Azo Dye Removal in Two-stage Anaerobic Sequential Batch Reactor with Starch as a Primary Electron Donor

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Abstract

Two-stage anaerobic system (S1) and its controlled one-stage system (S0) were established to investigate the effect of phase separation on the removal of an azo dye orange II (AO7) with soluble starch as a primary electron donor. S1 consisted of two series-connected anaerobic sequencing batch reactors (ASBRs) (R1: acidogenic phase; R2: methanogenic phase), while S0 consisted of only one ASBR (R0). During the entire operation period, no statistical differences in final AO7 removals of the both two systems were observed. However, the batch assays suggested 2.7-fold and 1.7-fold of pseudo first-order rate constants for AO7 removal (k_{AO7}) and sulfanilic acid (SA) formation (k_{SA}). The beneficial effect of phase separation in S1 only emerged in the period with higher influent AO7 concentrations (> 2.14 mM). Otherwise, the longer HRT (5 d) and sufficient electron donor supply (1.0 g starch L^{-1}) could submerged the advantage of phase separation in AO7 reduction. Within S1, R1 only accounted for about 10% of the entire AO7 removal, and k_{AO7} during batch assay in R1 (0.172 h^{-1}) was much lower than that in R2 (0.503 h^{-1}). In contrast with previous studies, methanogenic phase rather than acidogenic phase was the main contribution to AO7 removal. Because the required electron donor for AO7 reduction was limited, volatile fatty acids (VFAs) accumulation was mainly attributed to the balance of hydrolysis-acidification and methanation processes. Within S1, effluent from R1 containing readily available electron donors was fed as the influent of R2, and methanogenic phase was operated in neutral condition (pH 6.5 to 7.0) in which AO7 was preferred to be reduced rather than that in acidogenic phase.

Keywords

Two-stage anaerobic system; Azo dye; Starch; Phase separation

INTRODUCTION

China and other Asian countries have been suffering heavy pollutions caused by textile and dyeing industries in the past decades (Ito et al., 2016). The commercially used azo dyes and other chemical additives contained in textile dyeing wastewater are not readily degradable in natural conditions, resulting in the potential harm to the environment and also to human health. Thus an adequate treatment is needed before textile dyeing wastewater discharges to the water environment. In recent years, although many chemical and physicochemical methods have been widely used for textile dyeing wastewater treatment, biological process is still the lowest cost and energy-saving option (Saratale et al., 2012). Traditional biological systems, which composed of series-connected anaerobic and subsequent aerobic processes, were mostly employed in the engineering applications (Amaral et al., 2014; da Silva et al., 2012a). The former anaerobic reaction in this combined process contributed to large part of colour removal and could improve the biodegradability of recalcitrant organic compounds, finally enhancing the COD removal in the following aerobic process (Frijters et al., 2006). However, anaerobic is the rate-limiting step of the whole process in treating textile dyeing wastewater due to the strong electron affinity of the –N=N– bond associated in the azo dyes. Therefore, to fully exploit the potential advantages of the combined bio-process during textile wastewater treatment, it is an urge to improve the operating performance of anaerobic process.

Up to date, many anaerobic systems such as up-flow anaerobic sludge blanket (UASB) and anaerobic sequencing batch reactor (ASBR) have been successfully applied (Huang et al., 2015a). In these systems, the acidogenic and methanogenic microorganisms are co-existed, and the former mainly contributed to the azo dye reduction, while methanogenic microorganisms were capable to conduct only in the presence of external electron shuttling substrates (Rodrigues da Silva et al., 2012). To avoid the imbalance between acidogenic and methanogenic microorganisms, two-stage

anaerobic process were always instead of traditional one-stage in treating high organic load rate (OLR) and/or toxic wastewaters (Ráduly et al., 2016; Saddoud and Sayadi, 2007). Two-stage anaerobic process was assembled of two series-connected reactors, separating the acidogenic and methanogenic phases. By this way, the conditions for different microbial consortia in two separated phases were optimized, and finally enhanced the removal of organic pollutants (Zuo et al., 2015). Firmino et al. (2010) have reported a more stable azo dye removal in two-stage anaerobic system as compared to that in one-stage. Some active substrates such as Fe^0 and redox mediators (RMs) were introduced to acidogenic phase to enhance the key enzyme activities and the pollutants removal, thence submerging the separation benefits of acidogenic and methanogenic phases (da Silva et al., 2012b; Liu et al., 2012). The above studies were all fed with ethanol or easily biodegradable electron donors for azo dye decolouration. However, starch-based sizing agent was usually used in sizing process of textile industry (Franca et al., 2015), thus real textile wastewater always contains starch but not ethanol. The molecular weight of starch was much higher, and the conversion of starch to ethanol and/or other readily biodegradable intermediates needs special condition and longer hydraulic retention time (HRT) (Bai et al., 2008).

When two-stage anaerobic system was used to treat real textile wastewater, the process characteristic in separated phases might be more complex than that with ethanol, volatile fatty acids (VFAs) and other readily biodegradable co-substrates. This would in turn affect the azo dye removal and system operation. Thus, it has an engineering significance to investigate the azo dye reduction with starch as a co-substrate in two-stage anaerobic process. In this study, ASBR-based two-stage anaerobic system was established to investigate the effect of phase separation on azo dye removal with soluble starch as a primary electron donor.

MATERIALS AND METHODS

Experimental set-up

Two lab-scale systems (S0 and S1) composed of plexiglass made ASBRs were set-up in this study. The schematic diagram of the operating systems are shown in Figure 1.

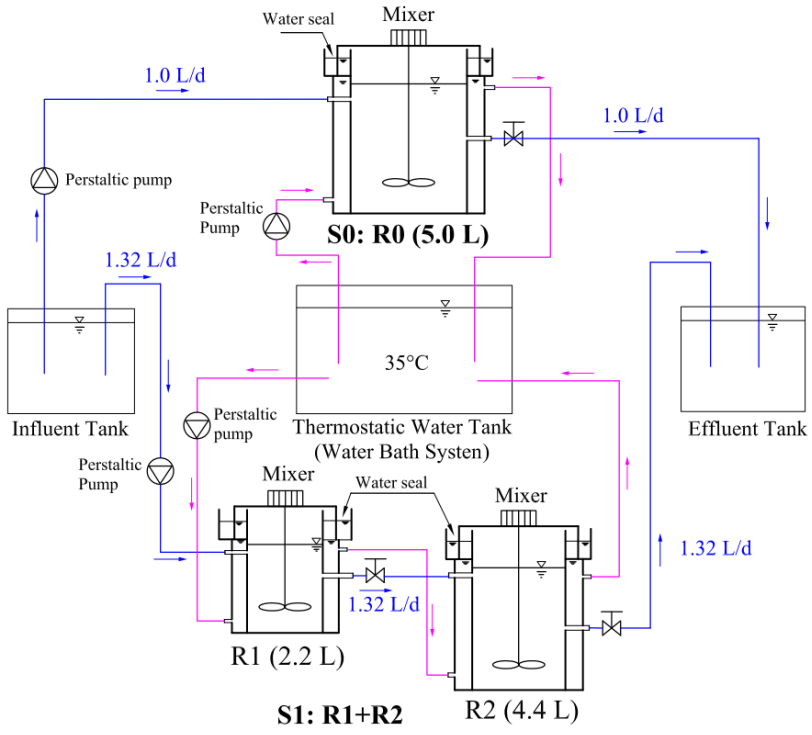

Figure 1. Schematic diagram of the one-stage (S0) and two-stage (S1) anaerobic systems.

System S0 was designed as one-stage anaerobic system (control), which consisted of only one ASBR (R0); while system S1 was designed as two-stage anaerobic system consisting of two series-connected ASBRs (R1: acidogenic phase; R2: methanogenic phase). The working volume of R0, R1 and R2 were 5.0 L, 2.2 L and 4.4 L, respectively. The water seals were located at the top of each ASBR to ensure that the reactors were airtight. The ASBRs were mechanically stirred (80 rpm) using a power-driven force mixer (HD 2004W, Sile Co., China) in water bath at 35 ± 1 °C. The two systems were operated with one cycle per day. Each cycle consisted of 22 h anaerobic stirring, followed by 1.5 h settling, 10 min decanting and 20 min feeding. The inoculated anaerobic sludge was obtained from a secondary sedimentation tank of a WWTP in Hangzhou city, China. Sludge concentration in any ASBR was 3 g VSS L^{-1}. The artificial textile wastewater was employed as the influent in this study. Typical azo dye orange II (AO7) and soluble starch acting as electron donor, were the main composition of the feed water. $MgCl_2 \cdot 6H_2O$ 20.3 mg L^{-1}, $CaCl_2 \cdot 2H_2O$ 14.7 mg L^{-1}, $FeSO_4 \cdot 7H_2O$ 2.28 mg L^{-1}, NH_4Cl 60 mg L^{-1}, KH_2PO_4 15 mg L^{-1} were supplied in the influent. Macro and trace metals and vitamins were supplied as described in our previous study (Huang et al., 2015b).

Continuous operation

During the entire operation period, the HRTs of S0 and S1 were both 5 d, which was determined according to a previous study when starch was employed as a co-substrate for azo dye removal (Manu and Chaudhari, 2003). To bring out a phase separation in S1, the designed HRTs of R1 and R2 were 1.67 and 3.33 d, respectively; and the designed pH in R1 ranged from 5.5 to 6.5, while that in R2 was 6.5 to 7.0 which was the same as that in S0 (R0). The designed pHs in each ASBRs were achieved by adjusting with stocked $NaHCO_3$ buffer solution (50 g L^{-1}). Soluble starch concentrations in the influent of S0 and S1 were both maintained at 1000 mg L^{-1} (equivalent to 1067 mg COD L^{-1}) during the entire operation, i.e., the starch loading rates were 0.2 kg m^{-3} d^{-1}. Whereas the influent AO7 concentrations were gradually increased from 0.43 to 2.57 mM, and finally stabilized at 2.14 mM after day 43, lasting to the end of the whole period (day 77). $NaHCO_3$ and AO7 in the influent of each system during different operational periods are shown in Table 1.

Table 1. $NaHCO_3$ and AO7 concentrations in the influent of each ASBRs during different periods

Marking	Time (d)	$NaHCO_3$ (mg L^{-1}) *			AO7 (mM) **
		R0	R1	R2	
a	1	300	0	300	0.43
b	5	400	400	400	0.57
c	6	500	300	400	0.57
d	7	600	300	400	0.57
e	8	600	300	400	0.71
f	9	750	500	500	0.71
g	10	1000	600	500	0.71
h	11	1000	600	400	0.86
i	12	1100	600	400	0.86
j	16	1100	600	400	1.14
k	20	1100	600	600	1.14
l	28	1100	600	600	2.14
m	34	1100	600	600	2.57
n	43	1100	600	600	2.14

Note: * $NaHCO_3$ concentrations in each reactor was different.

** Indicates the same influent AO7 concentrations in systems S0 and S1.

To obtain the designed HRT (5 d) of S0, 1.0 L of artificial wastewater was fed to R0 (working volume 5.0 L) per day. As to S1, it contained two series-connected ASBRs. i.e., R1 and R2. R1 was fed with fresh artificial wastewater, while R2 was fed with the effluent of R1. An HRT of 1.67 d in R1 (working volume 2.2 L) was obtained by replacing 1.32 L of fresh artificial wastewater per day; and an HRT of 3.33 d in R2 (working volume 4.4 L) was obtained by removing 1.32 L of the effluent in R2 and adding 1.32 L of the effluent in R1 every day. To monitor the operating performance of these systems, frequent analyses of effluent from each ASBR were conducted for pH, volatile fatty acids (VFAs), AO7 and its reduction product of sulfanilic acid (SA).

Batch assay

During the stabilization period after day 50, batch assays were conducted on day 74 to better illustrate the decolourization processes for the one-stage and two-stage anaerobic systems. The reaction kinetics of AO7 removal and SA formation were determined by varied concentrations as a function of time expanding. Within the batch assay cycle, 5 mL of mixture were withdrawn from R0, R1 and R2 at appropriate time intervals (0, 2, 4, 6, 8, 10, 12, 24 h). Modified pseudo first-order kinetic models were employed to analyze the AO7 removal and SA formation using the following equations (Shi et al., 2012):

$$C_{AO7(t)} = a + b\, e^{(-k_{AO7}\, t)} \quad (1)$$

$$C_{SA(t)} = a + b\, e^{(-k_{SA}\, t)} \quad (2)$$

where $C_{AO7(t)}$ and $C_{SA(t)}$ were the AO7 and SA concentrations (mM) at the time of t (h) during the batch assays; k_{AO7} and k_{SA} (h^{-1}) were the first-order rate constants for AO7 removal and SA formation, respectively; a is the possible minimum and b is the possible maximum concentrations of AO7 or SA (mM) during the batch assay.

Chemical analysis

All withdrawn samples were firstly centrifuged at 4000 × g for 20 min, and then membrane-filtered (0.45 μm). AO7 was measured spectrophoto-metrically at the wavelength of 484 nm. VFAs was composed of acetate, propionate, n-butyrate, n-valerate and their isomers. VFAs concentration was defined as the COD of all these components and the coefficient of them are 1.067, 1.512, 1.818 and 2.039. SA and VFAs components were simultaneously measured by a high performance liquid chromatography unit (HPLC, Agilent 1200, USA) equipped with an UV detector. A Shodex RSpak KC-811 analytical column following a Shodex RSpak KC-G guard column (Showa Denko, Japan) was used at 50 oC. The mobile phase was phosphoric acid solution (0.05%) at a flow rate of 0.7 mL min^{-1}, and the wave length for detection was 210 nm. pH was measured by a portable pH meter (SANXIN, SX751, China).

Statistical analysis

To compare the average levels of effluent AO7, SA and VFAs during the operational period between two experimental sets of S0 and S1, an analysis of Student's unpaired t-test was used and $P < 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

AO7 reduction in continuous operation

The effluent concentrations of AO7 and SA in one-stage system S0 (R0) and two-stage system S1 (R1 + R2) are shown in Figure 2. Under an HRT of 5 d, the effluent AO7 concentrations of both systems were in very low levels, and the final AO7 removal achieved 90% and 95%, respectively (Figure 2A). During the entire period, the maximum effluent SA concentrations of S0 and S1 were 1.79 and 2.00 mM (day 41), respectively; and during the stabilization period (after day 50), they kept at 1.6 ± 0.1 and 1.7 ± 0.2 mM, respectively (Figure 2B). Because SA and 1-amino-2-naphtol (1A2N) were the reductive products during AO7 decolouration, anaerobic reduction was the main AO7 removal pathway in this study. The statistical analysis of the above results suggested that better AO7 removal and SA formation in S1 than those in S0 were only found during days 34 to 49

($P < 0.05$); whereas no significant differences were observed during the entire and the stabilization periods ($P > 0.05$). After influent AO7 was resumed to 2.14 mM on day 45, final AO7 removal in S0 dropped from 94.0% to 88.7%, while it still kept at ~95% in S1. Therefore, S1 was more efficient than the S0 when influent AO7 was in relative higher concentrations (> 2.14 mM). On day 34, influent AO7 have increased to the maximum of 2.57 mM, which might need more available electron donor and reaction time for its reduction process. However, under lower influent AO7 concentration, the long HRT (5 d) and sufficient electron donor (1067 mg COD L^{-1}) could covered this enhanced effect of phase separation on AO7 removal. Similar results have been observed by Firmino et al. (2010) who found that the stable azo dye Congo Red removal in two-stage anaerobic process (UASB) is dependent on the ratio of available electron donor to azo dyes. Under sufficient electron donor supply, the competition between azo dye reduction and other electron-consuming biological processes such as methanation could be ignored, resulting in comparable azo dye removal efficiencies in one-stage and two-stage systems (da Silva et al., 2013). Under insufficient electron donor supply, the electron competition resulted in less electron flow shift to the azo dye reduction process (Firmino et al., 2010). Although the required electron donor for a complete reduction of maximum 2.57 mM AO7 in this study was only 82 mg COD L^{-1}, much lower than the added electron donor (1067 mg COD L^{-1}), it might limit the azo dye reduction kinetics by the smaller concentrations of available substrates (van der Zee and Villaverde, 2005). Two-stage anaerobic system separated the acidogenic and methanogenic phases and could might optimize the constituents of available electron donors and reaction kinetics for AO7 reduction, promoting more electrons shifted from the available electron donors to the final acceptor of AO7. Apart from this way, the addition of redox mediators (RMs) have also been reported to improve the reaction kinetic and finally make a sound impact of phase separation on azo dye reduction (da Silva et al., 2012b; Liu et al., 2012). Thus an kinetic study within one batch cycle should be further conducted to clearly illustrate the AO7 reduction process in one- and two-stage systems.

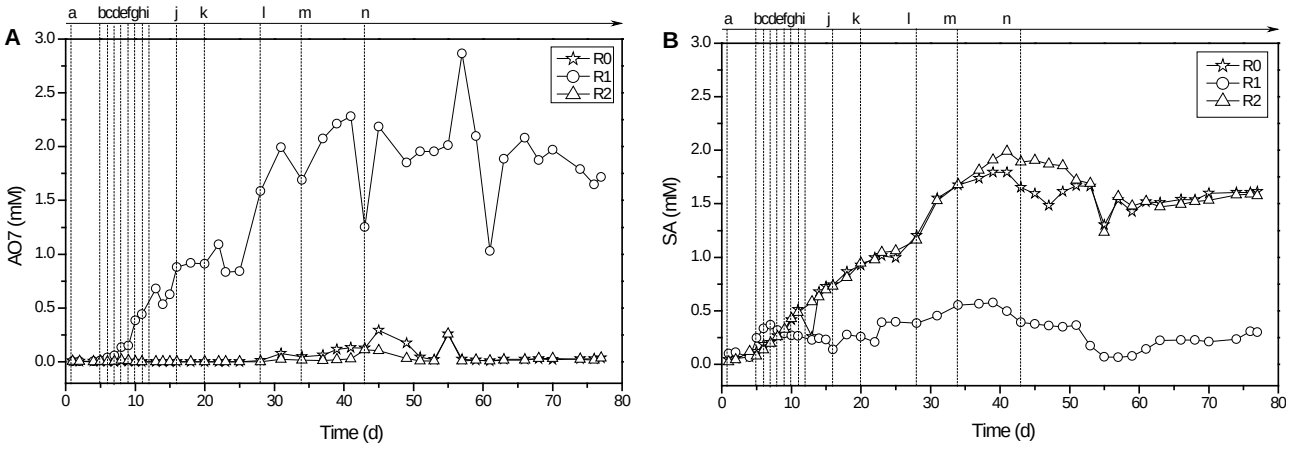

Figure 2. Effluent concentrations of AO7 (A) and SA (B) in system S1 (R0) and S2 (R1 + R2) during the entire operational period.

As mentioned to two-stage anaerobic system S1, it was found that effluent AO7 in hydrolysis-acidification phase (R1) was much higher than that in methanogenic phase (R2). The AO7 removal in R1 only accounted for about 10% of the entire AO7 removal within S1, implying that methanogenic phase had a greater contribution to AO7 removal in this study. This in turn led to a higher SA production in R2 as compared to that in R1. The result was in contrast with the previous publication, in which fermentative bacteria rather than methanogenic bacteria mainly contributed to the azo dye decolourization (Dos Santos et al., 2006). It was noticed that the used co-substrates in the previous study were all efficient electron donors such as glucose, methanol, acetate, formate and H_2/CO_2, for azo dye reduction (Hong et al., 2007). Whereas starch, which was

widely used for the sizing process of textile dyeing industry, was used in this study. As compared to those readily bio-available co-substrates, starch has a much higher molecular weight and complex structure, it should be first hydrolyzed to monosaccharide and then fermented to VFAs and H_2 in R1. This lagged process of available electron donor production might submerge the advantage of AO7 reduction in the acidification phase (R1). The detailed characteristics of reaction kinetic and electron donor conversion in two-stage anaerobic system and their effects on AO7 reduction will be discussed below.

AO7 reduction in batch assay

To compare the reaction kinetics of one-stage system S0 (R0) and two-stage system S1 (R1 + R2) in this study, AO7 and SA concentrations in R0, R1 and R2 at different time during one batch cycle on day 74 were measured. The obtained data were analyzed using Equations 1 and 2, resulting in the fitting curves shown in Figure 3. First-order kinetic models which well described the biological reduction process of azo dyes were used in this study (Koupaie et al., 2013; Yang et al., 2016). The calculated AO7 removal rate constants (k_{AO7}) and SA formation rate constants (k_{SA}) are shown in Table 2.

Table 2. Pseudo first-order rate constants of AO7 removal (k_{AO7}) and SA formation (k_{SA}) during the stabilization period (day 74) of systems S0 (R0) and S1 (R1, R2).

Operational stage	R0		R1		R2	
	k_{AO7} (h^{-1})	k_{SA} (h^{-1})	k_{AO7} (h^{-1})	k_{SA} (h^{-1})	k_{AO7} (h^{-1})	k_{SA} (h^{-1})
day 74	0.188	0.285	0.172	0.194	0.503	0.493

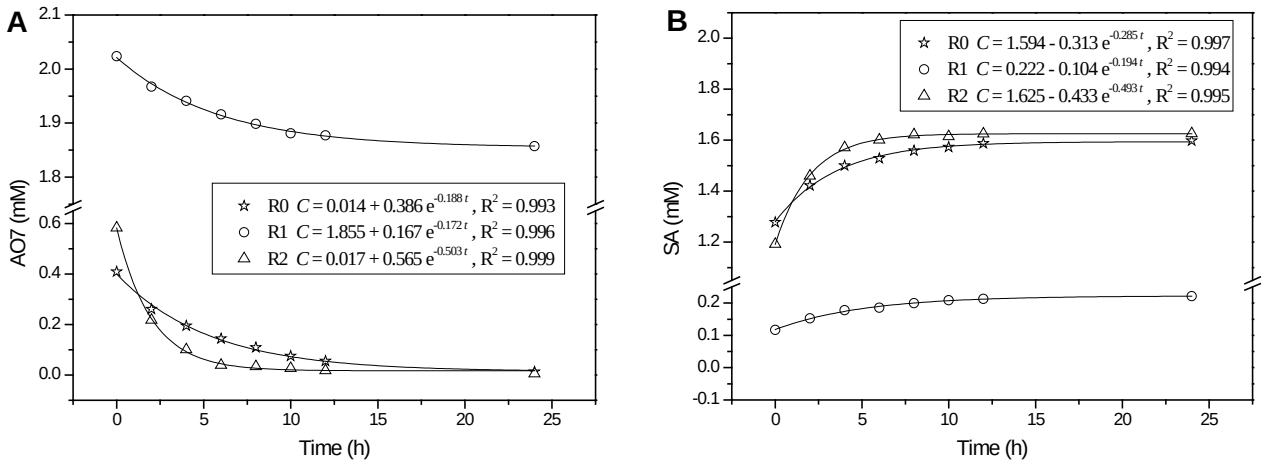

Figure 3. AO7 (A) and SA (B) concentrations at different time, and first-order kinetic fitting curves in one batch cycle of one-stage system S0 (R0, control) and one-stage system S1 (R1, R2).

Figure 3A shows that AO7 was completely removed within 6 h in the methanogenic phase (R2) of S1, while that in S0 (R0) need 12 h. Figure 3B shows that SA was simultaneously formed during the batch assays. k_{AO7} and k_{SA} from R2 were 2.7-fold and 1.7-fold of those from R0, respectively (Table 1). The above kinetic analysis from batch assays indicated that two-stage anaerobic system S1 was more efficient than S0, suggesting the benefit of phase separation to the reductive AO7 removal process. However, the longer HRT (5 d) used in this study have hidden this advantage, resulting in comparable AO7 removal efficiencies during the continuous operation as described in Figure 1. Under suitable operating conditions such as HRT and pH, two-stage anaerobic systems have been previously reported to be more efficient in pollutants removal and bio-energy production

than those in one-stage systems (Boonsawang et al., 2015). If the applied HRT was shortened, the beneficial effect of phase separation on AO7 removal during the long operation period could also be emerged. The shortened HRT would decrease the reactor size and save the cost for textile wastewater treatment (Aslanzadeh et al., 2014).

As mentioned to the AO7 reduction process within the acidogenic phase (R1) of S1, it was noticed that AO7 concentration was decreased from 2.02 mM to 1.86 mM during the batch assay, and k_{AO7} and k_{SA} were 0.172 and 0.194, respectively. Therefore, AO7 decolourization in this study was mainly finished in the methanogenic phase (R2). The result of batch assay was in consistent with the performance of continuous operation, but was in contrast with the previous publication (Dos Santos et al., 2006). The possible reasons of these results have been hypothesised in the above section, and the related mechanisms in respected to electron donor conversion will be discussed below.

VFAs accumulation in continuous operation

Soluble starch (1.0 g L^{-1}) was used as a primary electron donor in this study. Under anaerobic condition, starch can be hydrolyzed, fermented to VFAs, and then sank for AO7 reduction, methanation and biomass yield. To elucidate the electron donor conversion in this study, VFAs (the most important intermediates) in the effluent of controlled one-stage system S0 (R0) and two-stage system S1 (R1 + R2) during the entire operational period are shown in Figure 4.

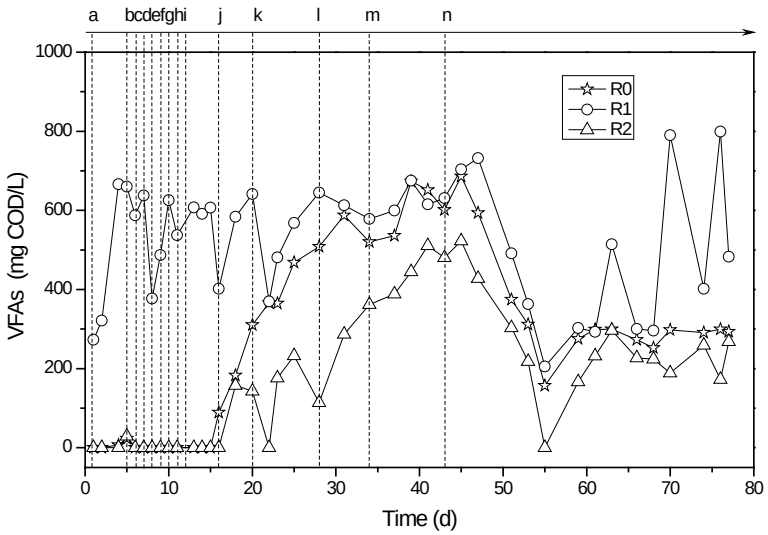

Figure 4. Effluent VFAs concentrations in system S0 (R0, control) and S1 (R1, R2) during the entire operational period.

In the initial accumulation period (days 1 to 15), influent AO7 concentrations were at a lower level (< 0.86 mM, 300 mg L^{-1}), and no VFAs was accumulated in the effluent of both systems. After then influent AO7 gradually increased to the ceiling of 2.57 mM, along with the increased VFAs in the final effluent of both systems. On day 45, the final effluent VFAs from S0 (R0) and S1 (R2) achieved to the maximum concentrations of 685 and 522 mg COD L^{-1}, respectively. Afterwards, influent AO7 resumed to 2.14 mM until the end of operational period (day 77), accordingly a decreased and relative stable VFAs accumulation for both systems were observed (150~300 mg COD L^{-1}). Therefore, the electron shift from the donor VFAs to the acceptor AO7 hardly contributed to the variation of effluent VFAs. This could also be reasonably explained that the stoichiometric requirement of the maximum 2.57 mM AO7 reduction in this study was only 82 mg COD L^{-1}, less than 10% of the total addition. Thus the VFAs accumulation was mainly attributed to the balance of hydrolysis-acidification and methanation processes. It was observed that

the acidogenic phase (R1) in S1 was always in relative higher VFAs accumulation; even in the initial stage, the VFA concentration have appeared nearly 600 mg COD L^{-1}. Therefore, the slow AO7 reduction in R1 was not due to the lack of electron donor; and the higher influent AO7 concentration would inhibit the methanogenic process to some extents but weakly to the acidogenic process. Thus, the consumption of the fermented VFAs by methanogenic microorganisms will decline along with the increase of influent AO7 concentrations, resulting in an increased VFAs accumulation in the effluent. This finding was in consistent with a previous study indicating that certain azo dye concentration (above 200 mg L^{-1}) would inhibit the methanogenic processes (Işik & Sponza, 2005); as a consequent, the accumulated VFAs was observed proportionally at a higher azo dye concentration.

Overall, it further found that the final effluent VFAs of S0 (R0) was significantly higher than those of S1 (R2), especially during the middle stage (days 20 to 50) of the operating period ($P < 0.05$). The separation of acidogenic and methanogenic phases in two-stage anaerobic system created a balanced condition for both metabolisms of acidogenic and methanogenic process (Ráduly et al., 2016; Saddoud & Sayadi, 2007). As a result, VFAs was favourably produced from starch fermentation in R1, and then efficiently converted to methane in R2, leading to a relative lower VFAs accumulation in the final effluent of S1.

Starch is one of the polysaccharides with higher molecular, itself can not be easily utilized as an efficient electron donor for azo dye removal (Albuquerque et al., 2005). When starch is employed as a sole electron donor, it should be first chemically or biologically hydrolyzed to reducing species such as glucose which can then be easily fermented to C_2~C_5 VFAs, including acetate, propionate, butyrate and valerate. Among which, C_3~C_5 VFAs could further acetified to intermediate H_2 and acetate, and then act as substrates for methanogenic process and/or served as direct electron donors for azo dye reduction (Manu and Chaudhari, 2002). This complex process might be the reason of long HRT (5 – 10 d) used in semi-continuous reactors with starch (1.0 g L^{-1}) as an electron donor in treating cotton dyeing wastewater (Manu and Chaudhari, 2003). The relative lower HRT (1 d) with tapioca starch as an electron donor only resulted in less than 60% removal of azo dye, much lower than that in this study and the above report with higher HRT (Chinwetkitvanich et al., 2000). Thus the microorganisms in R1 mainly participated in the hydrolysis-acidification process, and the operating conditions such as weak acid pH (5.5 to 6.5) was not suitable for AO7 removal in this study. Whereas in R2, effluent from R1 containing readily available electron donors was fed as influent, and it was operated in neutral condition (pH 6.5 to 7.0) in which AO7 was relative easier to be reduced than that in R1. This could be the explanation of the contrast result with ethanol as a primary electron donor, in which acidogenic phase was considered as the main contribution to azo dye removal (da Silva et al., 2013; Dos Santos et al., 2006).

CONCLUSIONS

During the continuous operation, the beneficial effect of phase separation in two-stage anaerobic system only emerged in the period with higher influent AO7 concentrations (> 2.14mM); while no statistical differences between two systems were observed in the entire operational period. However, k_{AO7} and k_{SA} in two-stage anaerobic system were 2.7-fold and 1.7-fold higher than those in one-stage system, indicating the potential benefit of phase separation to AO7 reduction. The longer HRT (5 d) and sufficient electron donor supply (1.0 g starch L^{-1}) could submerge the advantage of phase separation in AO7 reduction during the continuous operation. Within two-stage system, methanogenic phase accounted for about 90% of the entire AO7 removal, and the obtained k_{AO7} was 2.93-fold of that in the acidogenic phase. Methanogenic phase rather than acidogenic phase was the main contribution to AO7 removal. Within two-stage anaerobic system, effluent from acidogenic phase containing readily available electron donors was fed as the influent of methanogenic phase, in which AO7 was preferred to be reduced.

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