Evaluation of molasses concentration and anoxic-aerobic react period in biodecolorization and mineralization of tartrazine (acid yellow 23)

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Abstract. Dyes are widely used in the textile industry and 10–15% of these dyes are lost to effluent during the dyeing process. Dye reduction usually requires anaerobic or anoxic conditions, whereas bacterial biodegradation of aromatic amines is an exclusively aerobic process. Despite of its feasibility, increase efficiency of anaerobic color removal requires fast reductive processes with electron donor (organic co-substrate) which is usually the drawbacks of conventional biological process. The present study focuses on the evaluation of molasses concentration and anoxic-aerobic react period in biodecolorization and mineralization of tartrazine under integrated anoxic-aerobic react sequencing batch reactor (IAASBR). The IAASBR system was operated under anoxic-aerobic condition with 24 hours per cycle. Different molasses loading (0.5 g/L; 1.0 g/L) and anoxic/aerobic react periods (17/4 hours; 12.5/8.5 hours) were adopted, and their effects on microorganism growth, tartrazine decolorization and COD removal were determined. Removal efficiency of tartrazine dye and COD increased to around 50–70% and up to 95%, respectively, after the molasses concentration doubled from 0.5 g/L to 1.0 g/L. The MLVSS also increased from 3660 mg/L to 7700 mg/L. The presence of molasses promote the growth of biomass in the IAASBR system and improve the treatment efficiency of IAASBR in biodecolorization and mineralization of tartrazine. In addition, shorter anoxic react (12.5/8.5 hours anoxic/aerobic) exhibited higher COD reduction (up to 94.00 mg COD/L.hr) compared to 71.93 mg COD/L.hr for 17/4 hours anoxic/aerobic period. However, for biodecolorization of tartrazine dye, influence of the anoxic-aerobic react duration was insignificant but more consistent tartrazine removal was observed under 12.5/8.5 hours anoxic/aerobic period.

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1 Introduction

Water pollution due to improperly discharge of dye into environment from the effluents of dye utilizing industries has getting more serious concerns. More than 100 000 of different dyes exist in the world with the annual production of 700 000 tons has been reported [1]. Samanta et al. [2] found that around 280 000 tons of textile dyes are being released to nature annually, and azo dye was reported the largest group of synthetic dyes discharged from industrial effluent.

Azo dye reduction usually requires anaerobic/anoxic conditions, whereas bacterial biodegradation of aromatic amines is an almost exclusively aerobic process [3-6]. Most of the azo linkage cleavage occurs during the active bacterial growth [7]. In fact, some aromatic intermediates from the cleavage of azo linkages are found to be recalcitrant and resistant to further mineralization [8]. Bonakdarpour et al. [9] and da Silva et al. [10] found that biodecolorization of reactive black 5 up to 88% could be achieved by sequential anaerobic–aerobic sequencing batch reactor. For the same reactor configuration, 100% biodecolorization of Basic Red 46 [11] and 83% biodecolorization of Remazol Brilliant Violet 5R [12] were recorded.

Under anaerobic condition, bacterial growth is slow, so it is important to provide co-substrate such as carbon and nitrogen source to stimulate the reduction process and to maintain the vitality of the microorganisms. Garg et al. [13] explained that biodegradation of dyes is deemed to be difficult without the exogenous supplementation of carbon source. Ong et al. [7] found that addition of co-substrate apparently stimulates the reduction cleavage of azo bond. Bandary et al. [14] stated that glucose as primary substrate seemed to be a good co-substrate for *E.coli* while performing decolourization purpose under the presence of methylene blue (83.25 %) and methyl orange (84.24 %). Simple co-substrate like glucose, starch, acetate, yeast extract, molasses, fructose and a combination of complex organic source such as tapioca is an alternate growth substrate which can enhance the degradation of some pollutants that cannot promote microbial growth alone. Imran et al. [15] also reported that yeast extract as co-substrate will facilitate degradation of azo dye.

The aim of this study is to evaluate the performance of integrated anoxic-aerobic react sequencing batch reactor with respect to biodecolorization of tartrazine and COD removal with the presence of co-substrate. Factors of influencing such as different concentrations of co-substrate and anoxic-aerobic react period were also investigated on the biodecolorization and mineralization of tartrazine.

2 Methodology

2.1 Experimental set up and presence/absence of carbon source in activated sludge

A laboratory scale integrated anoxic-aerobic react sequencing batch reactor (IAASBR), with working volume of 3.5 L was set up. The acclimatization was operated in 24 hours cycle period comprised of 17/4 hours anoxic/aerobic react period for five weeks. Firstly, the activated sludge in the IAASBR acclimatized to base mix (ammonium chloride, magnesium sulphate, dipotassium hydrogen phosphate, iron (III) chloride, sodium bicarbonate) and carbon source (bacto-peptone and sucrose) for two weeks (Phase 1). Then, followed by second acclimatization phase where the activated sludge acclimatized to the tartrazine dye for three weeks (Phase 2). In phase 2, tartrazine dye was added to the system gradually (with 10 mg/L increment for every 3 to 4 days) until 50 mg/L to replace the original carbon source. Throughout the acclimatization phase 1 and phase 2, sludge volume index (SVI), mixed

liquor suspended solid (MLSS) and mixed liquor volatile suspended solid (MLVSS) of the system were observed to ensure the proper growth of microorganism.

2.2 Experimental design and treatment performance monitoring

After the acclimatization of activated sludge to tartrazine dye completed, the IAASBR system was used to study the effect of molasses dosage on biodecolorization and mineralization of tartrazine. Next, investigation on anoxic-aerobic react period on biodecolorization and mineralization of tartrazine with the presence of molasses was also conducted. The operational condition for the IAASBR system during the experimental period of 45 days (10 Jan – 21 Feb), defined as Set 1-3, are summarized in Table 1.

Table 1: Operational Condition

Set	Tartrazine Concentration (mg/L)	Molasses Concentration (g/L)	REACT Phase Duration (hours)	
			Anoxic	Aerobic
Set 1	50	0.5	17	4
Set 2	50	1.0	17	4
Set 3	50	1.0	12.5	8.5

For all the IAASBR operational conditions under studied, samples of effluent at the end of anoxic react and at the end of IAASBR cycle were collected and analysed daily except for weekend and public holiday. In addition, about a week duration was given to the system to adapt and stabilize (no sample collection and analysis) when there was a shift in different operational condition between set 1, 2 and 3 experiment. Thus, there are some gaps in data presentation in the figures. Treatment efficiency of the IAASBR was assessed in term of COD (closed reflux, titration method (5220 C)) [16] and tartrazine removal (double beam UV-Vis spectrophotometer). Besides, SVI, MLSS and MLVSS analysis were carried out (according to standard method of 2710D, 2540D and 2540E, respectively) [16] in order to monitor the growth condition of the sludge in the IAASBR.

3 Results and discussion

3.1 Influence of carbon source and tartrazine on sludge growth

Fig. 1 and Fig. 2 illustrate the effect of carbon source and tartrazine dye on biomass concentration during the acclimatization process. MLSS and MLVSS increased over time gradually from 5820 to 7124 mg/L and 4582 to 5598 mg/L, respectively at the end of acclimatization phase 1 (Fig. 1), indicating that the microorganisms were well grown by consuming organic carbon. The MLVSS to MLSS concentration ratio was in the range of 0.74 to 0.79 throughout the acclimatization phase 1, indicating high sludge activity. In municipal wastewater treatment plants, the typical optimum MLVSS/MLSS ratio is often believed to be about 0.75 [17].

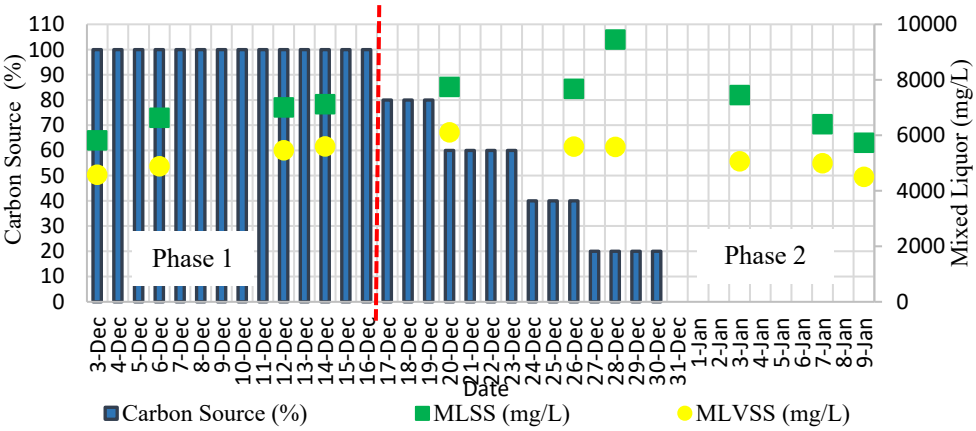

Fig. 1: Effect of carbon source on biomass growth in acclimatization phase

In acclimatization phase 2, tartrazine dye was added gradually until 50 mg/L to replace bactopeptone and sucrose as carbon source. In the absence of external carbon source together with the toxicity from the tartrazine dye, the MLVSS reduced gradually from 5584 to 4504 mg/L (Fig. 2). The result evidences the important of carbon source in the growth and survival of microorganism. Nonetheless, the MLVSS/MLSS ratio at the end of acclimatization was 0.79, indicates high percentage of viable sludge in the IAASBR system. Throughout the acclimatization process, the SVI value was below 80 mL/g. Seong [18] reported that sludge with good settling and compaction characteristics should have SVI of less than 100 mL/g.

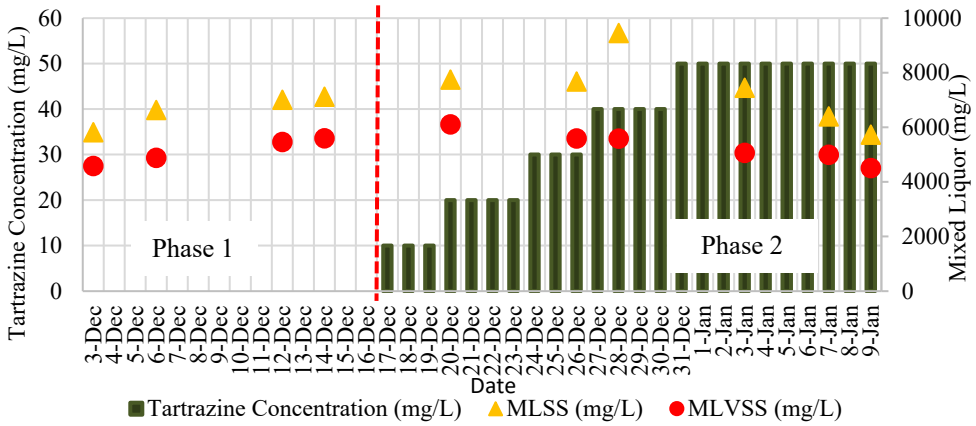

Fig. 2: Effect of tartrazine dye on biomass growth in acclimatization phase

Fig. 3 exhibits the growth of sludge at different molasses concentrations (between Set 1 and 2) and at different anoxic-aerobic react periods (between Set 2 and 3). At low molasses concentration of 0.5 g/L, a noticeable drop of MLVSS from 4504 to 3660 mg/L was observed (Set 1), leading to the low MLVSS/MLSS ratio of 0.57. When molasses concentration doubled to 1.0 g/L, MLVSS increased gradually regardless of anoxic-aerobic react period and stabilized at around 7000 - 7700 mg/L (Set 3). The results evidence the presence of molasses promotes the growth of biomass and to maintain vitality of microorganism in the sludge system.

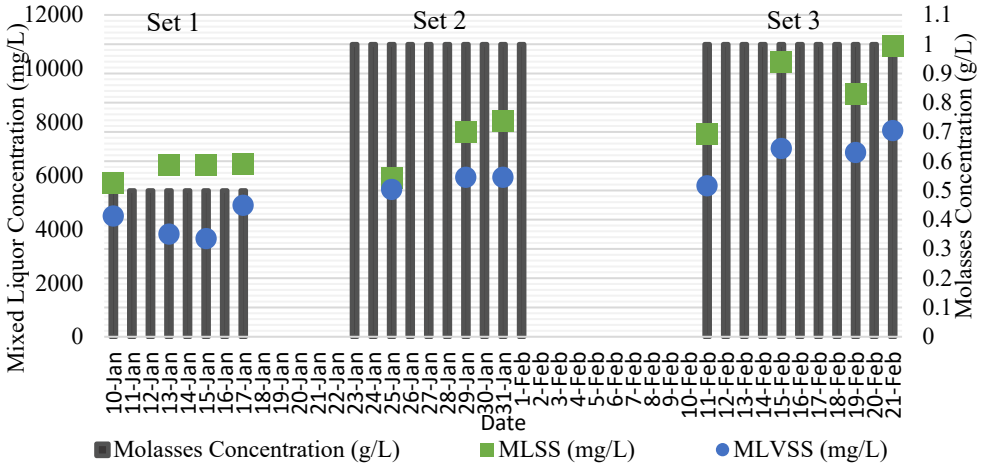

Fig. 3: Variation of MLSS and MLVSS in Set 1, Set2 and Set 3 experiment

3.2 Effect of molasses concentration on biodecolorization and mineralization of tartrazine

As co-substrate provides carbon and energy for organism growth and acts as electron donor that essential for cleavage of azo linkage, Set 1 and Set 2 experiments were conducted to investigate the effect of molasses (co-substrate) concentration on the reduction rate of COD and tartrazine in the IAASBR system. Two different molasses concentration were tested; 0.5 g/L and 1.0 g/L. Both COD and tartrazine removal efficiency were determined at the end of anoxic react and at the end of cycle (Fig. 4 and 5).

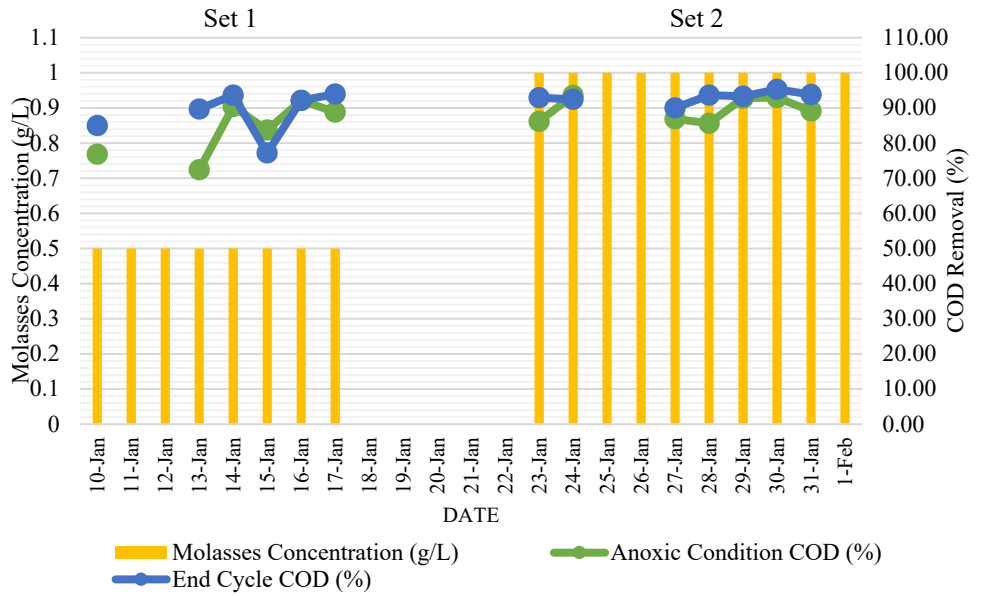

Fig. 4: Effect of molasses concentration on COD removal

As illustrated in Fig. 4, with molasses concentration of 0.5 g/L, the COD removal at the end of each cycle were slightly increased from 85% to 93.97%, with an average COD removal efficiency of around 88.59% over 8 days from 10 Jan to 17 Jan. When the molasses loading increased to 1.0 g/L, the COD removal increased to 93.06% averagely (from 23 Jan to 1 Feb) with the highest detected at 95.29%. These results showed that the COD can be almost completely removed with the presence of co-substrate 1.0 g/L molasses. In biological process, microorganism growth occurs concurrently with the oxidation of organic or inorganic compounds. The observed increased of MLSS and MLVSS in Fig.3 (Set 2) supported the explanation. Besides, it was also observed that COD reduction was predominantly occurred during the anoxic phase of the react mode (Fig. 4).

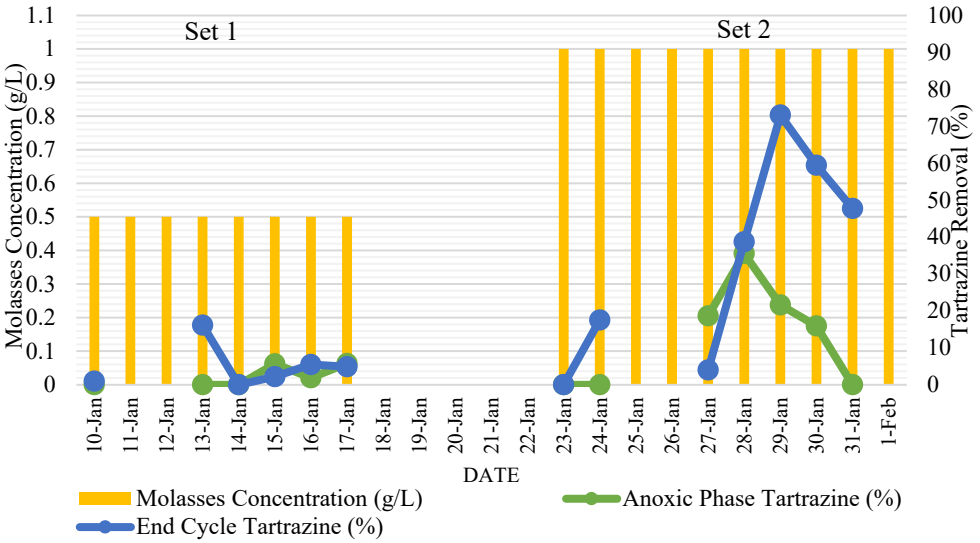

Fig. 5: Effect of molasses concentration on tartrazine removal

No significant removal of tartrazine was observed with 0.5 g/L molasses (Fig. 5), neither at the end of anoxic react nor end of cycle. Only about 4.88% of tartrazine decolorization was obtained even after 8 days (10 Jan to 17 Jan) of the operation. Noticeable tartrazine decolorization was only noted after molasses loading increased to 1.0 g/L (Set 2) for about a week (23 Jan to 27 Jan). The observed removal efficiency of tartrazine at the end of anoxic react and end of cycle was unstable and fluctuated around 5 – 20% and 50 – 70% (29 Jan to 31 Jan), respectively. The results also revealed that assimilation of tartrazine was mainly occurred during the aerobic phase of the react mode. As molasses is a more readily biodegradable and less toxic carbon source, hence it was assimilated first during the anoxic react phase (evidenced by COD removal in anoxic react phase) then only followed by decolorization and mineralization of tartrazine in aerobic react phase. The increase in molasses concentration has resulted in an increase of electron donors in IAASBR, which are required in the reduction cleavage of azo bond. A similar result was also reported by Ong et al. [7], who found that increased concentration of co-substrate enhanced Orange II decolorization and concluded that reduction of azo dyes took place when reducing environment prevailing in the reactor. In short, IAASBR system required a co-substrate at certain concentration to preserve the highly reducing environment in order to promote the biodecolorization capability.

3.3 Effect of anoxic-aerobic react period on biodecolorization and mineralization of tartrazine

In order to determine the optimum anoxic-aerobic react time for tartrazine dye and COD removal, experiment Set 3 was conducted by doubling the aerobic react phase to 8.5 hours and reducing the anoxic react phase from 17 to 12.5 hours while maintaining the concentration of tartrazine and molasses at 50 mg/L and 1.0 g/L, respectively, in synthetic wastewater. Fig. 6 and 7 illustrate the different anoxic-aerobic react period on COD removal and biodecolorization of tartrazine, respectively.

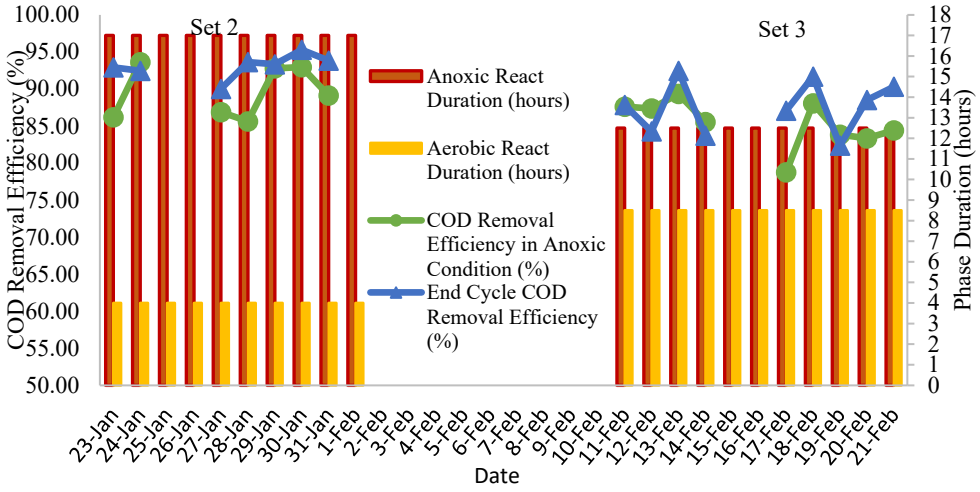

Fig. 6: Effect of anoxic-aerobic react phase on COD removal

As illustrated in Fig.6, the end of anoxic react COD removal efficiency achieved in Set 2 were fluctuated between 85.63% and 92.94% (23 Jan – 1 Feb), and for Set 3 between 78.74% and 89.32% (11 Feb – 21 Feb). In term of equivalent COD reduction rate in anoxic react phase, the COD was removed at the rate ranged from 66.27 to 71.93 mg COD/L.hr in Set 2 and at the rate ranged from 82.87 to 94.00 mg COD/L.hr in Set 3. The results show that to achieve COD removal efficiency of roughly 80% at the end of anoxic phase, shorter anoxic react period (Set 3) is sufficient to achieve similar COD reduction. Therefore, it can be concluded that extended anoxic react time did not improve the performance of the IAASBR in terms of COD removal. This finding is not in agreement with others researcher where in general, a better COD and dye removal could be achieved with extended anaerobic or anoxic phase [4, 11, 19, 20]. Al-Amrani et al. [4] also revealed that an anoxic-aerobic reaction ratio of 3:1 (16 hour: 4 hours) is the best strategy in degrading Acid Orange 7(AO7)-containing wastewater with respect to COD removal and biodecolourization.

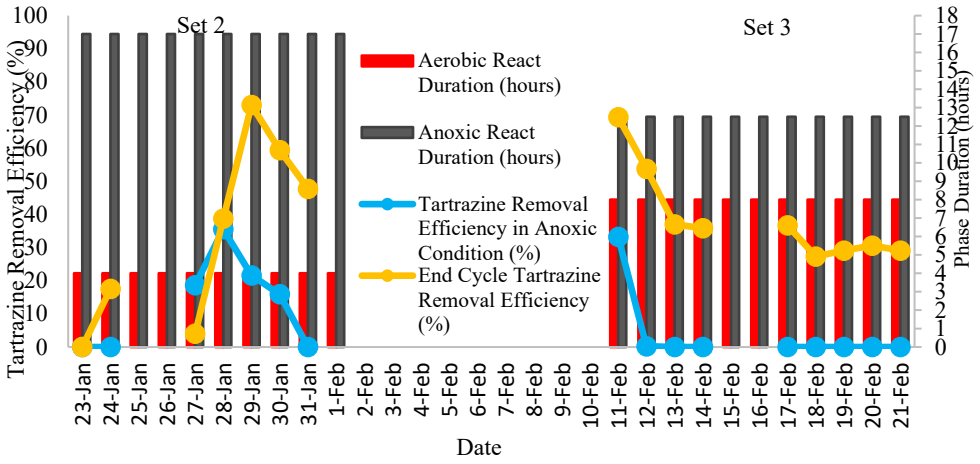

Fig. 7: Effect of anoxic-aerobic react phase on tartrazine decolorization

Fig. 7, experiment Set 3 exhibits the biodegradation of tartrazine was mostly occurred in aerobic react phase and was negligible at anoxic react phase. For anoxic/aerobic react period of 12.5/8.5 hr (Fig. 7, Set 3), tartrazine removal efficiency reduced gradually from 69.33% to 37.00 % at the early stage (11 Feb – 13 Feb) and maintained within the range of 27.29% to 30.59% after that, where the steady state condition had achieved (18 Feb – 21 Feb). Extended aerobic react duration did not show any progress with respect to tartrazine decolorization, except more consistent tartrazine removal efficiency was observed in Set 3. So, it can be concluded that longer aerobic react phase with shorter anoxic react phase was able to provide a more stable removal of tartrazine dye in this operation system.

Several studies have reported that azo dyes were decolorized under anaerobic condition and mineralized under aerobic condition [11, 21, 22]. However, in the present study, the main tartrazine decolorization phase in this operation system was aerobic phase and the contribution of anoxic phase to tartrazine removal was basically negligible (Fig. 7, Set 3). The reason for that can be explained by the easily biodegraded molasses was first degraded by microorganism in the anoxic condition, then followed by tartrazine dyes as carbon and energy source, and resulted in tartrazine decolorization in aerobic phase. The explanation further supported by COD reduction predominantly occurred in anoxic phase (Fig. 6). Tartrazine dye and base-mix contribute only 7-9% from the total influent COD of 1315.56 mg/L in the synthetic wastewater, means large amount of COD that being removed in anoxic react phase was mainly contributed by molasses.

4 Conclusion

An integrated anoxic-aerobic react IAASBR activated sludge system was successfully set up to evaluate biodecolorization and mineralization of tartrazine dye with the presence of molasses. COD removal efficiency of average 93.06% was achieved with molasses concentration of 1.0 g/L. In addition, removal efficiency of tartrazine dye increased from 4.88% to around 50 – 70% after the molasses concentration doubled from 0.5 g/L to 1.0 g/L. The results proved that higher molasses concentration resulted in an increase of reducing equivalents in the system, which are necessary for the reduction of azo bond in tartrazine

dyes. The presence of molasses promote the growth of biomass and to maintain vitality of microorganism in the sludge system as evidenced in the increase of MLVSS.

As for the factor influencing of the anoxic-aerobic react period, effective removal of COD (>80%) in both 17/4 hours anoxic/aerobic react duration and 12.5/8.5 hours anoxic/aerobic react duration was observed. Comparatively, 12.5/8.5 hours anoxic/aerobic period exhibited higher COD reduction (up to 94.00 mg COD/L.hr) at the end of anoxic phase. For biodecolorization of tartrazine dyes, influence of the anoxic-aerobic react duration was insignificance but more consistent tartrazine removal was observed under 12.5/8.5 hours anoxic/aerobic duration. In both influence of molasses concentration and anoxic-aerobic react duration studies, it was noticed that COD reduction was mainly occurred during the anoxic phase while biodegradation of tartrazine was in aerobic phase.

In summary, increased molasses concentration was able to promote the growth of microorganism in IAASBR and thus enhanced the COD and tartrazine removal capability. Besides, the 12.5/8.5 hours anoxic/aerobic react duration ensures a more stable biodegradation of tartrazine as compared to 17/ 4 hours anoxic/aerobic react duration.

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