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Effect of intermittent operation of lab-scale upflow anaerobic sludge blanket (UASB) reactor on textile wastewater treatment

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ABSTRACT

Different intermittent phases were introduced during the continuous operation of upflow anaerobic sludge blanket reactor treating simulated textile wastewater using single dye. The reactor was operated at an organic loading rate of 2 kg COD/m^3 d and hydraulic retention time (HRT) of 24 h to optimize the non-feeding period of reactor for maximum chemical oxygen demand (COD) and color removal rates. The optimized combination was then operated for mixed dyes solution. Feeding period of 12 h and non-feeding period of 12 h (12F/12NF) gave COD and color removal of 57.5% and 71.0%, respectively. The similar cycle was operated using mixed dye feed with increase in dye concentration to 30 mg/L at same operating conditions. Maximum COD and color removal efficiency of 47.8% and 38.3% was achieved. The decreased removal rates for mixed dyes were due to the presence of intermediate metabolites produced by chromogenic breakdown of dyes. The above optimized condition was extended to 48 h HRT (12F/12NF/12F/12NF) with dyes concentration of 50 mg/L. Improved COD and color removal rates of 67.0% and 77.8% were achieved.

Keywords: Dye adsorption; Intermittent operation; Textile wastewater; Upflow anaerobic sludge blanket reactor

1. Introduction

Dyes manufacturing companies emphasize on production of complex structured dyes that have high fixation rates on variety of cellulosic fibers with minimal usage of dyes. These dyes are designed to avoid the natural biodegradation by sunlight, water, chemical or microbial action and tend to attain long lasting bright colors with enhanced stability [1]. Though the finest dye merchandizers claim to achieve more than 80% of dye fixation from light to dark shades of fabric, the amount of dye usage exponentially increases for darker shades. This also decreases the probability of their fixation rates on various cellulosic fibers. Also, most of the dyes, which are used in hydrolyzed form to increase their solubility, lose their adsorption properties to fibers. Almost 40% of initial dyes used are in unfixed hydrolyzed state [2]. Poor exhaustion properties of some dyes result in 10%–20% of dye residuals in effluent. The fixation efficiency also varies with class of dyes used, which is 98% for basic and 50% for reactive dyes. Thus, it was observed that degree of fixation rate is never complete in textile dye baths [3] resulting in chemically hazardous effluent and making it one of the severely polluted wastewater that needs to be treated.

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Diluted textile wastewater contains various dyes based on their chemical structure (azo, anthraquinone, azine, xanthene, nitro, phthalocyanine, etc.) or application methods used in dyeing process (acid, basic, direct, reactive, etc.) [4]. These dye classes are reported to be effectively treated by aerobic biological treatment technologies, that is, activated sludge process. These dyes are testified to get adsorbed on weak negatively charged aerobic sludge, converted into flocs due to their insoluble nature (disperse and vat dyes) and their large molecular weight (direct or basic dyes having cationic nature). However, dyes having 60%–70% of market share [5,6] used for printing and dyeing are hydrophilic, reactive and electron deficient in nature, that is, anionic acid dyes, which do not get adsorbed on aerobic sludge easily. They are less liable to oxidative catabolism and hydrolyzed easily having less affinity for fiber [7]. This results in unfixed reactive dyes [8].

Combined anaerobic process with post aerobic treatment is reported as effective method in literature for effective color and organic load removals from textile wastewater [4]. Azo reactive dyes are reduced via a four-electron reduction process at azo dye linkage bond and primary aromatic amines (PAAs) as intermediate metabolites are generated under strict anaerobic conditions [9]. Electron donating organic (carbon) sources such as starch, volatile fatty acids (VFAs) or glucose provides required electrons for azo bond reduction. Methanogenic and acetogenic bacteria in anaerobic biomass contain distinctive reduced enzymes cofactors, such as F430 and vitamin B12 that have the tendency to potentially reduce azo bonds [5]. It is generally reported in literature that intermediate metabolites produced by anaerobic reduction of azo bonds cannot be further assimilated into simpler by-products. Thus, they can only be completely oxidized by post aerobic treatment options [10–12]. However, some specific studies indicated the possibility of complete removal of dyes and dye metabolites under anaerobic conditions [11] without requirement of aerobic post treatment. Colorless PAAs produced as dye metabolites, having hydroxyl and carboxyl groups, are reported to be completely assimilated under strict anaerobic methanogenic conditions.

Intermittent or stabilization period involves the interruption of continuous operation of reactor for a certain amount of time keeping the operating conditions same as during the continuous (feeding) period [13]. This condition was introduced by Lettinga and Hulshoff [14] for treating dairy wastewaters. During intermittent (non-feeding) period, time is allowed for complete biological degradation of adsorbed substrate on biomass. This eliminates or reduces the accumulation of organic matter on sludge bed [15]. The beneficial effect of intermittent period is attributed to forced adaptation of anaerobic biomass to substrate type which is resilient to biodegradation. Couras et al. [13] described the positive consequence of intermittent period during upflow anaerobic sludge blanket (UASB) reactor operation to maximize methanization rate by sufficient biogas production and its capacity to handle the operational shocks encountered due to change in HRT, OLR or temperature. However, no specific investigation is reported in literature, concerning the influence of time duration of intermittent period on UASB reactor treating complex dyes present in textile wastewaters [12].

In this study, UASB reactor was operated as stand-alone treatment option for the simulated textile wastewater. The aim of this research was to evaluate the effect of intermittent feeding on the performance of reactor in terms of COD and color removal efficiencies. Single dye simulated textile wastewater was used for optimization of intermittent feeding period and then the optimized conditions were applied for UASB operation with mixed dye solution.

2. Materials and methods

2.1. Lab-scale reactor

A laboratory scale anaerobic UASB reactor (Fig. 1), made of transparent acrylic plastic sheet was used in the study. The total volume of reactor, having 0.60 m height and 0.16 m internal diameter, was 10.87 L with effective volume of 9.98 L. The reactor was provided with hopper bottom of 0.12 m length and a feed inlet pipe of 1 cm diameter to avoid choking during operation. Gas liquid solid separator is placed at the top of reactor occupying 20% of reactor volume with inclined walls at 45°. Two peristaltic pumps were used. The first peristaltic was used to pump synthetic feed into reactor and second peristaltic pump was used for effluent recirculation. The effluent recirculation flow rate was adjusted with influent flow to maintain the upflow velocity of 0.5 m/h in the reactor. Peristaltic pumps were controlled by digital timers (DH48S-S) and temperature of reactor was maintained at mesophilic range of 35°C by heating system containing a heating rod, thermocouple, magnetic contractor and thermostat.

2.2. Seed collection and preparation studies

A partially granulated anaerobic sludge was acclimatized from aerobic sludge which was obtained from pilot scale membrane bioreactor (MBR) wastewater treatment plant at NUST, Islamabad, Pakistan. It was used as inoculum for experimentation studies. Almost 0.5 L of cow dung obtained from local cattle farm was used as a seed to accelerate the granulation of 4 L of anaerobic sludge. The amount of sludge added to reactor was 3.60 L, that is, approximately 1/3 of total reactor volume. Initial MLSS concentration of partially granulated anaerobic sludge was 4,400 mg/L. The batch studies were continued for almost 2 months till the time steady state condition persisted for a week, that is, COD reduction up to 85% was attained and semi granulated, dark black color anaerobic sludge with characteristic odor was achieved.

2.3. Simulated textile wastewater

Real textile wastewater (RTWW) was obtained from a textile mill in Rawalpindi, Pakistan. Samples were characterized so that a matching synthetic wastewater can be prepared. The results of RTWW analysis are shown in Table 1. The composition of simulated wastewater is given in Table 2. 10 mg/L concentration of Bezaktiv Cosmos Orange (reactive) dye was used during single dye feed studies for optimization of non-feeding period for UASB reactor. Mixed dye feed was prepared by using Bezaktiv Cosmos Orange (reactive), Foron Red S3BS (disperse) and Novasol Navy DB (vat) at dye concentration of 30 and 50 mg/L at optimized conditions.

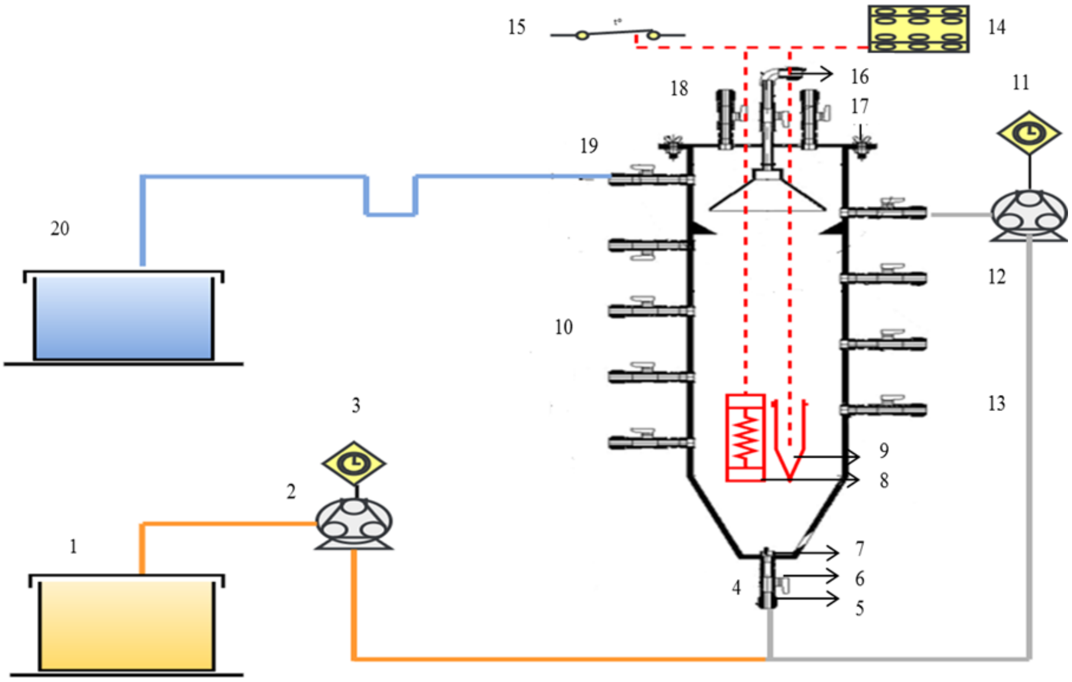

Fig. 1. Schematic configuration of lab-scale UASB reactor. (1) Influent tank, (2) peristaltic feed pump, (3) timer 1, (4) influent port, (5) connector, (6) isolation valve, (7) threaded nipple, (8) heating rod, (9) thermocouple, (10) sampling ports, (11) timer 2, (12) peristaltic effluent recirculation pump, (13) effluent recirculation pathway, (14) magnetic contractor, (15) thermostat, (16) threaded elbow, (17) butterfly bolt and nut, (18) pressure relief valve, (19) effluent port and (20) effluent tank.

Table 1
Characterization of textile mill effluent

Parameters analyzed	Average values[a]
COD (mg/L)	2,540.0
Orthophosphates (mg/L)	22.27
Nitrogen –ammonia (mg/L)	26.31
Total Kjeldahl nitrogen (mg/L)	25.45
Chlorides (mg/L)	745.50
TDS (mg/L)	1,870.0
EC (mS/cm)	3.27
pH	5.35

[a]Number of samples (n) = 6.

Table 2
Simulated textile wastewater composition

Components	Formula	Quantity used
Dextrose (glucose)	$C_6H_{12}O_6$	2 g
Ammonium chloride	NH_4Cl	0.38 g
Potassium dihydrogen phosphate	KH_2PO_4	0.109 g
Magnesium sulfate	$MgSO_4.7H_2O$	10 mg
Calcium chloride	$CaCl_2$ granular	20 mg
Sodium bicarbonate	$NaHCO_3$	0.5 g
Ferric chloride	$FeCl_3$ anhydrous	5 mg
Zinc chloride	$ZnCl_2$	1 $mg/L_{reactor\ volume}$
Cobalt chloride	$CoCl_2.6H_2O$	1 $mg/L_{reactor\ volume}$

2.4. Experimental procedure

After achieving a steady state for start-up period of reactor, the simulated textile wastewater was introduced in the reactor with 10 mg/L concentration of Bezaktiv Cosmos Orange dye at HRT of 24 h and OLR of 2 kg COD/m^3 d to optimize the length of non-feeding period. Later, Foron Red and Novasol Navy were added in feed to give mixed dye concentration at 30 mg/L and HRT of 24 h for Run 6 and 7. For Run 8, the dye concentration was increased to 50 mg/L at increased HRT of 48 h (Table 3).

2.5. Analytical parameters

Effluent samples were analyzed for COD, color, TSS, VSS, alkalinity, TKN and orthophosphates while sludge samples were analyzed for MLSS and MLVSS in accordance to Standard Methods for the Examination of Water and Wastewater [16] using procedures 5220C, 2120C, 2540D, 2320B, 4500-Norg C and 4500-P C, respectively.

3. Results and discussion

3.1. Optimization of different continuous/intermittent combinations

3.1.1. COD and color removal rates

A total load of 2 kg COD/m^3 d was applied for each continuous (feeding) and intermittent (non-feeding) combination at 24 h HRT with a minimum dye concentration

Table 3
Experimental conditions for UASB reactor operation

Operating conditions		OLR (kg COD/m^3 d) during continuous cycle	Number of stable operational days (d)
Single dye feed at 2 kg COD/m^3 d OLR, 24 h HRT and 10 mg/L dye concentration (Phase 1)			
Run 1	Continuous cycle (24 h)	2	37
Run 2	Continuous cycle (18 h) Intermittent cycle (6 h)	2.67	35
Run 3	Continuous cycle (12 h) Intermittent cycle (12 h)	4	30
Run 4	Continuous cycle (6 h) Intermittent cycle (18 h)	8	28
Run 5	Continuous cycle (6 h) Intermittent cycle (6 h) Continuous cycle (6 h) Intermittent cycle (6 h)	2 2	33
Mixed dyes feed at 2 kg COD/m^3 d OLR, 24 h HRT and 30 mg/L dye concentration (Phase 2)			
Run 6	Continuous cycle (18 h) Intermittent cycle (6 h)	2.67	29
Run 7	Continuous cycle (12 h) Intermittent cycle (12 h)	4	25
Mixed dyes feed at 2 kg COD/m^3 d OLR, 48 h HRT and 50 mg/L dye concentration (Phase 3)			
Run 8	Continuous cycle (12 h) Intermittent cycle (12 h) Continuous cycle (12 h) Intermittent cycle (12 h	1 1	31

of 10 mg/L. This was done to optimize the intermittent period for maximum COD and color removal. The length of each run was dependent on biomass adaptation to complex substrate in the feed and sludge characteristics. Effluent recirculation was used in reactor to maintain the reactor's hydrodynamic conditions and maximum adsorption of complex dyes onto biomass granules to achieve complete contact between them. The effluent was recirculated in adjustment with influent flow for each operating run.

Initially the reactor was operated with continuous feeding for 24 h HRT at input OLR of 2 kg COD/m^3 d (Run 1). COD and color removal efficiencies of 46.7% and 58.69% were achieved as shown in Fig. 2. High COD concentration in effluent (1,281 mg/L) was due to large suspended solids concentration in non-granulated anoxic sludge with low retention capacity. Gradual formation of granulated sludge leads to decrease in effluent suspended solids. Initially, due to low acclimatization of sludge to influent substrate, the steady state was maintained for a week at low COD and color removal rates. Higher COD and color removal was not achieved though continuous effluent recirculation was applied. This indicated that mass transfer is not a limiting factor for dyeing wastewater. Recirculation of the effluent kept on recycling the solubilized material in reactor keeping the COD and dye residual concentration stable and high. This had no considerable effect on reactor process.

The intermittent period was introduced for the fact that dyes are complex organic source for biomass and need stabilization period for complete assimilation. Shorter non-feeding period of 6 h was introduced first to increase the biomass adaptation to complex substrate with 18 h continuous feeding at 2.67 kg COD/m^3 d of OLR and 24 h HRT (Run 2). The increased OLR of 2.67 kg COD/m^3 d during continuous period is the extra amount of food provided to biomass during non-feeding period. The COD and color removal rates were measured before and after the non-feeding period to analyze the effect of provision of stabilization phase on substrate biodegradation. Results showed a positive effect of intermittent phase with increase in total COD removal from 45.92% to 51.5% and color removal from 54.8% to 69.04%. During feeding period, not all the substrate were bio-assimilated and mostly got desorbed due to overloading. This verified the theory that intermittent period forces the microbes to feed on the complex substrate when easily degradable glucose is not available anymore. Since dyes act as a complex organic source, the feeding on dyes led to their assimilation into harmless end products. It is to be noted here that non-feeding period of run contributed less in total COD removal as compared with continuous period. This indicates that main substrate removal mechanism is adsorption (physical entrapment) followed by microbial degradation of adsorbed substrate in non-feeding period [15,17].

Nadais et al. [15] operated five lab-scale UASB reactors at different intermittent conditions for treatment of dairy wastewater and found out that shorter non-feeding period is not sufficient for complete biodegradation of accumulated complexes on sludge bed. Thus, the non-feeding period was extended to achieve better assimilation of substrate. This was done by providing 12 h continuous feeding followed by 12 h non-feeding period. A load of 4 kg COD/m^3 d OLR was

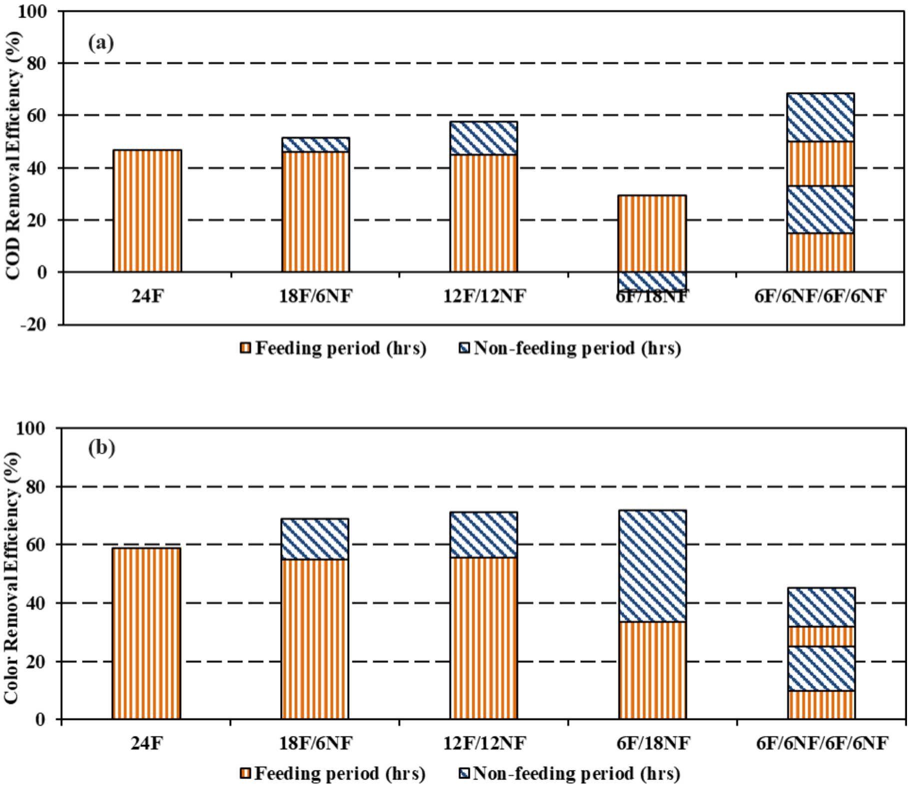

Fig. 2. COD and color removal efficiency at continuous/intermittent combinations.

applied during continuous operation of reactor at 24 h HRT (Run 3). This loading did not exceed the adsorption capacity of substrate on biomass, leading to better assimilation of complex substrate and increasing the COD and color removal rates to 57.5% and 71.0%, respectively. Coelho et al. [17] also revealed that among two UASB reactors treating simulated dairy effluent, one with longer non-feeding period of 9 h produced better methanization rate as compared with UASB reactor with shorter non-feeding period. UASB reactor underwent problems such as reactor leakage, power failure and faulty heating equipment during Run 3. These problems were reflected in the effluent COD and dye residual concentrations. The reactor was preceded with same operating conditions and the run was operated till steady state was achieved.

Since extension of non-feeding (intermittent) period showed marked positive response on complex substrate removal rates, the 12 h non-feeding period was extended to 18 h at increased OLR of 8 kg COD/m^3 d during continuous feeding for 24 h HRT (Run 4). Sudden increase in OLR from 4 (Run 3) to 8 kg COD/m^3 d (Run 4) reduced the total COD removal efficiency to 21.8%. Further 18 h non-feeding period forced the microbes to starve for food for long and put the biomass into endogenous phase. Productions of soluble microbial products by bacteria were reflected in effluent COD concentration. Though increased color removal of 71.8% was observed for Run 4, color was only removed by adsorption phenomena and not due to assimilation. This shows that continuous high load for shorter HRT leads to exhaustion of sludge retention capacity resulting in a dropped reactor performance.

Variation in total COD removal rate for each day was observed throughout the experimental Run 4. This was due to uneven assimilation of dyes in addition to glucose as substrate that leads to higher COD removal rates for some days while sometimes dye residuals in effluent increased the effluent COD concentration. In order to avoid the error due to variations occurred; adequate amount of substrate availability with stabilization period may improve reactor performance. Thus, a combination of 6F/6NF/6F/6NF was operated at 4 kg COD/m^3 d OLR during feeding periods for 24 h HRT (Run 5). This was equally extended at 6 h feeding with 6 h non-feeding periods. The maximum COD removal of 68.5% was obtained with lowest color removal rate of 45.14%. Low color removal was due to excessive overloading of biomass leading to desorption of already accumulated dye concentration with equally less stabilization period for complex dyes. Glucose was readily available to biomass and was assimilated by microbes during short non-feeding period giving increased COD removal efficiency. This observation was similar to Couras et al. [13] who observed the effect of fat shock by raising the feed fat from 110 to 261 mg/L in intermittent systems and 63 to 130 mg/L in continuous systems. Intermittent systems showed no significant effluent COD or TSS removal efficiency however, COD removal rate was reduced during continuous system.

3.1.2. UV-visible spectrum analysis

The color removal rates can be further observed by significant changes in peaks in UV-visible spectrum of influent and effluent samples during single dye feed studies.

Fig. 3 shows the structural changes due to biodegradation of single influent dye used during 12F/12NF (Run 3). The influent spectrum had a visible peak at 488 nm in visible region (350–1,100 nm) while effluent showed the absence of peak where the curve linearly dropped. The disappearance of absorbance peak at 488 nm reflected as evidence of decolorization and breakdown of chromophoric group of Bezaktiv Cosmos Orange dye. The linear curve started at higher absorbance giving indication of peak below 350 nm in UV region. The presence of peak at lower wavelength showed the occurrence of colorless aromatic amines which are not properly assimilated [12,18]. This shows that biodegradation was mainly due to adsorption of dyes on biomass and not due to microbial assimilation. The biodegradation pathway proposed by Manu and Chaudhari [2] and Ong et al. [19] under anaerobic conditions mentioned that Orange II upon reduction produces 1-amino-2-napthol and sulfanilic acid. The FTIR spectrum (not shown) of dried Bezaktiv Cosmos Orange dye powder also confirmed the presence of sulfonic group which upon biodegradation must have generated the intermediate sulfanilic acid observed at 329 nm. The minimal traces upon degradation can also be detected by spectrum obtained from effluent sample.

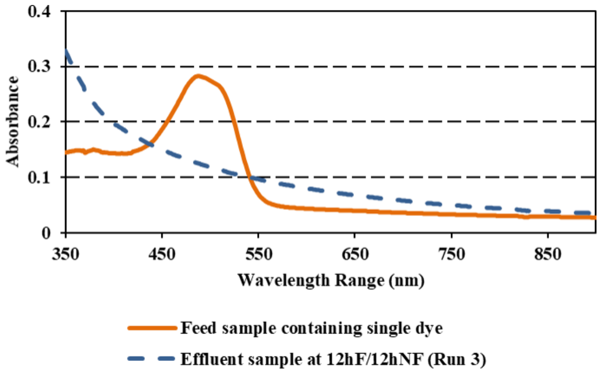

Fig. 3. UV-visible spectrum of single dye in influent and effluent (Run 3).

3.1.3. Reactor stability parameters

Effluent sample was analyzed for VSS/TSS, VFA/alkalinity and pH which depicts the stable operating conditions of the whole reactor (Fig. 4). At the beginning of Run 1, heavy flotation of biomass was observed at top section of reactor due to continuous upflow regime of reactor and presence of non-settled granules. Thus, the VSS/TSS ratio exceeded the desired range to 0.67. However, with adaptation of sludge to substrate nature and improved retention of acclimatized granules, non-feeding period reduced the biomass washout. During Run 2, the VSS/TSS ratio was stabilized between 0.4 and 0.6 which was maintained for the operational cycle of Run 3. During Run 4, a slight increase in VSS concentration was found in effluent due to increased inflow velocity as they drag the fine solids to outflow, however, the overall VSS/TSS ratio was well around 0.56. With effluent recirculation, the effect of decrease in HRT was clear as it was dependent on inflow/recirculation ratio. However, this ratio was disturbed again to 0.63 when consecutive feeding of 6 h was done after small non-feeding period of 6 h in Run 5.

Another stability parameter to optimize the anaerobic treatment is pH which must be in range of 6.6–8.2 [18,20]. Due to presence of dyes toxicity in feed, an acidogenic and facultative methanogenic culture was found in microbial consortia which if buffered properly, will help to achieve better color removal rates. Here, not all the H_2 and acetic acid formed by acidogenic and acetogenic bacteria were converted to methane. Due to higher concentration of VFAs in reactor, carbonate alkalinity generated by biomass was consumed and pH was not in the desired range. To maintain the pH in reactor, external bicarbonate alkalinity in the form of sodium bicarbonate was added as per requirement to get the pH in desired range. The pH of 6.75 was found during continuous feeding at 24 h HRT. With extension of non-feeding periods from Run 2 to Run 3 and incapability of biomass to generate enough carbonate alkalinity, a drop in pH till 6.10 was observed due to VFAs accumulation in reactor. This drop in pH can be related with corresponding decrease in COD and color removal rates and high VSS/TSS ratio due to sloughing of biomass. External

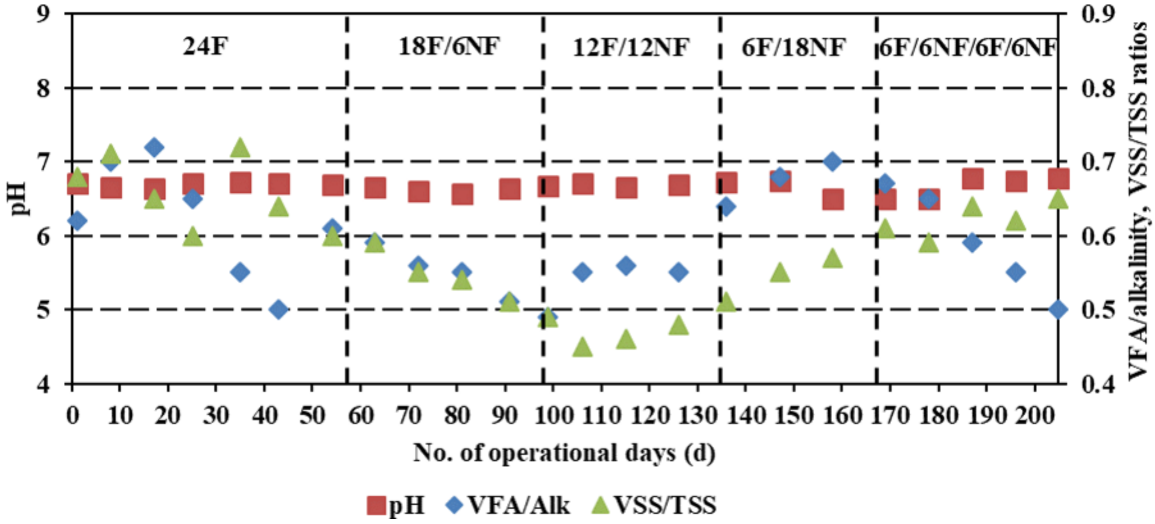

Fig. 4. Reactor stability parameters under single dye feed conditions.

addition of 0.5 g of sodium bicarbonate improved the reactor performance while maintaining the pH in the desired range. pH was largely dropped during Run 4 due to occurrence of endogenous phase for biomass, low microbial activity and build-up of VFAs in sludge, leading to addition of external alkalinity twice a day.

COD removal efficiency decreases as alkalinity decreased from 250 mg/L. It was reported in literature that 0.68 VFA/alkalinity ratio is obtained when alkalinity concentration is less than 250 mg/L indicating system unstable. The intermittent period is suitable for forced assimilation of VFA concentration found in reactor if sufficient alkalinity of 1,000–5,000 mg/L is available to biomass. The system alkalinity was maintained by recirculation of treated effluent [20]. However, during experimentation the reduction in VFA concentration was gradually achieved due to conversion of acidogenic consortia into facultative methanogenic culture. Introduction of dyeing wastewater in anaerobic biomass led to accumulation of VFAs up to 1,052 mg/L with average VFA/alkalinity ratio of 0.65. This ratio was decreased to 0.60 at VFA concentration of 578 mg/L but reduced alkalinity generation of 963 mg/L during Run 2. 12F/12NF (Run 3) further stabilized the reactor by reducing the ratio to 0.55 at 525 mg/L of VFAs and 954 mg/L of alkalinity concentration. Run 4 led to acidic conditions in reactor with high accumulation of VFAs up to 1,233 mg/L concentration at high VFA/alkalinity ratio of 0.71. Run 5 gave optimum VFA/alkalinity ratio of 0.52 at VFA concentration of 425 mg/L which is the minimum concentration of VFAs recorded.

3.1.4. Biomass concentration

Fig. 5 shows that MLVSS/MLSS ratio of initial sludge was 0.43 at MLSS concentration of 4,400 mg/L in Run 1. The actual growth was observed at the end of Run 2 and start of Run 3 to 7,300 mg/L at MLVSS/MLSS ratio of 0.73. The increase indicates higher concentration of biomass. It was reported in a study by Nadais et al. [21] that biomass developed under intermittent conditions has better degradation capacity of complex substrate. The drop in MLVSS (microbial concentration) to 6,900 mg/L was observed during Run 4 with increased VSS concentration of 183 mg/L at VSS/TSS ratio of 0.56. This indicates the washout of inactive microbes during endogenous phase due to prolonged non-feeding period.

3.2. Mixed dyes feed at optimized intermittent conditions

3.2.1. Mixed dyes removal rates

The optimum operating condition of 18F/6NF was examined for the treatment of mixed dyes simulated textile wastewater. The simulated feed consisted of three dyes of different classes (reactive, disperse and vat) which were chosen randomly to imitate any real textile wastewater characteristics. Each dye was used at 10 mg/L concentration giving 30 mg/L of mixed dye solution at 24 h HRT and 2.67 kg COD/m^3 d OLR. This mixed dye feed in simulated textile wastewater was used during the feeding period of cycle (Run 6). The reactor showed stress in its performance due to increase in dyes concentration and presence of mixed dyes. Bezaktiv Orange (reactive dye) gave reasonable removal of 32.5% where its influent concentration of 10 mg/L was decreased to 6.74 mg/L in effluent as biomass was previously acclimatized to the dye's chemical nature (Fig. 6).

Novasol Navy (vat dye) gave highest removal rate of 49% at 5.19 mg/L residual concentration indicating that it was readily adsorbed on biomass at short retention period of 6 h. Among three dyes, Foron Red (disperse dye) gave the lowest removal rate of –28% (12.82 mg/L). This resulted in decreasing the overall COD and color removal efficiency to 37.50% and 17.6%, respectively. Due to intractable nature of Foron Red dye, a high speed stirrer was used to dissolve the dye in simulated textile wastewater feed. This partially hydrolyzed dye covered the granule surface, reduced the biodegradability efficiency of biomass and desorbed at overloading point. This also resulted in less availability of adsorption space for incoming substrate. Since the dye was not completely assimilated into colorless amines, it contributed in overall COD and color removal rates. Senthilkumar et al. [22] reported that

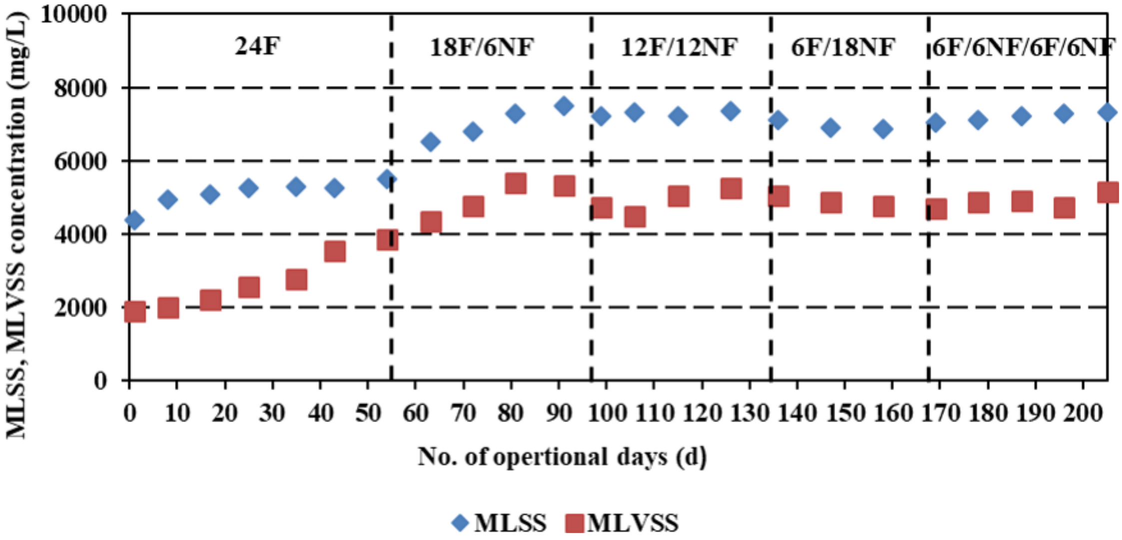

Fig. 5. Biomass profile under single dye feed conditions.

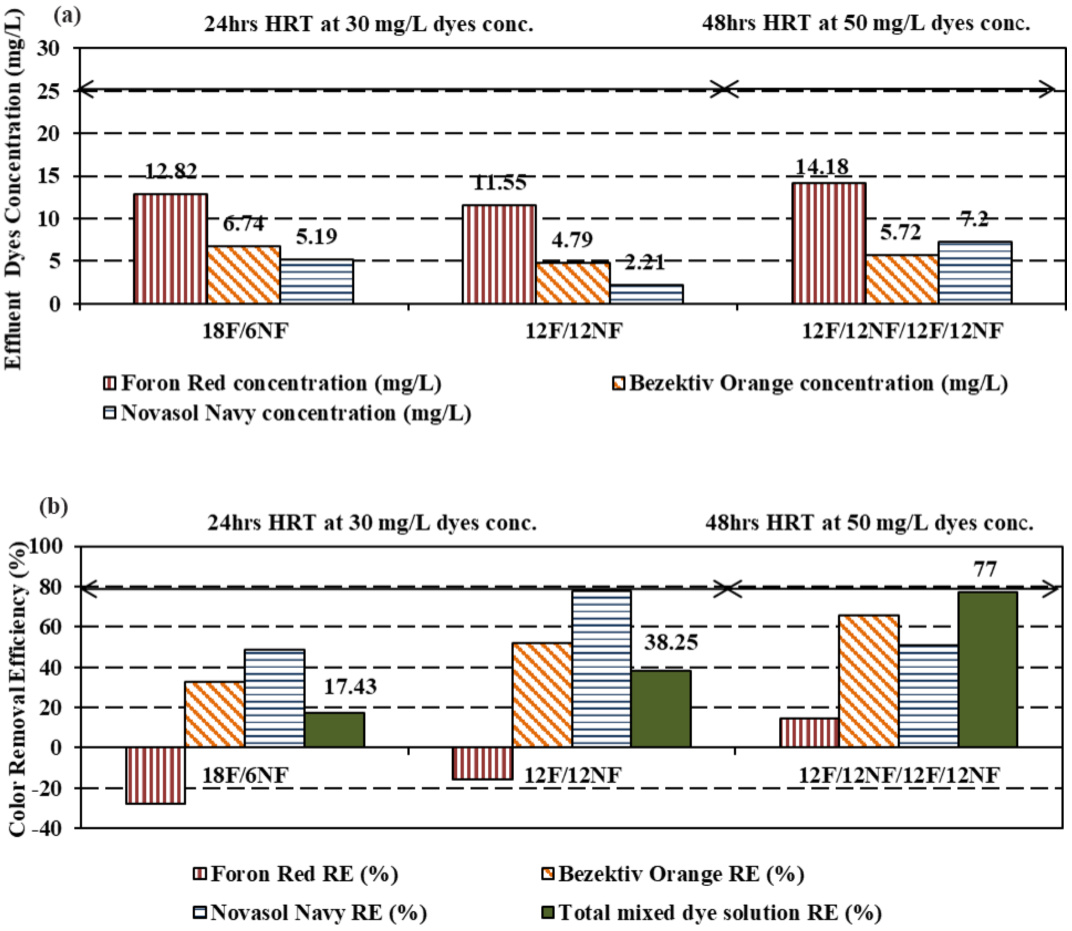

Fig. 6. Color removal efficiency at optimized intermittent combinations under mixed dyes feed conditions.

increase in dye concentration increases the pH but lowers the COD removal efficiency and affects the dye metabolic rate. There was also a high reproducibility of dye at high pH especially if the dye is partially hydrolyzed. Although external sodium bicarbonate addition helped in maintaining the pH of reactor which was in acidic to neutral range, large amount of unassimilated Foron Red dye concentration was seen in the effluent.

The operating condition was shifted to 12F/12NF with increase in OLR to 4 kg COD/m^3 d during continuous feeding period of cycle at 24 h HRT (Run 7). Bezaktiv Orange and Novasol Navy gave further reduced effluent residual concentration to 4.79 and 2.21 mg/L, giving 52% and 78% removals, respectively, in total color removal rate (Fig. 6).

Foron Red was retained on granule surface where its effluent residual concentration was reduced to 11.55 mg/L. This showed the desorption of dye in effluent but in lesser concentration as compared to previous run giving improved color removal efficiency of –15.50%. The overall color removal was improved to 38.3% (18.50 mg/L) but COD removal was slightly enhanced to 47.8% (1,044 mg/L). Thus, increase in non-feeding period helped in recovery from the overloading faced during previous run. Increase in OLR from 2.67 to 4 kg COD/m^3 d indicated that adequate substrate was needed as electron donor for effective color removal. This also showed that 30 mg/L of mixed dyes concentration did not behave as suitable electron acceptors for effective COD and color removals at 24 h HRT. Işik and Sponza [12] reported 77% COD and 67.2% color removal rate in UASB reactor treating Procion Red H-37B at 3.1 kg COD/m^3 d OLR and 24 h HRT and reported the removal efficiency to be insufficient. Thus, to increase the removal efficiency, increase in HRT was required.

In Phase 3 (Run 8), individual dye concentration was increased to 16.5 mg/L giving 50 mg/L of mixed dyes solution in simulated textile wastewater feed. Increase in dyes concentration also gave an overall increase in residual dye concentration in effluent. This also resulted in high decolorization rates when cycle was extended to 48 h HRT with 12F/12NF/12F/12NF operating run (Run 8). To avoid the endogenous phase at same OLR of 2 kg COD/m^3 d, consecutive feeding and non-feeding periods for 12 h were implemented. This helped in assimilation of more substrate to end products. Somasiri et al. [18] reported the residual dye concentration of 0.4 mg/L at 10 mg/L influent dye concentration while 18 mg/L persisted at 300 mg/L dye concentration. This gave an increase in color removal efficiency to 71% but increased residual dye concentration of 14.12 mg/L in effluent. Similar results were obtained for Bezaktiv Cosmos Orange and Novasol Navy dyes whose removal efficiency was improved to 65.5% and 56.5%, respectively. Foron Red dye also gave better removal efficiency of 14.5% but with residual concentration of 14.18 mg/L. Lourenço et al. [23] reported the effect of operational parameters on dyes biodegradation in SBR with increase in Remazol Brilliant Violet 5R dye concentration from 60 to 100 g/L at SRT of 10–15 d and described that bacteria in anaerobic/aerobic biomass bring exhaustion of carbon source in feed early, favoring expansion of dye metabolizing microbial consortium. This supported the results for better Foron Red dye removal rates. Longer HRT of 48 h enabled the granules to adsorb the sudden increase in dye concentration and change in dye characteristics. Ong et al. [19] also reported the COD and Orange II removal efficiencies to be increased from 27% to 35% and from 82% to 97% by increasing the HRT from 24 to 48 h and dye loading rate from 0.06 to 0.30 g COD/L d at 30°C in lab-scale UASB-SBR system.

3.2.2. UV-visible spectrum scan for mixed dyes feed

The effluent samples from Run 7 and 8 were also analyzed for spectrum obtained from UV-visible spectrophotometer in visible range (350–1,100 nm) as shown in Fig. 7. This was done to analyze the degradation pathway adapted by mixed dyes feed solution. The influent curve from Run 7 (30 mg/L at 24 h HRT) showed two peaks at 401 and 577 nm in visible range. Similar peaks were observed for influent sample from Run 8 (50 mg/L at 48 h HRT) but at lower absorbance values. The effluent curve for Run 7 show the linear curve with disappearance of corresponding influent peaks. It showed the rupture of chromophoric structure of dye and production of small amount of colorless dye metabolites near UV range at 371 nm. With increase in HRT to 48 h for Run 8, the effluent peak starts at the same absorbance point as influent peak showing the possibility of absence of any aromatic amines and their assimilation into end products. One peak at 589 nm showed the possible dye residuals obtained after Foron Red reduction (528 nm) while other curves indicated dye residuals in effluent at 488 nm.

3.2.3. Biomass adaptation to mixed dyes feed

A drop in MLSS from 6,780 to 5,900 mg/L was observed due to increase in dye concentration as a shock. A quick recovery was observed when non-feeding period was extended to 12 h and better sludge acclimatization to substrate was achieved. Here, MLSS was maintained at 6,300 mg/L (Fig. 8).

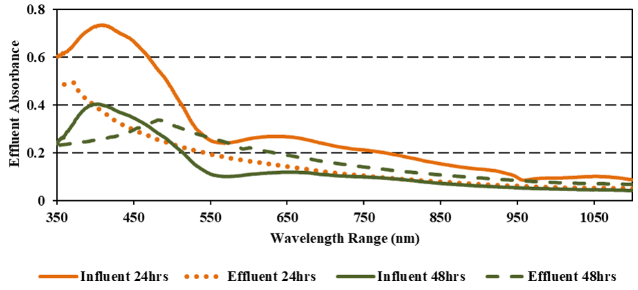

Fig. 7. UV-visible spectrum of mixed dyes in influent and effluent (Run 7 and 8).

A better MLVSS/MLSS ratio of 0.85 was observed with high biomass concentration when HRT was extended from 24 to 48 h. This was accompanied by stable pH between 6.70 and 6.98 and VFA/alkalinity ratio of 0.49 at 370 mg/L VFA and 755 mg/L alkalinity concentration (Fig. 9).

3.2.4. Total Kjeldahl nitrogen and orthophosphates removal

Total Kjeldahl nitrogen (TKN) and orthophosphates concentration was analyzed in effluent samples for last three runs operated for mixed dyes solution. Generally, textile wastewater has low levels of nitrogen compounds in wastewater stream so it was supplemented to meet the biomass nutrient requirements. Dyes reduction via organic carbon source (dextrose) led to the growth of acetogenic/methanogenic microbes in anoxic-anaerobic environment. Denitrification is basically the reduction of nitrates and nitrites to nitrogen gas. Different microbial species are required to mediate the denitrification and methanogenic process. Due to negligible presence of nitrogen compounds in influent initially, methanogenic culture was likely to dominate the denitrification process. Thus, this resulted in removal of minimal particulate nitrogen compounds via filtration, sedimentation and accumulation of nitrogen compounds mostly in the form of nitrates and ammonia onto the biomass. Small amounts of nitrates were converted into ammonia which cannot be further degraded in anaerobic conditions. This was because of abundance of ammonia formers in anaerobic biomass as compared with denitrifies [24].

This accumulated ammonia (nitrogen compounds) reached their over-loading point where they get desorbed and their increased concentration was found in effluent with TSS. Thus, low removal efficiency of nutrient was observed

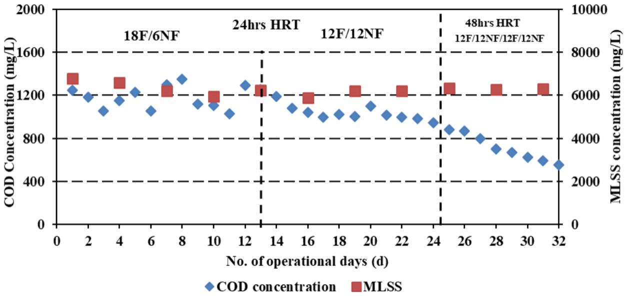

Fig. 8. Biomass profile at optimized intermittent combinations under mixed dyes feed conditions.

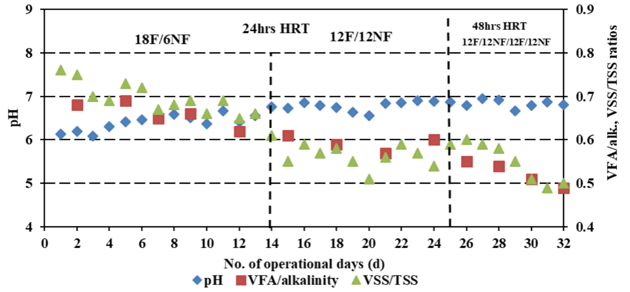

Fig. 9. Reactor stability parameters at optimized intermittent combinations under mixed dyes feed conditions.

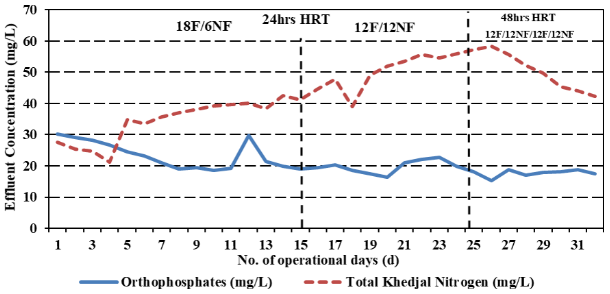

Fig. 10. Nutrient removal rate at optimized intermittent combinations under mixed dyes feed conditions.

with high concentration of TKN in Run 6 which kept fluctuating between 28 and 37 mg/L. During Run 7, minimal TKN removal was observed as effluent continued to rise till 56 mg/L (Fig. 10) indicating no denitrifers were present in biomass to assimilate the nitrates and ammonia into safer components. This might also be due to limited organic carbon source for denitrifers and inappropriate pH range (6.5–7.5) in which they thrive [25]. At increased HRT of 48 h consecutive feeding and non-feeding periods (Run 8), better MLSS concentration (biomass growth) was observed. This was physically observed by good dense settled granules at the bottom of reactor indicating the change and growth of biomass. Analytically, reduced TKN effluent concentration of 42.35 mg/L was measured which was expected to continue to decrease throughout the run. Further decrease in VFA concentration in effluent from Run 6 to Run 8 indicated that denitrifers used VFAs for their carbon requirement.

The source of orthophosphates in the reactor synthetic feed was potassium dihydrogen phosphate which was one of basic nutrients for biomass growth. No significant orthophosphate removal was observed in reactor due to negligible biomass growth in reactor throughout the experiment. Due to negligible demand of phosphorus by anaerobic bacteria, a little accumulation of phosphorus was observed. Mainly a balance was maintained in reactor with total phosphorus in and out of reactor to be same at effluent concentration of 17–18 mg/L [26]. This observation was also indicated by various studies showing conservation of nutrients (nitrogen and phosphorus) in anaerobic reactors. A little removal efficiency may due to filtration and sedimentation by dense sludge granules at the reactor bottom. However, it is also reported that ammonia and nitrate accumulation in anaerobic biomass also hinders the phosphate removal process [27].

4. Conclusion

In the present study, the anaerobic treatability of simulated textile wastewater containing dyes of different nature was examined under the intermittent operation of UASB reactor. The reactor performance (pH, VFA, alkalinity, COD and color) was evaluated under five different intermittent (non-feeding) conditions at similar operating conditions of 24 h HRT and 2 kg COD/m^3 d OLR. Optimum intermittent (non-feeding) condition of 12 h feeding with 12 h non-feeding (12F/12NF) gave the maximum COD and color removal rates of 57.5% and 71.0%. This optimal run was operated for the mixed dyes solution of 30 mg/L. Non-feeding period gave an incremental COD and color removal rates with gradual acclimatization of dyes in laboratory scale UASB reactor under strict anaerobic conditions. Due to the complex nature of dyes, incomplete anaerobic digestion occurs resulting in acidogenic phase in reactor and presence of intermediate metabolites of dyes. Thus, with increase in dyes concentration in feed from 30 to 50 mg/L and change in dye's nature (disperse, vat and reactive dyes), the hydrogen and acids produced were not completely assimilated by methanogens under anaerobic conditions. To improve the reactor's performance and avoid endogenous phase, consecutive 12 h of feeding and non-feeding period (12F/12NF/12F/12NF) was operated. This improved the individual dye removal rate with overall color removal efficiency of 71% and better acclimatization of dyes to anaerobic consortia. With external addition of 500 mg/L of sodium bicarbonate, the reactor achieved the minimum VFA/alkalinity ratio of 0.49. Limited TKN removal efficiency was observed with no phosphates removal indicating the accumulation of organics onto the sludge bed and poor biomass growth.

It is important to have a detailed nutrients removal and biogas production study by treating real textile wastewater. This will help to analyze complete matrix effect on color removal and other process indicators being affected by various chemicals present in it. This study can also help to further consider the biogas production rates with detailed granulated sludge examination during non-feeding (intermittent) phase of anaerobic treatment operation. Integrated methanogenic/denitrification process operated at optimum intermittent conditions can have the greatest potential for textile wastewater treatment where organic carbon can be fully assimilated since denitrification can be ensured before methane production.

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References

[1] S. Wijetunga, X. Li, C. Jian, Effect of organic load on decolourization of textile wastewater containing acid dyes in upflow anaerobic sludge blanket reactor, J. Hazard. Mater., 177 (2010) 792–798.

[2] B. Manu, S. Chaudhari, Anaerobic decolorization of simulated textile wastewater containing azo dyes, Bioresour. Technol., 82 (2002) 225–231.

[3] A. Verma, P. Bhunia, R. Dash , R. Tyagi, R. Surampalli, Effects of physico-chemical pre-treatment on the performance of an upflow anaerobic sludge blanket (UASB) reactor treating textile wastewater: application of full factorial central composite design, Canad. J. Chem. Eng., 93 (2015) 808–818.

[4] W. Somasiri, X. Li, W. Ruan, C. Jian, Evaluation of the efficacy of upflow anaerobic sludge blanket reactor in removal of colour and reduction of COD in real textile wastewater, Bioresour. Technol., 99 (2008) 3692–3699.

[5] C. Couras, V. Louros, T. Gameiro, N. Alves, A. Silva, M. Capela, L. Arroja, H. Nadias, Anaerobic degradation of

dairy wastewater in intermittent UASB reactors: influence of effluent recirculation, Environ. Technol., 36 (2015) 2227–2238.

[6] S. Farajzadehha, S. Mirbagheri, S. Farajzadehha, J. Shayegan, Lab-scale study of HRT and OLR optimization in UASB reactor for pretreating fortified wastewater in various operational temperatures, APCBEE Procedia, 1 (2012) 90–95.

[7] A. dos Santos, F. Cervantes, J. van Lier, Review paper on current technologies for decolourisation of textile wastewaters: perspectives for anaerobic biotechnology, Bioresour. Technol., 98 (2007) 2369–2385.

[8] G.A. Amin, L. Vnens, Optimization of up-flow anaerobic sludge blanket reactor for treatment of composite fermentation and distillation wastewater, Afr. J. Biotechnol., 13 (2014) 1136–1142.

[9] P. Firmino, M. da Silva, F. Cervantes, A. dos Santos, Colour removal of dyes from synthetic and real textile wastewaters in one- and two-stage anaerobic systems, Bioresour. Technol., 101 (2010) 7773–7779.

[10] A. Tawkif, D. Zaki, M. Zahran, Combination of Up-flow Anaerobic Sludge Blanket and Down-flow Hanging Sponge System for Removal of Color from Reactive Dyes Wastewater, 17th International Water Technology Conference, Istanbul, 2013.

[11] K. Muda, A. Aris, M. Salim, Z. Ibrahim, A. Yahya, M. van Loosdrecht, A. Ahmad, M. Nawahwi, Development of granular sludge for textile wastewater treatment, Water Res., 44 (2010) 4341–4350.

[12] M. Işik, D. Sponza, Anaerobic/aerobic treatment of a simulated textile wastewater, Sep. Purif. Technol., 60 (2008) 64–72.

[13] C. Couras, V. Louros, A. Grilo, J. Leitao, M. Capela, L. Arroja, M. Nadais, Effects of operational shocks on key microbial populations for biogas production in UASB (Upflow Anaerobic Sludge Blanket) reactors, Energy, 73 (2014) 866–874.

[14] G. Lettinga, L. Hulshoff Pol, UASB-process design for various types of wastewaters, Water Sci. Technol., 24 (1991) 87–107.

[15] H. Nadais, I. Capela, L. Arroja, A. Duarte, Optimum cycle time for intermittent UASB reactors treating dairy wastewater, Water Res., 39 (2005) 1511–1518.

[16] APHA/AWWA/WEF, Standard Methods for the Examination of Water and Wastewater, 2012, 541 p.

[17] N. Coelho, A. Rodrigues, L. Arroja, I. Capela, Effect of non-feeding period length on the intermittent operation of UASB reactors treating dairy effluents, Biotechnol. Bioeng., 96 (2007) 244–249.

[18] W. Somasiri, W. Ruan, L. Xiufen, C. Jian, Decolourization of textile wastewater containing acid dyes in UASB reactor system under mixed anaerobic granular sludge, Electron. J. Environ. Agric. Food Chem., 5 (2006) 1224–1234.

[19] S. Ong, E. Toorisaka, M. Hirata, T. Hano, Decolorization of azo dye (Orange II) in a sequential UASB-SBR system, Sep. Purif. Technol., 42 (2005) 297–302.

[20] M. Latif, R. Ghufran, Z. Wahid, A. Ahmad, Integrated application of upflow anaerobic sludge blanket reactor for the treatment of wastewaters, Water Res., 45 (2011) 4683–4699.

[21] H. Nadais, M. Barbosa, A. Almeida, A. Cunha, I. Capela, L. Arroja, Intermittent Operation of UASB Reactors as a Strategy to Enhance Anaerobic Degradation of Dairy Wastewaters, Proc. 6th WSEAS International Conference on Environment, Ecosystem and Development, 2008, pp. 38–43.

[22] M. Senthilkumar, G. Gnanapragasam, V. Arutchelvan, S. Nagarajan, Treatment of textile dyeing wastewater using two-phase pilot plant UASB reactor with sago wastewater as co-substrate, Chem. Eng. J., 166 (2011) 10–14.

[23] N. Lourenço, J. Novais, H. Pinheiro, Effect of some operational parameters on textile dye biodegradation in a sequential batch reactor, J. Biotechnol., 89 (2001) 163–174.

[24] H. Hendriken, B. Ahring, Integrated removal of nitrate and carbon in an upflow anaerobic sludge blanket (UASB) reacctor: operating performance, Water Res., 30 (1996) 1451–1458.

[25] J. de Sousa, K. Santos, I. Henrique, D. Brasil, E. Santos, Anaerobic digestion and the denitrification in UASB reactor, J. Urban Environ. Eng., 2 (2008) 63–67.

[26] P. Ndegwa, D. Hamilton, J. Lalman, H. Cumba, Effects of cycle-frequency and temperature on the performance of anaerobic sequencing batch reactors (ASBRs) treating swine waste, Bioresour. Technol., 99 (2008) 1972–1980.

[27] G. Yilmaz, R. Lemaire, J. Keller, Z. Yuan, Simultaneous Nitrification, Denitrification, and phosphorus removal from nutrient-rich industrial wastewater using granular sludge, Biotechnol. Bioeng., 100 (2008) 529–541.