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Anaerobic biogranulation in a hybrid reactor treating phenolic waste

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Abstract

Granulation was examined in four similar anaerobic hybrid reactors 15.5 L volume (with an effective volume of 13.5 L) during the treatment of synthetic coal wastewater at the mesophilic temperature of $27 \pm 5\,^{\circ}C$. The hybrid reactors are a combination of UASB unit at the lower part and an anaerobic filter at the upper end. Synthetic wastewater with an average chemical oxygen demand (COD) of 2240 mg/L, phenolics concentration of 752 mg/L and a mixture of volatile fatty acids was fed to three hybrid reactors. The fourth reactor, control system, was fed with a wastewater containing sodium acetate and mineral nutrients. Coal waste water contained phenol (490 mg/L); *m*-, *o*-, *p*-cresols (123.0, 58.6, 42 mg/L); 2,4-, 2,5-, 3,4- and 3,5-dimethyl phenols (6.3, 6.3, 4.4 and 21.3 mg/L) as major phenolic compounds. A mixture of anaerobic digester sludge and partially granulated sludge (3:1) were used as seed materials for the start up of the reactors. Granules were observed after 45 days of operation of the systems. The granules ranged from 0.4 to 1.2 mm in diameter with good settling characteristics with an SVI of 12 mL/g SS. After granulation, the hybrid reactor performed steadily with phenolics and COD removal efficiencies of 93% and 88%, respectively at volumetric loading rate of 2.24 g COD/L d and hydraulic retention time of 24 h. The removal efficiencies for phenol and *m*/*p*-cresols reached 92% and 93% (corresponding to 450.8 and 153 mg/L), while *o*-cresol was degraded to 88% (corresponding to 51.04 mg/L). Dimethyl phenols could be removed completely at all the organic loadings and did not contribute much to the residual organics. Biodegradation of *o*-cresol was obtained in the hybrid-UASB reactors.

Keywords: Anaerobic; Granulation; Morphology; Hybrid reactor, Coal effluent

1. Introduction

Developing countries are giving priority to their fast industrial growth. Rapid industrialization has resulted in the dwindling reserves of petroleum and natural gas. Coal conversion processes (coal liquefaction and coal gasification) are promising alternatives for the production of gaseous and liquid fuels. Coal conversion wastewaters contain toxic compounds that not only resist degradation, but also inhibit the degradation of other constituents of the wastewaters. Among the organic constituents of coal conversion wastewaters, phenolic compounds primarily phenols, methyl phenols and C_2-phenols constitute 60–80% of the organic content. In addition to their suspected mutagenicity and carcinogenity, phenolic compounds are reported to be either toxic or lethal to fish at concentration of 5–25 mg/L [1].

Biological processes are very effective for treating aqueous waste streams containing mixtures of organic constituents. Aerobic biological processes with or without pre-treatment for the reductions of ammonia and phenolics have been proposed for the treatment of coal wastewaters [2,3]. Despite long aeration times and high energy consumption, many undesirable constituents of coal conversion wastewaters including phenol, cresols and dimethyl phenols are not removed in the aerobic processes. Recent developments have demonstrated that anaerobic processes might be an economically attractive alternative for the treatment of different types of industrial wastewaters. Suidan et al. [4] examined the treatment of coal gasification wastewater with a process train consisting of a berl saddle packed anaerobic filter followed by an expanded bed activated carbon anaerobic filter. Khan et al. [5] demonstrated that a granular activated carbon packed anaerobic reactor could treat synthetic wastewater containing relatively high influent concentration of phenol and catechol efficiently with methane production playing a significant role. Among the high rate anaerobic technologies, upflow anaerobic sludge blanket (UASB) process has been widely applied due to its simplicity, low cost and higher efficiency. Literature on the upflow anaerobic sludge blanket reactors suggested that the reactors could be modified by adding an anaerobic filter

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in the upper zone [6]. The modified system provides enhanced biomass holding capacity and a higher solid retention time.

Anaerobic digestion in UASB's centres on the anaerobic sludge which maintains superior settling characteristics for the stabilization of organic matter. The formation of anaerobic granular sludge can be considered as the major reason of the successful treatment of industrial effluents [7]. In UASB reactors, microorganisms agglutinate to form compact granules with high biological activity and settleability. With the development of good quality of granular sludge maximum COD removal efficiency even at higher organic loading rate can be achieved. Moreover, the high granular biomass accumulation and layered granular structure protect bacteria from exposure to toxic compounds by providing better mass transfer resistance to substrate diffusion inside the granules. Recent studies have revealed that granular sludge based technology is effective for the treatment of industrial wastewaters containing toxic phenolic compounds, such as benzoate [8], phenol [9] and [10] and nitrophenols [11]. However, majority of these studies were conducted using single phenolics like phenol or *p*-cresol. The biological treatment of complex phenolic wastewater has often been studied utilizing activated carbon in anaerobic reactors, where activated carbon served to adsorb the toxic pollutants and acted as a carrier for bacterial growth [12,13]. There are fewer studies on the continuous anaerobic treatment of mixed phenolic compounds without any dose of activated carbon [14–16]. The successful treatment of coal conversion effluents will require that the major phenolic substrates (phenols, cresols, and dimethyl phenols) should be degraded simultaneously. Therefore, biodegradation studies should evaluate mixture of phenols.

Degradation of phenol, *o*- and *p*-cresol by methanogenic consortia is well established [17–21]. Phenol and *o*-cresol are first carboxylated to *p*-hydroxy benzoic acid and 4-hydroxy 3-methyl benzoic acid, respectively. These compounds are then decarboxylated into benzoic acid and *m*-toluic acid, respectively [18]. *P*-cresol degradation proceeds by oxidation to produce 4-hydroxy benzaldehyde and 4-hydroxy benzoic acid [22]. Dimethyl phenols undergo demethylation reactions to form their corresponding methyl phenolic isomers and follow the methanogenic degradation pathway to form hydroxyl benzoic acid. All the intermediates are transformed into a common metabolite benzoic acid, which is then reduced, cleaved and transformed into acetate and propionate and finally methane [23].

Start up of the anaerobic reactors is more time consuming and is subjected to disturbances more than that of aerobic reactors. Many researchers [24–26] reported long start up periods of 2–3 months to 1 year (or even more) for the anaerobic reactors. The start up of the anaerobic process is still considered a major area of research. Considerable efforts have been made to study the granulation process but the mechanism involved in the formation of granular sludge is still unknown. There is lack of information and data on the effects of various operating parameters on the process of granulation and morphology of the granules. The emphasis of the present work has been to correlate the effect of various operating parameters on the granulation occurring in anaerobic hybrid UASB reactors using a simulated coal wastewater. Due to the toxic nature of coal conversion waters, the present work has been carried out in the hybrid reactor system—a two phase system (consisting of suspended growth and attached growth). The specific objective was to study the biomass granulation process in anaerobic hybrid systems fed with wastewater containing a high content of aromatic toxic compounds.

2. Materials and methods

2.1. Experimental setup

For the study, four identical bench scale hybrid reactors (R1, R2, R3 and R4) of total volume of 15.5 L (with an effective volume 13.5 L) each were designed as per the guidelines given by Lettinga and Hulshoff Pol [27] and fabricated. The schematic diagram of the hybrid reactor is given in Fig. 1. The reactors were constructed from transparent acrylic plastic sheet with inner dimensions of 0.1 m × 0.1 m, length of 1.5 m and wall thickness of 6 mm. The reactors were provided with hopper bottom of 0.15 m length and a feed inlet pipe of 2.5 cm diameter to avoid choking during operation. An outlet was provided at the top (1.5 m), which is connected to the effluent tank. Gas solid separator (GSS) device was of square pyramid with bottom dimensions 80 mm × 80 mm. The reactors were provided with six equidistant ports along its height to facilitate sampling. A filter media of length 30.48 cm was provided at the middle of the reactor. The filter media consisted of polyvinyl chloride

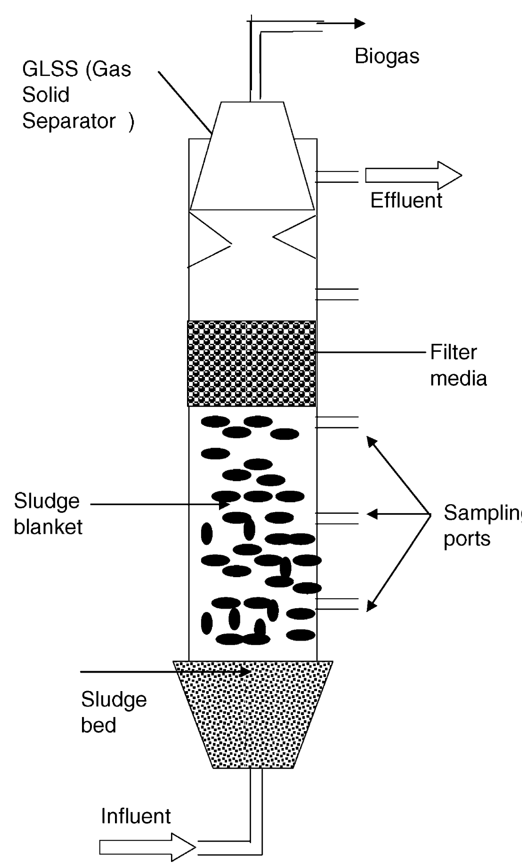

Fig. 1. Schematic diagram of the hybrid UASB reactor.

(PVC) rings. About 215 rings were packed in all the four reactors for consistency. The surface area of each ring was 6.28 cm^2 and the total surface area occupied by the packing was 1350.2 cm^2. Reactors were seeded with a mixture of digested and partially granulated sludge (3:1). Digested sludge (median bio-particle diameter of 0.025 mm and total solids content of 24.2 g/L) was obtained from Mahanadanda Dairy works, Goregaon, Mumbai. Partially granulated sludge (total solids content of 62.1 g/L) was obtained from the bench scale UASB reactor treating phenolic compounds. The quantity of seed sludge was estimated as per the guidelines mentioned in the literature [28]. The reactors were operated at mesophilic temperature (27 ± 5 °C) and they were examined for early start up and granulation.

2.2. Analytical procedures

Alkalinity, pH, COD, SVI, SS, VSS and TSS were analyzed according to the Standard Methods for the Examination of Water and Wastewater [29].

Phenolic compounds were determined by gas chromatograph (Agilent Model: 6890 No. G1530, USA). The fluid sample was filtered through a 0.45 μm membrane filter, and extracted with dichloromethane (1:2) prior to injection into the column using fast injection technique and directly analyzed in gas chromatograph furnished with capillary column (CP-SIL 5 CB, 50 m × 0.25 mm). Helium was used as a carrier gas and FID was used as a detector. Injector temperature was 200 °C. Detector temperature was 250 °C. The initial temperature of the column was 70 °C followed with a first ramp of 10 °C/min to the temperature of 140 °C for 2 min and a second ramp of 10 °C/min and a final ramp of 170 °C for 2 min.

Methane was quantified with the gas chromatograph with a thermal ionization detector. Column, injector and detector temperature were maintained at 55, 90 and 90 °C, respectively. Nitrogen was employed as a carrier at a flow rate of 20 mL/min. Volatile fatty acids (VFA) in the effluent was measured by injecting 2 μL of filtered acidified samples through gas chromatograph (Perkin-Elmer, Sigma 2000, USA) equipped with Flame Ionization Detector using a 10% free fatty acid phase (FFAP) on (60/80) Chromosorb WHP/0.1% orthophosphoric stainless steel column. FFAP is cross-linked and bonded to resist the damage that can occur when injecting water based samples and can be used at operating temperatures at 60–250 °C. The analysis was carried out at an oven temperature of 150 °C, injector temperature of 180 °C and detector temperature of 250 °C. Hydrogen and zero air were used to fuel the flame, while nitrogen as carrier gas was applied at the rate of 20 mL/min.

Scanning electron microscope (SEM) (Zeiss model DSM-960) was employed to study the morphology of the granules. The size of the granules was determined by optical microscope (Nikon SMZ, USA). Mineral analysis was carried out by drying the granular sludge samples in an oven at 105 °C, and digestion in microwave Labstation (mls 1200 mega, Milestone, Italy). Digested sludge samples were analyzed for different elements using argon plasma in inductively coupled plasma atomic emission spectrophotometer (ICP-AES, 8440 MO Plasma Lab, GBC, Australia). The size distribution of granules was estimated in an image analysis system (Quantimet 500 Image Analyzer, Leica, Germany) using a commercial statistical software package (SAS Institute Inc., Cary, NC).

2.3. Strategy of operation

The start up was carried out following a procedure modified from that of Bull et al. [30] using stepped organic loading to produce most rapid biomass development. Organic loadings were increased by increasing influent COD concentration upon attainment of pseudo-steady state. Pseudo-steady state was defined based on the consistent COD removal efficiency.

The continuous experiments were conducted in four laboratory scale identical hybrid UASB reactors and were operated on a continuous mode for a period of 120 days to study the start up and granulation of biomass. During the whole period of study, the hybrid UASB reactors were operated continuously with a constant hydraulic retention time (HRT) of 24 h. The start up operation was divided into five phases. The total phenolics concentration in the feed was increased stepwise during the five phases as follows: 226, 376, 451, 602, and 752 mg/L. The corresponding COD concentrations were 530, 860, 1440, 2000, and 2240 mg/L, respectively. At the end of phase 5, the reactors were operated at steady state for a period of 40 days (days 80–120) at an influent total phenolic concentration of 752 mg/L. At each loading condition, the reactors were closely monitored until a quasi steady state was reached. Three reactors were fed with an influent containing a mixture of volatile fatty acids and phenols and the control reactor was fed with sodium acetate and mineral nutrients. The synthetic wastewater used in the experiment contained 490 mg/L phenol; 58.6 mg/L *o*-cresol; 123.0 mg/L of *m*-cresol; 42.0 mg/L of *p*-cresol; 4.2 mg/L of 2,4-dimethylphenol; 4.2 mg/L of 2,5-dimethylphenol; 6.3 mg/L of 3,4-dimethylphenol and 21.3 mg/L of 3,5-dimethylphenol as major phenolic compounds. Volatile fatty acids in the synthetic wastewater were represented by 28.0 mg/L acetic acid; 16.0 mg/L propionic acid; 5 mg/L butyric acid and 3.5 mg/L valeric acid, respectively. Major nutrients in the feed included 280 mg/L ammonium chloride; 100 mg/L calcium chloride; 250 mg/L dipotassium hydrogen ortho phosphate; 100 mg/L magnesium sulphate; 20 mg/L iron sulphate; 1000 mg/L sodium bi carbonate. Trace metal solution was prepared in distilled water by dissolving per liter 0.05 mg zinc chloride; 0.05 mg cupric chloride; 0.03 mg manganous sulphate; 0.05 mg ammonium molybate; 0.05 mg aluminium chloride; 0.05 mg cobaltous chloride and 0.05 mg nickel chloride. One milliliter of this solution was added per liter of the feed solution. These compositions are comparable to those used by Kosaric et al. [31] in their study on granulation in UASB reactors.

The reactors were started up with the same amount and proportions of seed of the two different biomasses. Moreover, the reactors were operated maintaining the same operating conditions. The operational conditions including influent concentration of phenolic compounds, COD, alkalinity, VFA, effluent suspended solids, biogas production, and volatile solids in the reactors were measured through out the acclimation period. During the operational period (120 days), pH and

COD were measured daily. Phenolic concentrations in the effluent and methane percentage were measured weekly. Biogas production, methane production, SS in the effluent and VSS in the reactors were measured biweekly. Morphological and physico-chemical characterization of sludge was carried out at each organic loading. Size distribution of granules was carried out by sampling granules from the upper and lower portions of the sludge bed. Effect of organic loading on the morphology and other physical parameters of the granules were studied at each loading condition. The start up of the reactors in this case was defined as the time taken by the reactors to achieve quasi steady state. The granulation period was defined based on the first appearance of granules in the reactors.

3. Results and discussion

3.1. Effect of step-loading of phenolics on the performance of the reactors

The time dependent response of the hybrid reactors during the start up period is presented in Fig. 2.

During phase 1 (days 1–15) of start up operation, the synthetic solution containing a mixed phenolic concentration of 226 mg/L (530 mg COD/L) was fed to the reactors for 15 days (Fig. 2a). The biogas production was not observed till day 3. During the first 5 days of phenolics feeding, a low COD removal of only 5–30% was achieved. Then the sludge started to recover and adapt to the phenol substrate at a fast pace. By day 8, the reactors were capable of producing more than 0.5 L/d of biogas with greater than 60% COD removal efficiency achieved. No VFA other than acetate were detected in the effluent. The acetate concentration in the reactor effluent increased to 50 mg/L on day 5, which then subsided to a level of 11–13 mg/L after day 10. The COD removal efficiency of the reactors at the end of the phase 1 ranged between 70% and 77% (Fig. 2b) with a biogas production of 2.1–2.5 L/d, having a methane content of 37.5–41.5%

Phase 2 (days 15–25) of the start up operation lasted 20 days; the feed mixed phenol concentration was maintained at 376 mg/L (860 mg COD/L). The biogas production rate increased two-fold after only 8 days of operation and remained at 5 L/d through out the remainder of phase 1. The COD removal efficiency was greater than 85% in all the reactors by day 20. Little accumulation of VFA was detected even during the transitional period. Acetate was the only VFA detected in the effluent with the concentration in the effluent fluctuating, but remaining at less than 15 mg/L for phase 2.

During phase 3 (days 25–40) of the start up operation, phenolics concentration in the feed solution was increased to 451 mg/L (1440 mg COD/L). When the phenol loading was increased on day 25, the COD removal efficiency steadily decreased from 85% to 35%. However, COD removal efficiency recovered to 77% in 5 days and reached 90–92% in all the reactors by day 32. The residual phenolics concentration in the effluent was 200 mg/L during the first 3 days, but dropped rapidly to a steady 20 mg/L in 12 days in all the reactors. Acetate was the sole volatile fatty acid detected in the effluent at a concentration of 25 mg/L during the first 3 days of operation and a concentration of less than 10 mg/L for the reminder of the phase in all the reactors. This is the stage where small granules were formed in the sludge bed.

During phase 4 (days 40–55) of the start up operation, the feed phenolics concentration was increased to 602 mg/L (2000 mg COD/L). The response of the reactor to the increase in feed phenol concentration was monitored closely during the early stage of the transitional period. The daily biogas production rate increased immediately after feed phenol concentration was increased. No prolonged acclimation period was needed for bacteria to cope with increased phenol load. Total phenolics concentration in the effluent increased rapidly with increased influent phenolics concentration and was as great as 450 mg/L on day 43. Thereafter, it subsided quickly and fluctuated between 120 and 125 mg/L in all the reactors. Total phenolics concentration in the effluent averaged 27 mg/L (corresponding to 95% removal efficiency) during the final 10 days of phase 4 operation. Acetate concentration in the effluent exhibited a similar trend with a peak of 45 mg/L on day 42 and 25 mg/L during the last 13 days of phase 4. No other volatile acids except for acetate were detected in the effluent during phase 4. The granules started accumulating at a rapid pace during this phase.

During final phase of the start up, phase 5 (days 55–80), phenolics concentration in the feed was increased to 752 mg/L (2240 mg COD/L) for a continuous operational period of 25 days. The response was similar to that during phase 4 except for a greater phenol concentration in the effluent. The daily biogas production gradually increased to 8.0 L/d in all reactors in approximately 12 days after the phenol feed increased. Effluent phenolics concentration stabilized at approximately 55 mg/L in

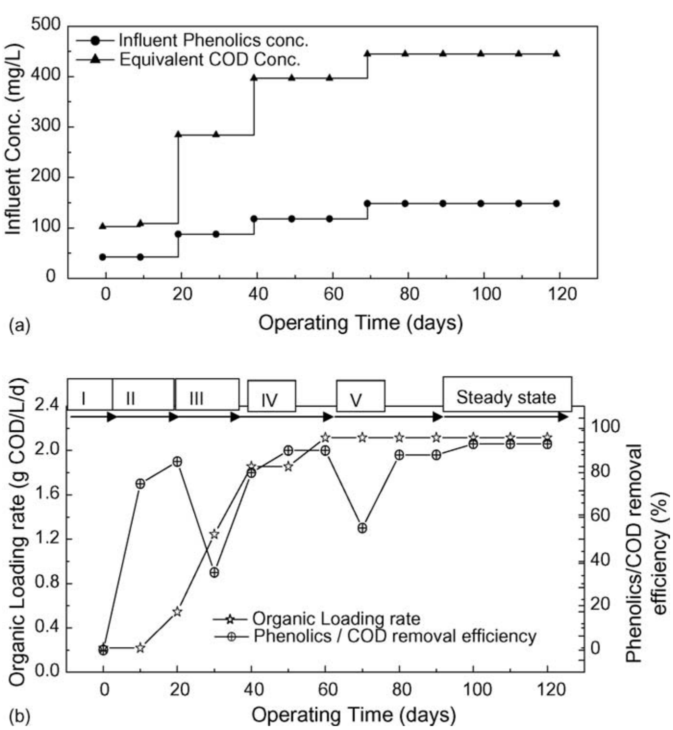

Fig. 2. Operating parameters and reactor performance during start up and steady state operation: (a) influent phenol and COD concentration, mg/L; (b) OLR, g COD/L d and phenol and COD removal efficiency, %.

all the reactors which corresponded to a COD removal efficiency of 88%. Concentration of acetate in the effluent was as high as 150–180 mg/L on day 68, with an average of 75–78 mg/L for the remaining days and subsided to 25 mg/L at the end of the operational phase. Phenol and acetate in the effluent were accumulated in this phase, with concentrations significantly greater than in other phases. Granulation proceeded steadily in this phase and granules of size 1.2 mm diameter were formed.

During the entire operational period, with a wide range of feed phenol concentrations, phenolics degradation and COD removal efficiencies exhibited a similar trend. Acetate was the only low molecular weight volatile acid detected in the effluent and its concentration increased with increased phenolic loading concentration.

Results showed the removal of all the phenolic compounds, phenol, cresols (*o*-, *m*- and *p*-) and dimethylphenols (2,4-, 2,5-, 3,4- and 3,5-). Phenol concentration in the effluent stabilized to 39.2 mg/L (corresponding to 92% removal), while *p*-cresol and *m*-cresol concentrations remained at 8.6 mg/L in the effluent (corresponding to 93% removal), while *o*-cresol started accumulating in the reactors during phase 4 on day 50 and it finally stabilized to 7.06 mg/L by day 80 (corresponding to 88% removal). Dimethyl phenols could be removed completely at all the organic loadings and did not contribute much to the residual organics. The results were comparable to that of the observation of Cross et al. [32] in which in an anaerobic GAC filter system, the maximum COD loading rate was 2.5 g/L/d with 93% phenol removal efficiency using a 10-fold diluted coal gasifier effluent (phenol = 560 mg/L). Though the scope of the study was not to observe the effect of one phenolic compound on the removal of other, yet it was observed that, among the phenolics, *m*-cresol and *o*-cresol accumulated in the effluent and contributed to the residual organics. Residual *m*-cresol concentration can be attributed to the fact that *p*-hydroxybenzoate, an intermediate in the *p*-cresol degradation pathway inhibited *m*-cresol metabolism. This is similar to the observation of Ramanand and Suflita [33]. The reactors at the end of acclimation phase were removing 752 mg/L of complex phenolic mixture representing the major fraction of the synthetic wastewater.

o-Cresol is the most toxic isomer among the cresols and complete mineralization of this isomer from industrial wastes has not been reported till date. Removal of *o*-cresol upto 51.04 mg/L could be achieved in this study. Higher degree of bio-conversion could be possible due to the enhanced biomass holding capacity and process stability of the hybrid reactor. The biodegradation of *o*-cresol in a hybrid-UASB reactor treating a mixture of 8 phenolic compounds is an important observation in the anaerobic biodegradation of toxic compounds. Razo-Flores et al. [34] observed the biodegradation of *o*-cresol in continuous UASB reactor treating a mixture of 3 phenolic compounds.

For 1 month from days 90 to 120 the reactor was considered to be operating at a pseudo-steady state condition with a feed phenol concentration of 752 mg/L (2240 mg COD/L), HRT of 24 h, OLR of 2.24 g COD/L/d- and SLR of 0.75 g COD/g VSS/d. Statistical operational data are shown in Table 1. Average COD removal efficiency was 88%. During this period, the reactor produced 10 L of biogas with 67% methane content daily. Based on GC analysis, the effluent on an average contained 25–50 mg/L of phenolics. Acetate was the only volatile acid detected in the effluent, with an average concentration of 25 mg/L. Benzoate concentration was below the detectable level.

Table 1
Steady state operational data of the hybrid UASB reactors

Parameter	Value	Unit
Operating conditions	–	–
Feed phenol concentration	752	mg/L
Equivalent COD concentration	2240	mg/L
HRT	24	h
OLR	2.24	g COD/L d
SLR	0.75	g COD/g VSS d
Effluent quality	–	–
Phenolics	55	mg/L
COD	220 ± 42	mg/L
Acetate	25 ± 18	mg/L
Other VFAs	Nil	
Removal efficiency	–	–
Phenolics	93 ± 1	%
COD	88 ± 1	%
Biogas	–	–
Biogas production	10 ± 0.1	L/d
Methane content	67 ± 2	%
Methane yield	0.5 ± 0.01	L CH_4/g COD removed
Sludge production	0.05 ± 0.001	kg VSS/kg COD_{rem}

3.2. Sludge granulation course

The bio-particle size at different operational periods under various organic loadings was used for the characterization of the granulation of sludge. The bio-particles grew slowly during the initial 20 days of operation. This might be due to the fewer bacterial populace present in the reactor which were getting slowly acclimated to the new substrate. After day 25, small initial granules were formed in the lower part of the reactor and began to accumulate at a rapid pace. Bio-particle growth increased steadily and on day 40, formation of small initial granules was observed. Granule initiation has been reported to be crucial for the successful granulation in anaerobic systems [35]. This initial granular growth progressed for a period of 80 days until granules were formed with diameters in the range of 1–1.2 mm. SVI values for the granules ranged from 16 to 20 mL/g SS. With initial granules developed sludge settleability improved progressively as a result of which higher organic loading could be applied and this in turn promoted the granular growth. This led to the compaction of sludge which is evident from the decrease in SVI from 20 to 12 mL/g SS in all the reactors. Fig. 3 represents the granular growth course observed in the hybrid reactors.

3.3. Sludge mass growth course

The course of sludge mass growth in terms of average sludge concentration (VSS) in the sludge bed of the reactors as a result of biomass synthesis is shown in Fig. 3. Initially, biomass concentration increased slowly in the reactors, because of extremely unfamiliar substrate introduced and subsequently the effluent

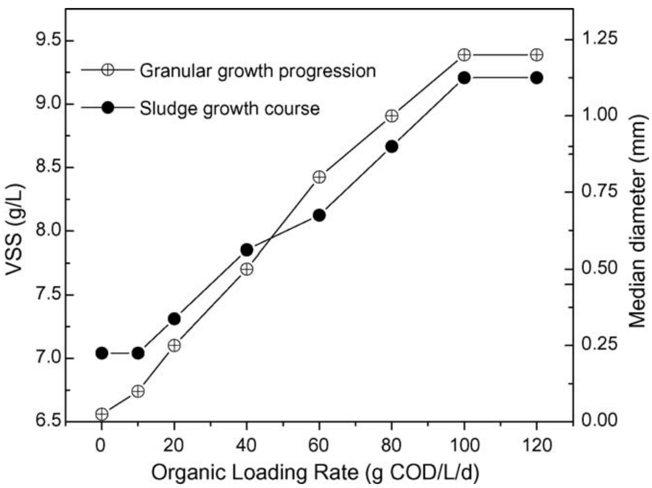

Fig. 3. Granular growth course and sludge mass growth course.

suspended solid concentration was high due to the low loading rate applied and poor settleability of the seed sludge. The sludge concentration in the reactors gradually reached 9.5 g VSS/L in all the reactors at the final stage of operation.

3.4. Characteristics of granular sludge

At the end of the 120-day operation, granular sludge was sampled from the reactor for the measurement of its characteristics. The granular sludge had a median diameter of 1.2–1.4 mm (typically ranging between 1.0 and 4.00 mm) and was well settleable with a SVI of 12 mL/g SS.

Morphological characterization of the sludge was carried out using the scanning electron microscope. Visual examination of granular biomass revealed a black color with a spherical shape. Slight irregular projections were also seen on the surface of the granules. SEM photographs of the granules showed that overall surface of granules were rough and uneven. Filamentous microorganisms were predominantly observed not only on the surface, but also in the centre of the granules. The spherical granule had numerous cavities, which represent the hollow cores formed as a result of substrate limitations inside the biological matrices. Bridging effect of inorganic elements like calcium and iron could be seen on the granule surface.

The mineral content of sludge revealed that relatively higher percentage of iron and calcium and a lower percentage of other inorganic components including sodium, potassium, zinc, cobalt, copper, molybdenum and nickel have been incorporated in the granules in the present study. Inorganic precipitates of calcium, iron and phosphorus play an important role as support materials essential for sludge granulation and are stimulated by the alkaline pH due to the degradation of acidic substances in the phenolic wastewaters [37].

To study the size distribution of granules in the sludge bed the granules from the upper and lower portions of the sludge bed were characterized. The granular diameter distribution in the upper and lower portions of the sludge bed is shown in Fig. 4a and b. Granules of smaller diameter (0.025–0.5 mm) dominate the upper portion of the sludge bed while the granules of larger

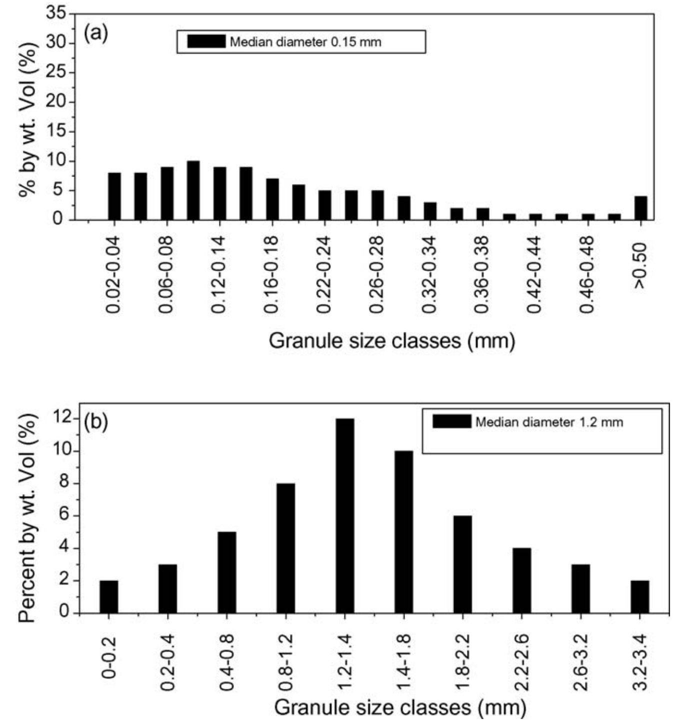

Fig. 4. Granular sludge distribution curve: (a) granular sludge distribution at the upper portion of the sludge bed; (b) granular sludge distribution at the lower portion of the sludge bed.

diameter (1.0–1.2 mm) occupied the lower portion of the sludge bed and were very scanty. It is thus certain that the granules are formed in the bottom active zone and breaks up in the upper zone. This study demonstrated that granules would have been essentially formed at the bottom active zone and they decrease in size due to shear forces and selective pressures acting on them, supporting the theory of Wentzel et al. [38]. High partial pressure of hydrogen, existing upto the bottom most active zone of the sludge bed plays a key role in the pelletization of biomass in the form of granules [36]. Majority of bio-particles in the form of smaller aggregates (<0.5 mm) play their role in maintaining the dynamic granular balance and structural stability.

The physico-chemical characteristics of granules were studied during the start up and steady state operation at various organic loading rates. SVI of the sludge in the reactors decreased from a value of 16–20 to 11–12 mL/g SS in the entire reactor. The plausible reason for the decrease in the SVI is the increased settleability of the sludge due to the pelletization of sludge occurring in the reactor.

Increase in organic loading rate resulted in an increase in the sludge bed height. The sludge bed height increased from 14% to 18% at 0.8 g COD/m^3/d to about 32–33% at 2.24 g COD/m^3/d in all the reactors.

Aggregation of granules in the sludge bed led to the pelletization of smaller granules into a larger size, which is also evident from the increase of percentage granulation with increase in organic loading rate. About 55% of granulation was achieved at the end of the operational period.

Ash content of the granules decreased with increase in the organic loading. Ash content of the granules decreased from

14–18% at 0.8 g COD/m^3/d to 11–13% at 2.24 g COD/m^3/d. Similar results were reported by Chang et al. [20] on the characteristic of anaerobic methanogenic sludge grown on phenol.

The VSS/SS ratio of sludge increased marginally from 0.4 to 0.55 with increase in the organic loading. This shows that the ratio was less than 0.7–0.8 as given in the literature for the good quality granular sludge.

4. Conclusions

Based on the findings of the above study, the following conclusions can be drawn:

- Hybrid UASB reactors could successfully degrade synthetic coal waste water with an influent phenolics concentration of 752 mg/L at an organic loading rate of 2.24 g COD/L/d and a HRT of 24 h at mesophilic temperature (27 ± 5 °C).
- Removal efficiencies for phenol and *m*/*p*-cresols reached 92% and 93% (corresponding to 450.8 and 153 mg/L), while *o*-cresol was degraded to 88% (corresponding to 51.04 mg/L). Dimethyl phenols could be removed completely at all the organic loadings and did not contribute much to the residual organics.
- Biodegradation of *o*-cresol in a hybrid-UASB reactor treating a mixture of 8 phenolic compounds is an important observation in the anaerobic biodegradation of toxic compounds.
- Granulation was successful in the hybrid UASB reactors with the combined inoculum (consisting of partially granulated sludge and anaerobic digester sludge) using phenolic compounds as sole carbon source. The granules were observed after 45 days of operation.
- Granules developed using phenolic mixtures ranged from 0.4 to 1.2 mm in diameter, were well settlable with an SVI of 12 mL/g SS. Respective phenol degradation and COD removal efficiencies of 93% and 88% was achieved.
- Morphological examination of the granules revealed the predominance of *Methanosarcina* and *Methanothrix* on the surface of the granules.
- Particle size distribution in the sludge bed revealed that the granules of smaller diameter dominate in the upper portion of the sludge bed, while granules of larger diameter occupy the lower portion of the sludge bed and are very scanty.
- The physico-chemical parameters like size of granules, percentage of granules, and VSS/SS increased with an increase in the organic loading rate, while SVI and the ash content of the granules decreased.

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