mg L^{-1}. This means that the biomass acclimated to a feed of a particular R/C ratio experiences either inhibition or is limited due to its degradation potential when it is subjected to a different R/C ratio. This phenomenon seemed to be more pronounced while decreasing the feed of higher R/C ratio to a lower R/C ratio.

The EDAX results also show that the amount of Si, Al, S, Fe, Mg and Na increased and that the amount of Ca, P and K decreased. Hulshoff Pol [16] reported that the presence of iron and sulphur in significant quantities on the surface of granules possibly contributed to the aggregation of biomass. In the present study, the ratio of S/Fe was 1.4 and 0.42 for the granules grown on catechol alone and the binary mixture of catechol and resorcinol, respectively, and that the black color of the granules intensified when the reactor was switched over to the binary mixture of catechol and resorcinol. This observation is reinforced with the findings of Kosaric et al. [15] who proposed that the granules grown on a synthetic VFA medium appear to be black when the ratio of S/Fe is small. The black colour of granules probably appears due to ~41% increase in sulphur content from 4.01 to 6.02% and an increase of ~3.50% in iron content from 0.6 to 2.71% in the form of iron sulphides and related compounds.

3.3. XRD analysis

XRD analysis was carried out to understand the crystalline structure of the sludge acclimated and grown on catechol and, thereafter, on the binary mixture of catechol and resorcinol as shown in Fig.2. The analysis

Fig. 2



of the crystals observed in the interstitial spaces of the sludge indicated the existence of graphite (C) as the major constituent. It is already known from EDAX analysis that granules consist of predominantly carbon. The EVA software used for the XRD analysis shows only one sharp peak (2θ=26°) which corresponds to (002) plane of graphitic structure. The intensity of peaks does not show major changes except for the disappearance of peak at ~ 49.3° (2θ). A rise in the peak at ~ 25.55° (2θ) after exposure to binary mixture indicates minor changes occurring in the sludge constituents. Inorganic precipitates localized in the sludge granules are identified as vuagnatite ($CaAlSiO_4(OH)$) and struvite ($MgNH_4PO_4(H_2O)_6$. This indicates that the major fraction of the granular sludge comprised organic matter and that the inorganics are in the form of precipitates of calcium, aluminium and magnesium salts.

3.4. FTIR spectroscopy analysis

Fig. 3 shows the FTIR spectra of the granules grown on catechol and the binary mixture of catechol and resorcinol. The spectra exhibited similar absorbance peaks at similar wave numbers but the relative intensity of the absorbance changed after the treatment of the binary mixture. In particular, the peak at about 2925 cm^{-1} increased very slightly which indicated that the characteristics of VFAs and other aliphatic components remained unchanged during the treatment period. The disappearance of the peak at ~2360 cm^{-1} after the treatment of binary mixture indicates biodegradation of amines in the sludge. The increase in the absorbance intensity at around 1040 cm^{-1} was very small after the treatment of binary mixture. This indicates that the

accumulation of aromatic ethers and polysaccharides in the sludge sample was very small. Changes after the treatment affected only band intensities, indicating that the mineralization of the binary feed containing catechol and resorcinol does not effect significant changes in the surface functionalities of the granular sludge. The sludge sample contains primarily proteins (amino acids), fats (aliphatic esters) and cellulose, as organic substances [17].

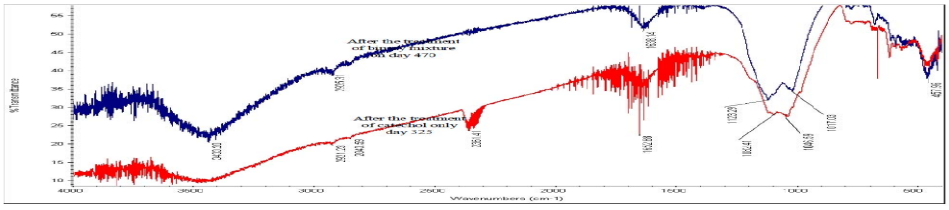


Fig. 3

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