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Low-biodegradable composite chemical wastewater treatment by biofilm configured sequencing batch reactor (SBBR)☆

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Abstract

Biofilm configured system with sequencing/periodic discontinuous batch mode operation was evaluated for the treatment of low-biodegradable composite chemical wastewater (low BOD/COD ratio ∼0.3, high sulfate content: 1.75 g/l) in aerobic metabolic function. Reactor was operated under anoxic–aerobic–anoxic microenvironment conditions with a total cycle period of 24 h [fill: 15 min; reaction: 23 h (aeration along with recirculation); settle: 30 min; decant: 15 min] and the performance of the system was studied at organic loading rates (OLR) of 0.92, 1.50, 3.07 and 4.76 kg COD/cum-day. Substrate utilization showed a steady increase with increase in OLR and system performance sustained at higher loading rates. Maximum non-cumulative substrate utilization was observed after 4 h of the cycle operation. Sulfate removal efficiency of 20% was observed due to the induced anoxic conditions prevailing during the sequence phase operation of the reactor and the existing internal anoxic zones in the biofilm matrix. Biofilm configured sequencing batch reactor (SBR) showed comparatively higher efficiency to the corresponding suspended growth and granular activated carbon (GAC) configured systems studied with same wastewater. Periodic discontinuous batch mode operation of the biofilm reactors results in a more even distribution of the biomass throughout the reactor and was able to treat large shock loads than the continuous flow process. Biofilm configured system coupled with periodic discontinuous batch mode operation imposes regular variations in the substrate concentration on biofilm organisms. As a result, organisms throughout the film achieve maximum growth rates resulting in improved reaction potential leading to stable and robust system which is well suited for treating highly variable wastes.

Keywords: Composite chemical wastewater; Low-biodegradable; Sequencing batch biofilm reactor (SBR); Periodic discontinuous batch mode operation; Organic loading rate; Substrate degradation rate; Biofilm

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1. Introduction

The wastewater generated from chemical units are characteristically different with respect to quality and quantity and is considered to be relatively complex due to the presence of soluble organic materials, inorganic chemicals, suspended solids, priority pollutants, heavy metals, toxic organic, refractory substances, volatile matter, color, inorganic salts, etc. [1–6]. Regular process variation and consumption of large quantity of chemicals experience rapid and substantial change in the composition and concentration of the wastewaters and upset the biological treatment process. Conventional biological treatment processes are seldom capable of achieving required degree of performance because of the complex nature of this wastewater and prevailing shock loads [4,7–9]. Effective treatment of highly variable and complex wastewater requires system which can adapt to changing influent conditions. For treating highly variable and complex wastewater, a large reaction potential and ability to rapidly assimilate compounds is required, which prevent inhibitory accumulation of substrate if a shock load enter the system [10]. Also for efficient biological conversion of complex wastewater it requires the activity of microbial communities

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Abbreviations: BOD, biochemical oxygen demand (5-day test); *C*, concentration of substrate (COD); COD, chemical oxygen demand (closed refluxing titrimetric procedure) cum-m^3; DO, dissolved oxygen (mg/l); DNA, deoxyribonucleic acid; GAC, granular activated carbon; HRT, hydraulic retention time; OCR, oxygen consumption rate; OLR, organic loading rate; ORP, oxidation reduction potential; RNA, ribonucleic acid; SBBR, sequencing batch biofilm reactor; SDR, substrate degradation rate; SDR$_T$, non-cumulative substrate degradation rate; SEM, scanning electron microgram; SS, suspended solids; *t*, time; VSS, volatile suspended solids; ξ_{COD}, COD (substrate) removal efficiency

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with vast metabolic ranges and need a complex suite of interaction between the native microflora, which differs greatly in growth and yield rates [11,12]. Sequencing batch reactor technology (SBR), a periodic discontinuous process can be considered for this type of wastewater treatment and application of this process was successfully documented for various types of wastewater treatment (domestic wastewater, medium and low strength landfill leachates, specific organic pollutants, various types of industrial wastewaters and contaminated soils) using diverse types of reactor configurations [13–30]. Periodic operation imposes regular substrate and oxygen gradients on the treatment organisms that overwhelm natural variations in waste strength and composition [10] and further maintain effective cultures within the reactor [31–33]. The resulting environment offers robust microflora capable to persist and metabolize at extremely adverse and diverse conditions [11,12]. The time oriented nature of operation also makes it easy to alter SBR operating cycles in response to waste variations that occur when industries change production process and this level of process control is not easily matched in continuous flow systems [10]. To the best of our knowledge, there are no experimental studies reported so far on the treatment of composite chemical wastewater by biofilm configured sequencing batch/periodic discontinuous process operation.

Earlier we have reported the successful application of the periodic discontinuous batch process for the treatment of non-biodegradable composite chemical wastewater using suspended growth [4] and GAC-biofilm [9] configurations. Reactor configuration is one of the important factors, which govern the performance of any biological system. It is evident that the biofilm configured systems are well suited for the treatment of wastewater containing poorly degradable compounds [34,35]. Immobilization of microflora on substrate as biofilm results in high biomass hold up, which enables the process to be operated significantly at higher liquid throughputs and OLR. Attached biofilm acts as buffer to reduce the concentration of toxic chemicals during process operation thereby providing advantage for the treatment of low biodegradable industrial wastewater containing recalcitrant compounds [36]. Biofilm systems are generally less energy intensive and more resistant to shock loads to which wastewater treatment systems are frequently subjected [37]. Biofilm systems are particularly useful where high hydraulic loading variations occur and where slowly growing organisms with special metabolic capacities are to be protected from washout [10,38].

In the present communication we have reported the biofilm configured periodic discontinuous system for the treatment of low-biodegradable composite chemical wastewater. The performance of the reactor was evaluated by varying OLRs. An attempt is made to correlate the experimental data to arrive at a best possible configuration for achieving the process optimization. Low-biodegradability was assigned to the composite chemical wastewater being studies due to presence of low BOD/COD ratio of 0.3. This value suggests the fact that the wastewater contains only 30% of the total carbon source which is amenable for biodegradation. Based upon the figures this wastewater was considered as low-biodegradable.

Table 1
Characteristics of wastewaters used as feed

Parameters	Concentrations
pH	7.83 ± 0.24
TDS (g/l)	11 ± 0.98
Suspended solids (mg/l)	900 ± 181
Oil and grease (mg/l)	14 ± 0.42
COD (mg/l)	6000 ± 342
BOD_5 (mg/l)	2600 ± 108
Chlorides (mg/l)	5000 ± 96
Sulfates (mg/l)	1750 ± 47
Phosphates (mg/l)	360 ± 24
Total nitrogen (TKN) (mg/l)	125 ± 11
Nitrogen (ammonical) (mg/l)	35 ± 2
Total alkalinity (mg/l)	950 ± 30

2. Experimental

2.1. Composite chemical wastewater

Composite chemical wastewater was used as feed to evaluate the reactor performance. The wastewater was a combined mixture of effluents from about 110 chemical-based industries. The wastewater was a composite one aggregated from chemicals, drugs, pharmaceuticals, pesticides and various chemical process units. All these wastewater was sent to a common effluent treatment (CETP) plant in Hyderabad, India. We have collected aggregated wastewater from the equalization tank of the CETP. After collection, the wastewater was transferred immediately to the laboratory and stored at 4 °C. The strengths and detailed characteristics (in average values) of the composite chemical wastewaters used as feed in this experiment are presented in Table 1. The complexity of the selected composite chemical wastewater could be assessed from its characteristics by the presence of a low BOD/COD ratio (∼0.31), high sulfate content (1.75 g/l) and high TDS concentration (12.4 g/l). The wastewater was not corrected for trace elements deficiency.

2.2. Biofilm reactor configuration

The bench scale reactor with biofilm configuration was fabricated using ‘perplex’ glass material. The reactor consisted of leak proof sealing with proper inlet and outlet arrangement (total volume: 1.4 l; working volume: 1.3 l). A schematic representation of the bioreactor is depicted in Fig. 1. The reactor with internal diameter of 7.5 cm was operated in the upflow mode (Table 2). Inert stone chips with size 2.5 cm × 1.5 cm (void ratio: 0.49) were used as fixed bed packing material for supporting the biofilm formation. Air was supplied through a fabricated diffuser network arrangement provided at the bottom of the reactor. Feed was introduced from the bottom of the reactor to achieve better mixing during reaction phase. Preprogrammed timers (ETTS, Germany) were used to regulate the feed, recirculation, aeration and discharge operations. Peristaltic pump (Watson Marlow 101U/R) controlled by electronic timer was used for supplying feed to the reactor and for providing recirculation. The controller was programmed to operate on a repeating 24 h cycle

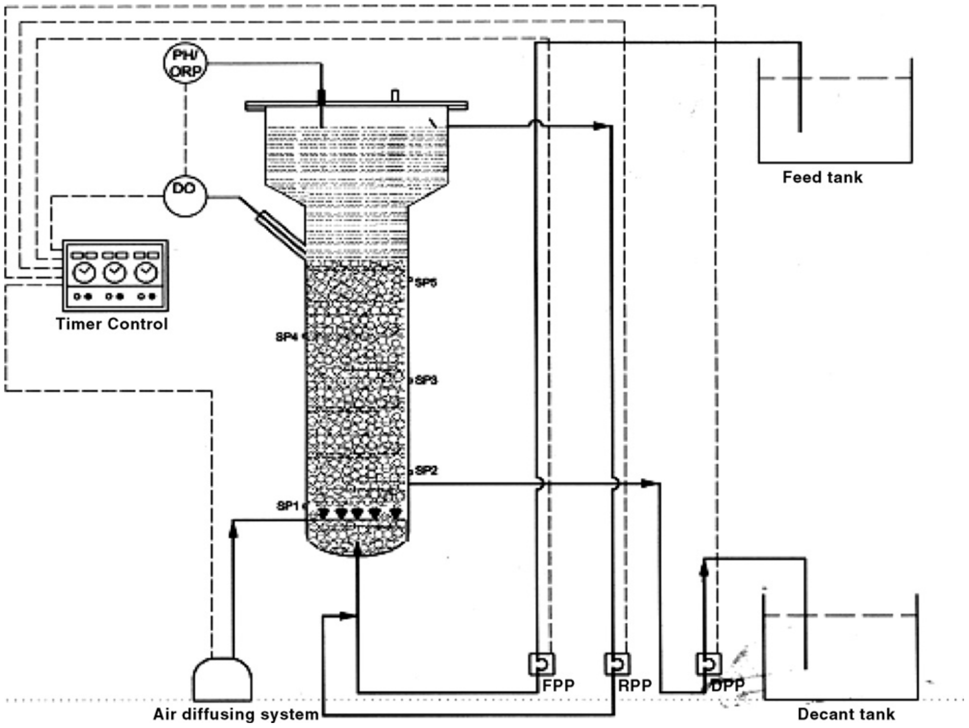

Fig. 1. Schematic details of SBBR (SP: sampling ports; RPP: recirculation peristaltic pump; DPP: decanting peristaltic pump; FPP: feeding peristaltic pump).

with a sub-program and the out put dedicated to the operation of each controllable element. Throughout the study recirculation rate (recirculation volume to feed volume ratio) of the two was maintained to achieve a homogeneous distribution of the substrate and requisite consortia along the reactor depth.

2.3. Reactor startup and operation

Biofilm configured system was operated under aerobic metabolic functions with a total cycle period of 24 h (retention time) consisting of 15 min of fill phase, 23 h of reaction (aerobic) phase with recycling, 30 min of settle phase and 15 min of decant phase (Table 3). The reactor was inoculated with aerobic biomass acquired from the activated sludge unit treating composite chemical wastewater in our laboratory for the past 1 year. The mixed liquor from the aeration chamber (VSS: 3 g/l) was inoculated at a ratio of 1:4 (v/v) with reactor volume and the reactor was operated with designed synthetic feed (g/l) (glucose: 1.0 g/l; sodium acetate: 1.0 g/l; Na_2HPO_4: 0.3 g/l; pH 7.0) to support effective biomass formation on stone chips. Initially after the successful start up (12 days), the reactor was operated with composite chemical wastewater at an OLR of 0.923 kg COD/cum-day. After stable performance was achieved, the reactor was operated at higher OLRs of 0.923, 1.5, 3.07 and 4.76 kg COD/cum-day to assess the suitability of the reactor configuration for treating the composite chemical wastewater with 24 h of HRT. Subsequently, the reactor was also operated at OLR 4.76 kg COD/cum-day with 48 h of HRT. All experiments were carried out at room temperature (31 ± 2 °C). At the beginning of each cycle, immediately after withdrawal (earlier sequence), a pre-defined feed volume

Table 2
Reactor configuration details

Reactor internal diameter	7.5 cm
Height of the reactor	63 cm
Fixed bed height	50 cm
Void ratio of fixed bed	0.49
L/*D* ratio	6.66 (with fixed bed height); 7.46 (with liquid depth height)
Total volume of reactor	2.75 l
Liquid (working) volume	1.4 l
Height of decant outlet	12.5 cm
Reactor liquid level	56 cm
Upflow velocity	0.023 m/h
Hydraulic loading rate	2.0 cum (liquid)/cum-day
Operating temperature	31 ± 3 °C
Recirculation ratio (*R*/*F*)	2
Flow direction	Upflow
Fixed bed supported media	Stone chips (2.5 cm × 1.5 cm)

Table 3
Details of sequence phases used in the designed experiments

Metabolic function	Total cycle period (h)	Sequence phase details			
		Phase	Period	Micro-environment	Air supply
Aerobic	24	Fill	15 min	Anoxic	Off
		React	23 h	Aerobic	On
		Settle	30 min	Anoxic	Off
		Decant	15 min	Anoxic	Off
	48	Fill	15 min	Anoxic	Off
		React	47 h	Aerobic	On
		Settle	30 min	Anoxic	Off
		Decant	15 min	Anoxic	Off

(1.0 l) was pumped into the system. At the end of the cycle, the treated wastewater was withdrawn from the reactor. During the reaction phase, aqueous phase dissolved oxygen (DO) was maintained in the range of 2.0–3.5 mg/l. The pH of the feed was adjusted to 7.0 ± 0.1 before wastewater dosing. During the settling phase, both feed and aeration were discontinued. During reactor operation under reaction phase (recirculation rate of 2), the upflow velocity in the reactor was around 0.023 m/h. Anoxic microenvironment in this study refers to the functioning of aerobic microbial consortia (operating in aerobic metabolic function) under oxygen limiting conditions.

2.4. *Analytical methods*

The performance of the reactor was assessed by monitoring COD removal throughout the operation. In addition, pH, ORP, sulfates, BOD, OCR and DO were also determined during the sequence phase operation. The analytical procedures for monitoring the above parameters (COD-closed refluxing titrimetric method (5220 C); BOD-5-day BOD test (5210 B); pH-electrometric method (4500-H+B); ORP-electrometric method (2580 B); sulfates-turbidimetric method (4500-SO4^{2-}E); DO-membrane electrode method (4500-0G)) were employed as outlined in the Standard Methods [39]. OCR was determined using DO probe (YSI 5100) by continuously monitoring the DO depletion in the reaction phase in the absence of air (Method-2710 B) [39] and OCR is evaluated as

$$\mathrm{OCR} = \frac{\mathrm{DO}_1 - \mathrm{DO}_2}{t_2 - t_1} \quad (1)$$

where OCR in mg O_2/min and DO_1 and DO_2 are the DO concentrations at time t_1 and t_2, respectively, in minutes. All the analytical determinations were made in duplicate and the average was taken. The biofilm formed on the stones were subjected to scanning electron microscopy (SEM, Hitachi S-520, Japan) imaging to observe morphology details after carefully dehydrating the biofilm (incubated in 3% glutaraldehyde for 2 h and serially dehydrated with 10–90% alcohol) without disturbing the actual morphology.

3. Results and discussion

3.1. *Reactor performance*

The performance of SBBR was evaluated by estimating COD (substrate) removal efficiency (ξ_{COD}) calculated using Eq. (2). C_{SO} represents the initial COD concentration (mg/l) in the feed and C_{S} denotes COD concentration (mg/l) in the reactor outlet:

$$\xi_{\mathrm{COD}} = \frac{C_{\mathrm{SO}} - C_{\mathrm{S}}}{C_{\mathrm{SO}}} \quad (2)$$

The reactor was initially operated at 0.923 kg COD/cum-day of OLR and the performance of the reactor with respect to the COD removal efficiency was assessed during the cycle operation (Fig. 2a). The reactor showed a COD removal of 88% accounting for a substrate degradation rate of 0.81 kg COD/cum-day at steady state condition. COD removal rate was relatively slow (2.4%) during the initial phase of sequence phase operation (up to 2 h) and with increase in sequence time, a relatively rapid COD removal was noticed. At 16 h of cycle operation, maximum COD removal of 88% was achieved and thereafter it remained more or less constant till the end of the cycle period. The reactor attained stable conditions within 15 days and remained more or less constant thereafter. Subsequently after achieving stable performance, the reactor was operated at higher OLRs to understand the performance (1.5, 3.07 and 4.76 kg COD/cum-day), respectively, keeping all other operating conditions the same (Fig. 2b). On the day 36 after startup, the reactor was fed with an OLR of 1.5 kg COD/cum-day. Immediately after raise in the organic load, the reactor showed an increase in the outlet COD concentration. At 1.5 kg COD/cum-day, the reactor showed 78.68% of COD removal efficiency with an SDR of 1.18 kg COD/cum day, and 3.07 kg COD/cum-day OLR, the reactor yielded 56.67% of COD removal (SDR of 1.74 kg COD/cum-day). At 4.76 kg COD/cum-day, the reactor showed about 55% of COD removal accounting for SDR of 2.62 kg COD/cum day. On the days 55 and 71 after the startup, the reactor was subjected to higher organic loading of 3.07 and 4.76 kg COD/cum-day, respectively (Fig. 2b). It is evident from the results that the SBBR reactor showed consistently good performance at higher OLR and stabilized within relatively less time. The BOD profile during the sequence operation showed comparably the same pattern as the COD profile (Fig. 3). BOD removal efficiency of 88.89% was observed at operating OLR of 0.923 kg COD/cum-day after the reactor attained steady state. BOD removal efficiencies of 84.21%, 76% and 77.45% were observed at operating OLRs of 1.5, 3.07 and

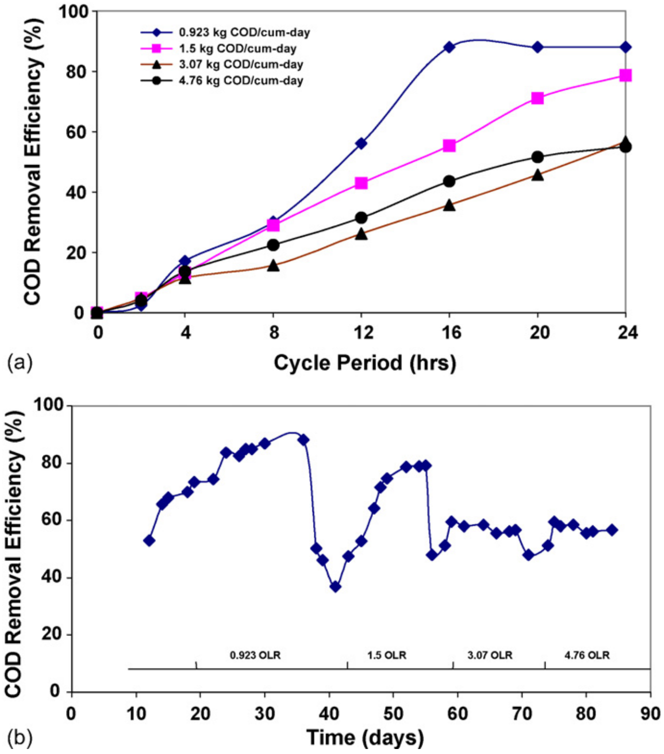

Fig. 2. COD removal efficiency during sequence phase operation of SBBR.

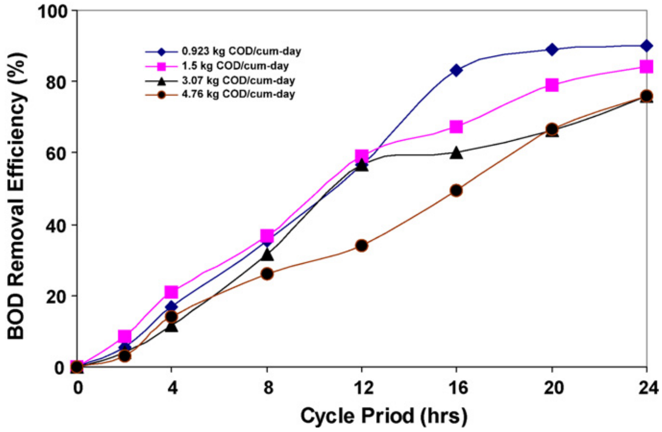

Fig. 3. BOD removal efficiency during sequence phase operation.

4.76 kg COD/cum-day, respectively, at steady state conditions. With continued operation, the reactor showed enhanced performance with respect to COD and BOD removal and after attaining stable conditions the performance remained more or less constant. It is evident from the experimental data that the reactor performance with respect to substrate removal was found to be influenced by the operating OLR. The SBBR performance during two consecutive cycle operations was shown for all the OLR studied at stable conditions (Fig. 4b).

Further to study the influence of HRT on the overall performance of SBBR, the reactor was operated with HRT of 48 h at OLR of 4.76 kg COD/cum-day. COD removal of 70.24% accounting for 3.38 kg COD/cum-day of SDR was observed at 48 h of operation (Table 4). About 15% enhancements in COD removal (with 0.76 kg COD/cum-day improvement in SDR) was observed with respect to 24 h increase in the HRT. A marginal improvement in sulfate reduction and BOD removal was observed due to the enhanced contact time. Rapid stabilized period was observed in SBBR for every change in organic loading with respect to COD removal efficiency. To achieve stable performance (with respect to carbon removal), the reactor required 15 days at OLR of 0.923 kg COD/cum-day and 14 days for OLR of 1.5 kg COD/cum-day. About 5 and 7 days were required to achieve stable performance at OLRs of 3.07 and 4.76 kg COD/cum-day, respectively.

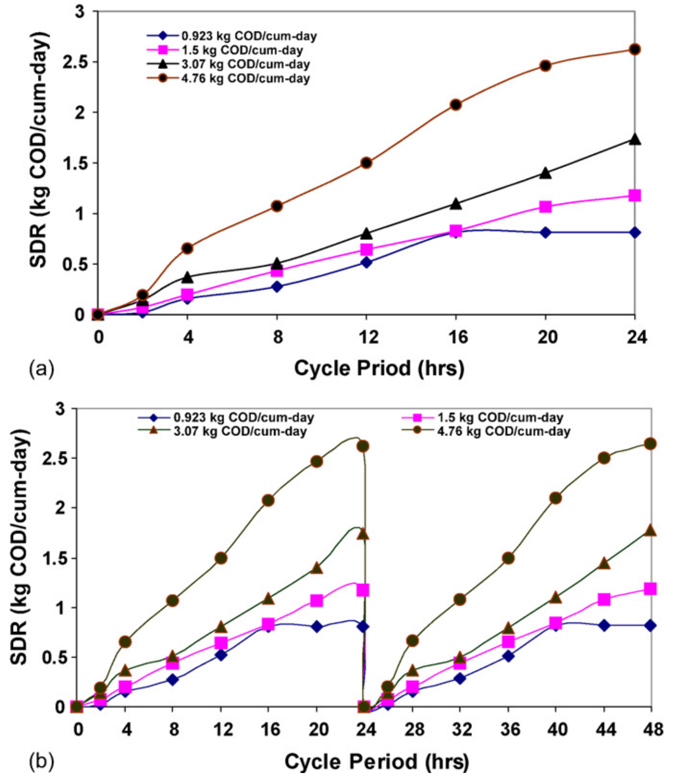

Fig. 4. (a) Variation in substrate degradation rate (SDR) during sequence phase operation at experimental variations studied. (b) SDR variation during two consecutive cycle operation.

3.2. *Non-cumulative SDR_T*

Substrate degradation rate (non-cumulative) (SDR_T) (kg COD/cum-h) was calculated to study the rate of substrate (COD) removal for a unit time during the sequence phase

Table 4
Comparative performance evaluation of SBBR with other reactor configurations studied

Configuration (reactor microenvironment)	OLR (kg COD/ cum-day)	HRT (h)	COD removal efficiency (%)	BOD removal efficiency (%)	SDR (kg COD/ cum-day)	Sulfate removal efficiency (%)	Reference
Biofilm (anoxic–aerobic–anoxic)	0.92	24	88.05	88.89	0.81	14.67	This work
	1.50	24	78.68	84.21	1.18	20.10	
	3.07	24	56.67	76.00	1.74	22.0	
	4.76	24	55.00	75.45	2.62	17.80	
	4.76	48	70.24	88.24	3.38	19.82	
Suspended GAC (anoxic–aerobic–anoxic)	1.70	24	67.34	86.21	1.13	13.21	[9]
	3.50	24	55.19	81.12	1.92	10.62	
	5.50	24	37.99	66.01	2.09	9.67	
Suspended (anoxic–aerobic–anoxic)	0.80	24	66.36	92.22	0.53	7.79	[4]
	1.70	24	47.01	72.67	0.80	8.26	
	3.50	24	25.34	57.00	0.88	8.31	

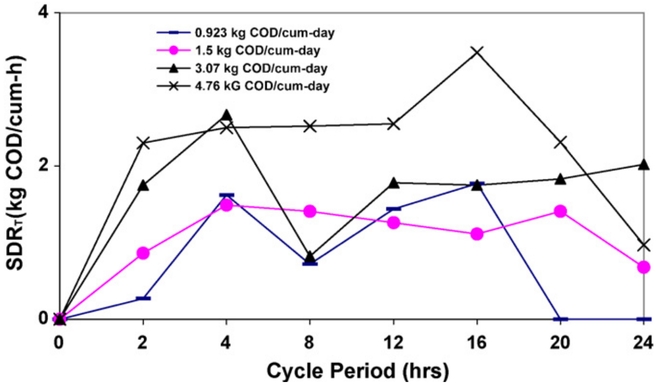

Fig. 5. SDR_T variation during sequence phase operation.

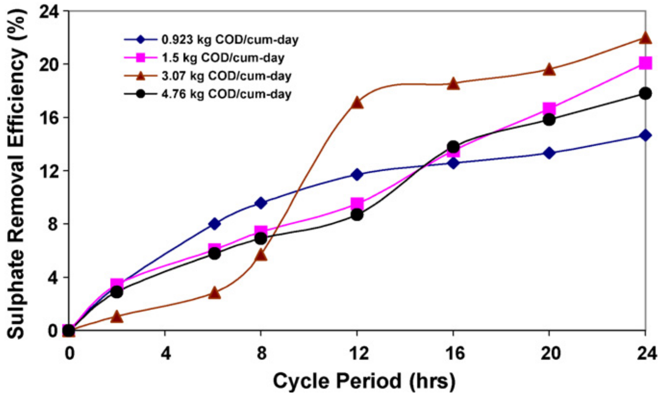

Fig. 6. Sulfate removal efficiency during sequence phase operation.

operation using the following Eq. (3), where, SDR_X and SDR_Y represents, the substrate degradation rate (kg COD/cum-day) at time X and Y, respectively, and t_X and t_Y denotes time (h) at X and Y, respectively:

$$SDR_T = \frac{(SDR_X - SDR_Y)24}{t_X - t_Y} \quad (3)$$

The non-cumulative substrate degradation rate profiles for all the four OLRs studied are depicted in Fig. 5. The profile for all the studied cases showed a consistent trend of increase in the substrate removal rate with the function of cycle period. Rapid substrate uptake was noticed during the initial 2 h of the cycle operation (0.27–1.62 kg COD/cum-h) and approached a maximum of 1.77 kg COD/cum-h at 16 h of cycle operation for the OLR of 0.923 kg COD/cum-day. Subsequent rise in the cycle period showed negligible substrate removal efficiency and remained same up to the end of the cycle period. Maximum substrate utilization was observed at 4 h (1.49 kg COD/cum-h) and 20 h (1.41 kg COD/cum-h) of the cycle operation in the case of 1.5 kg COD/cum-day of OLR. In the case of 3.07 kg COD/cum-day and 3.05 kg COD/cum-day maximum substrate utilization was observed at 4 h (2.67 kg COD/cum-h) and 20 h (1.84 kg COD/cum-h) of the cycle operation respectively. In the case of 4.76 kg COD/cum-day, the substrate utilization approached 2.30 kg COD/cum-h after 2 h of cycle operation and remained more or less constant up to the 16 h of the cycle period. Maximum substrate utilization of 3.45 kg COD/cum-h was observed at 16 h of cycle operation. Relatively low substrate utilization rate observed during the initial phase of the cycle operation might be attributed to the presence of high concentration gradient of the substrate in the reactor. With increase in the cycle period the system might have got acclimatized to the reactor volume leading to rapid substrate removal efficiency.

3.3. Sulfate removal

About 14% of sulfate reduction was achieved in SBBR operation at OLR of 0.923 kg COD/cum-day, while at the operating OLRs of 1.5, 3.07 and 4.76 kg COD/cum-day, sulfate reduction of 20.1%, 22% and 17.8% respectively was observed (Fig. 6 and Table 3). Effective sulfate transformation in the SBBR might be attributed to the prevailing anoxic environment in the internal layers of the biofilm and the induced anoxic conditions during the sequence phase operation. The biofilm floc immobilized on the stone chips had an anoxic micro-niche in the internal parts, which lead to sulfate transformation. The biofilm size has significant influence on the extent and presence of anoxic zone. The internal biofilm normally had anoxic environment that facilitates the sulfate. Biofilm floc size of 200 μm and above has induced anoxic micro-niches in the internal part of the thick flocs [41]. The persistent anoxic environment (15 min) during the feed phase and the subsequent settle and withdrawal phases (45 min) of the cycle operation facilitated a suitable environment for the sulfate conversion. The sulfate transformation in the periodic discontinuous process operation can be attributed to the induced anoxic microenvironment and to the prevailing anoxic zone in the internal layers of the biofilm [4,9].

3.4. Comparative evaluation with other reactor configurations

The performance of the corresponding suspended growth configuration at 1.7 kg COD/cum-day of OLR recorded 47.1% of COD removal efficiency accounting for SDR of 0.80 kg COD/cum-day [4]. At 3.5 kg COD/cum-day of OLR, only 25.3% of COD removal efficiency (0.875 kg COD/cum-day of SDR) was observed (Table 3). With increase in OLR a significant decrease in the substrate removal efficiency was observed and this correlated well with the reduction in mixed liquor VSS concentration from 1.8 g/l (1.7 kg COD/cum-day) to 0.9 g/l (3.5 kg COD/cum-day). Comparatively, GAC configured system showed effective performance over the corresponding suspended growth system [9]. The GAC configured system sustained its performance at higher OLRs (up to 5.5 kg COD/cum-day) without process inhibition whereas corresponding suspended growth system resulted in process failure at OLR of 3.5 kg COD/cum-day. On the whole, biofilm configured SBR showed relatively higher efficiency compared to corresponding suspended growth and GAC configured systems studied in the periodic discontinuous mode treating chemical wastewater and operated with similar experimental conditions in terms of the overall process performance (Table 3). A rapid COD removal during the initial phase of the cycle operation was observed in

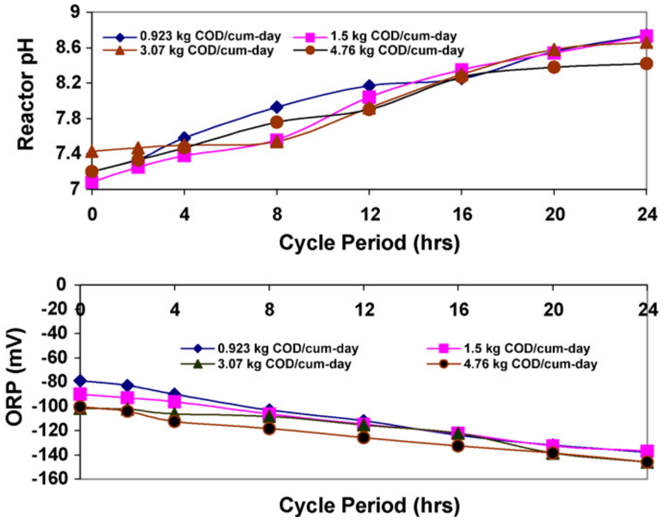

Fig. 7. pH and ORP variation during sequence phase operation.

biofilm configured system which is contrary to the observation made with the corresponding suspended growth and GAC configured systems. It is evident from the data, that with increase in organic load, the COD removal rate was reduced, however, SDR showed an increasing trend. Compared to suspended growth and GAC configured systems, the biofilm configured system evidenced effective sulfate reduction. On the whole, sustained performance was achieved in biofilm configured system when operating at higher loading rates along with enhanced sulfate reduction which can be attributed to the advantages of the biofilm configuration. Organic shock load has shown relatively less effect on the performance and stabilizing period in biofilm configured system. Biofilm configured system integrated with periodic operation imposes regular variations in substrate concentration on biofilm organisms [10]. Therefore, organisms throughout the film achieve maximum growth rates which results in improved reaction potential leading to stable and robust system well suited for treating highly variable wastes.

3.5. Process monitoring

Process was monitored during reactor operation to understand the on going biochemical process during sequence phase operation, by determining pH, ORP, DO, OCR, suspended solids (SS) and volatile suspended solids (VSS). The variation of pH and ORP during the sequence were also monitored and presented in Fig. 7. The influent pH was in between 7.0 and 7.4 for all the studied OLRs. At the initial stage of the cycle operation the reactor pH was near 7.9, which showed a gradual rise and approached 8.4 at the end of the reaction phase. ORP (mV) profile visualized a mirror image to pH profile.

VSS and SS were monitored throughout the study to assess the viability of the biomass during operation with complex chemical effluents. The viability of biomass (represented by the ratio of VSS/SS) was in the range 0.70–0.76 during the reactor operation at 0.923 and 1.5 kg COD/cum-day OLRs. With increase in operating OLR (3.5 and 4.78 kg COD/cum-day), a slight decrease in VSS/SS ratio (0.63 and 0.61) was observed. It can be presumed that increase in the operating OLR manifested increased substrate concentration in the reactor that led to reduced biomass viability. However, the performance inhibition was not observed at the studied OLRs in this system.

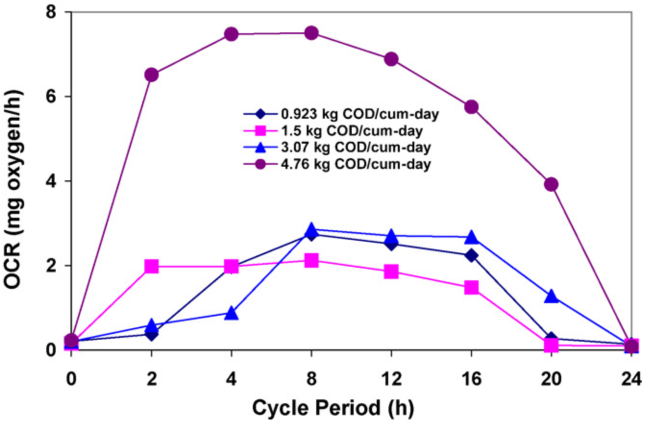

Fig. 8. OCR variation during sequence phase operation.

OCR was monitored during SBBR operation to assess the ability of self-immobilized biofilm to degrade complex substrate in the aerobic microenvironments. OCR increased gradually and approached a maximum value (2.74 mg O_2/h) at 8 h of the cycle operation and remained more or less constant up to 16 h and subsequent increase in cycle period showed gradual reduction in OCR (Fig. 8). The OCR was found to be dependent on the substrate utilization rate and resulted in maximum consumption figures during the course of the react phase of the cycle operation. The variation of OCR showed more or less similar trend for all the other OLRs studied.

Stone chips before using in the reactor and after self-immobilizing with aerobic biofilm were photographed separately (Fig. 9). The stone chips covered with biofilm were found to exhibit slight greenish brown color. Scanning electron micrograph (SEM) of the self-immobilized biofilm on the stone captured during stabilized operation of reactor at OLR of 3.07 kg COD/cum-day is depicted in Fig. 10. From the micrograph, the morphology of the biofilm was found to be heterogeneous in nature with well defined biomass clusters placed unevenly over the surface. Biofilm morphology showed to have irregular surface texture with good internal porous network.

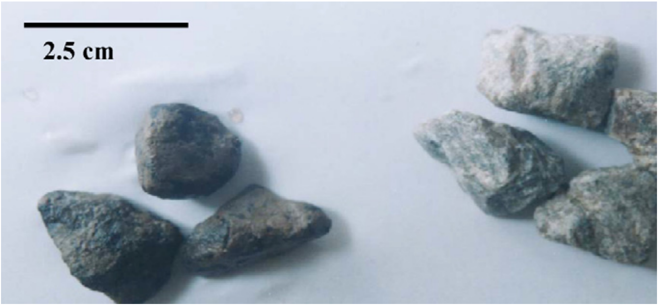

Fig. 9. Photograph of stone chips with self-immobilized biofilm acquired from SBBR along with the stone chips prior to adding to the reactor.

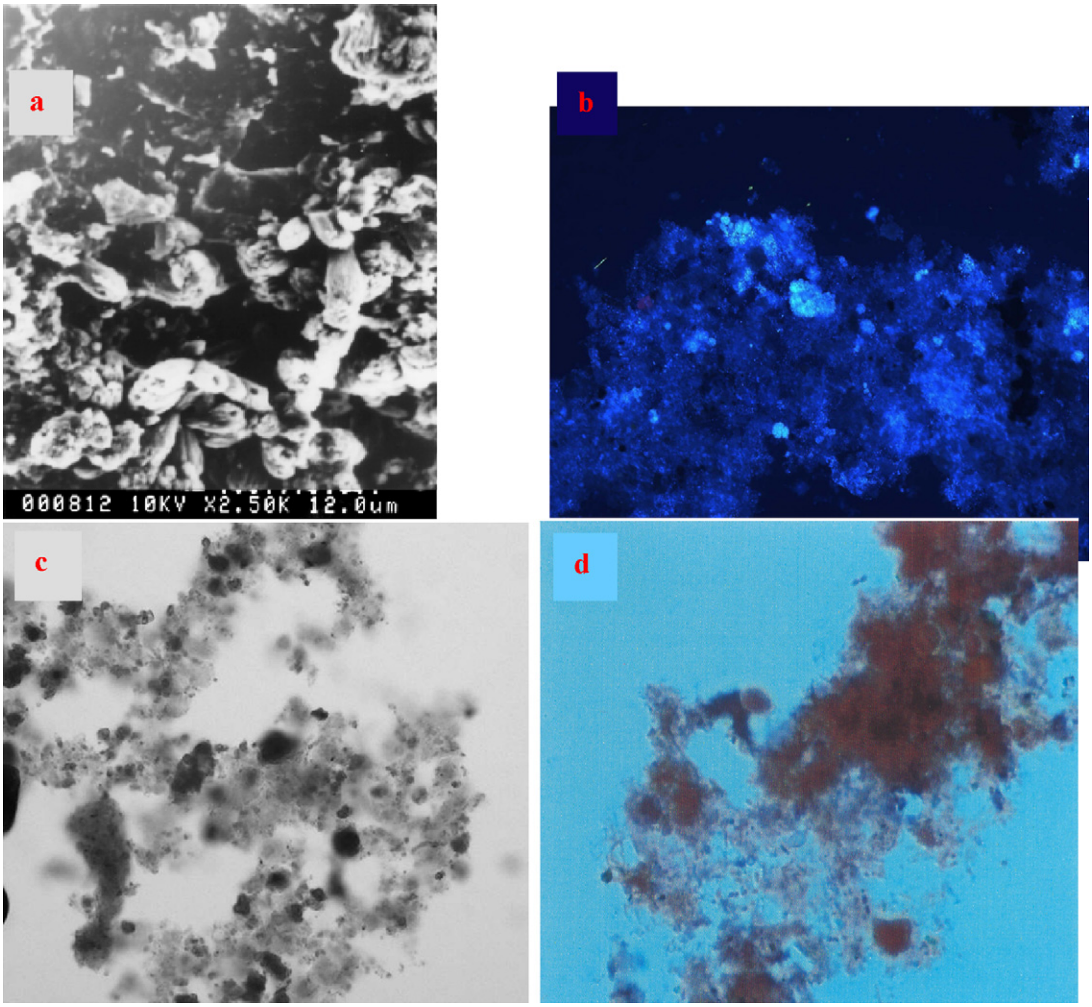

Fig. 10. Self-immobilized biofilm acquired from SBBR: (a) SEM image; (b) DAPI stain images (40×); (c) wet film image (light microscope: 10×); (d) gram stain (40×).

Self-immobilized aerobic biofilm formed on stone chips was further studied with light microscope for wet film analysis (10×), DAPI staining (40×) and gram staining (40×) (Fig. 10). The wet film microscopic studies revealed the presence of bacteria, protozoa and fungi. Both motile and non-motile organisms were seen along with protozoan. The dehydrated biofilm from SBBR was characterized and found to have total organic carbon (TOC): 2.49 mg/g; COD: 211.3 mg/g, VSS: 190 mg/l, pH 7.20 and ORP −32 mV. DAPI stains showed that clumps of microorganisms are involved in the biomass formation. To differentiate the microorganisms especially bacteria, gram staining was done. Gram stain smear (40× objective) showed to have mixed populations of both gram-positive and gram-negative microorganisms. In gram-negative most of the bacteria are in cocci and diplococci, whereas gram-positive bacteria are found in clusters. Genomic DNA quantified in SBBR showed to have 1.47 mg/g biomass wet weight, while suspended growth and GAC configured systems showed to have 0.79 mg/g biomass wet weight and 0.89 mg/g biomass wet weight respectively. Woolard suggested elevated RNA developed during periods of rapid growth increases the culture's ability to produce the enzymes necessary for the degradation of the inhibitory organics [10]. Continuous flow operation of biofilm reactors causes stratification and uneven biomass distribution which limits the ability of these reactors to respond to shock loads of substrates [10], while the periodic operation of biofilm reactor results in a more even distribution of the biomass throughout the reactor [10,38,40–42]. Periodic discontinuous process coupled with biofilm configuration combines the operational advantages of both the biofilm reactor and the periodic discontinuous operation, which maintains high biomass concentration, encourages the culture of slow growing organisms and can obtain homogeneous biomass distribution throughout the reactor [40–42]. Further, the selection of biomass particularly effective for the degradation of toxic and/or recalcitrant compounds is possible along with the maintenance of uniform biomass concentration along the whole height of the bed [38,43].

4. Conclusions

Experimental data revealed the efficacy of biofilm configured system over the corresponding suspended growth and GAC-biofilm configured system in treating low-biodegradable composite chemical wastewater. Sulfate removal efficiency of 20% was observed due to the prevailing anoxic microenvironment during the sequence phase operation and the existing internal anoxic zones in the biofilm. The reactor showed robustness and sustained its performance at higher organic loading rates. Periodic discontinuous batch mode operation coupled with biofilm configuration resulted in a more even distribution of the biomass throughout the reactor and imposed regular variations in the substrate concentration on biofilm organisms as a result, organisms throughout the film achieve maximum growth rates resulting in improved reaction potential leading to stable and robust system which is well suited for treating highly variable wastes. Biofilm configuration coupled with sequencing/periodic discontinuous batch mode operation appears to be promising option for the effective treatment of complex industrial wastewater containing poorly degradable compounds.

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