TREATABILITY OF AZO DYES IN ANAEROBIC/AEROBIC SEQUENTIAL PROCESSES

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by
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İZMİR

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ABSTRACT

In the framework of this Ph. D. thesis, the removal of azo dyes, which produced banned carcinogenic aromatic amines, was studied using batch anaerobic and continuous fed Upflow Anaerobic Sludge Blanket (UASB)/ Continuous Stirred Tank Reactor (CSTR) sequential reactor systems in synthetic, simulated and real textile wastewaters. Reactive Black 5, Direct Yellow 12, which are used in Izmir region, and Direct Brown 2, Direct Red 28 and Direct Black 38 which are banned azo dyes containing benzidine were degraded and decolorized rapidly at concentrations varying between 200 and 3200 mg/l. Meanwhile, the aromatic amines produced from the cleavage of azo bonds were accumulated in batch anaerobic conditions. The decolorization, and glucose-COD removals exhibited first order kinetics. The effect of operational conditions such as organic and dye loadings, hydraulic retention time (HRT), food to mass (F/M) ratio on the substrate removal rates, color removal efficiencies and methane gas productions was investigated in anaerobic (UASB)/aerobic (CSTR) sequential system using RB 5, DR 28 azo dyes, and DB 38 at a concentration varying between 0 and 3200 mg/l. It was found that HRT was more effective than the dye loading rates applied in UASB reactor for decolorization. The aromatics amines were effectively removed in CSTR reactor. Furthermore, the effect of co-substrate concentration (glucose) and alkalinity levels on the color removal of azo dyes was investigated. It was found the color of azo dyes was successively removed even if at lower substrate and alkalinity levels. The treatment of wool dyeing wastewater and a real wastewater taken from a Cotton Textile Mill were investigated in the same system. Color removal efficiencies varying between 80-91% and 40-77% were obtained in wool dyeing and cotton textile mill wastewaters, respectively, while the COD removal efficiencies were 83-96% and 39-82% in whole system. In order to obtain the kinetic coefficients, the UASB reactor was operated with simulated wastewater containing desizing agents, dye, salts etc. at

six different HRTs. Stover-Kincannon and Grau second order substrate removal kinetics were found to be the more appropriate models in lab scale UASB reactor treating simulated textile wastewater. Finally, synthetic wastewater containing DB 38 and DR 28 azo dye of 3200 mg/l, was decolorized using the anaerobic/aerobic sequential reactor system in order to monitor the toxicity and the intermediate products through decolorization of DB 38, and DR 28 azo dyes. Azo dyes were mineralized and the toxicity was decreased under anaerobic/aerobic conditions. HPLC-DAD and GC-MS analysis showed that the azo dyes could be mineralized using UASB/CSTR sequential system.

ÖZET

Bu doktora tezi kapsamında sentetik, simule ve gerçek tekstil atıksuları içinde bulunan yasaklanmış aromatik aminlerden oluşan azo boyarmaddeleri, kesikli anaerobik ve sürekli beslemeli anaerobik yukarı akışlı çamur yatak (YAÇYR)/ sürekli karıştırmalı aerobik reactör (SKTR) ile giderimi araştırılmıştır. Reaktif Siyah 5 (RS 5), Direkt Sarı 12 (DS 12), Direkt Kırmızı 28 (DK 28), Direkt Siyah 38 8 (DS 38), and Direkt Kahverengi 2 (DK 2) boyarmaddeleri İzmir'de kullanılan ve son üç tanesi benzidin içeren yasaklı azo boyarmaddeleridir. 200-3200 mg/l derişimlerde çalışılan azo boyarmaddeleri parçalanıp, hızlı bir şekilde rengi giderilirken, azo bağının kırılması ile oluşan aromatik aminler kesikli anaerobik koşullarda birikmiştir. Renk ve glikoz-KOİ giderim hızı birinci derece kinetiğe uymuştur. 0 ile 4000 mg/l arası derişimlerde RS 5, DK 28 and DS 38 boyarmaddelerinin anaerobik (YAÇYR)/aerobik (SKTR) sistemle arıtımında, substrat giderim hızı, renk giderim verimleri ve metan gazı üretimi üzerinde organik yükleme ve boyarmadde yükleme, hidrolik bekleme süresi (HBS) ve besi/mikroorganizma (F/M) oranları gibi işletme parametrelerinin etkisi araştırılmıştır. YAÇY reaktöründe HBS'nin uygulanan boyarmadde yüklemelerinden daha etkili olduğu bulunmuştur. SKTR ile aromatik aminler etkin bir şekilde giderilmiştir. Renk giderimi üzerinde yardımcı subsrat (glikoz) ve alkalinite derişimlerinin etkisi araştırılmıştır. Düşük substrat ve alkalinite seviyelerinde de rengin başarılı bir şekilde giderilebileceği saptanmıştır. Yün boyama atıksuyu ve pamuklu tekstil fabrikasının gerçek atıksularının arıtımı aynı sistemde araştırılmıştır. Renk giderim verimleri yün boyama ve tekstil fabrikası atıksuları için sırasıyla %80-91 ve %40-77 aralıklarında elde edilirken, KOİ giderim verimleri %83-96 ve %39-82 arasında elde edilmiştir. Kinetik katsayıları elde etmek için YAÇY reaktör haşıl maddesi, boyarmadde, tuz v.s içeren simule atıksuyu ile altı farklı HBS'de işletilmiştir. Stover-Kincannon ve Grau ikinci derece substrat giderim kinetik modelleri simule tekstil atıksuyunu arıtan sistem için daha uygun

bulunmuştur. Son olarak, arıtım süresince toksisite ve oluşan ara ürünleri izlemek için 3200 mg/l DR 28 ve DB 38 azo boyarmaddeleri içeren sentetik atıksu anaerobik/aerobik sistemle arıtılmıştır. Anaerobik/aerobik koşullar altında azo boyarmaddeleri mineralize edilmiş ve toksisite azaltılmıştır. HPLC-DAD and GC-MS analizleri YAÇYR/SKTR ardışık sistemle azo boyarmaddelerinin mineralize edilebileceğini göstermiştir.

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CHAPTER ONE
INTRODUCTION

1.1. Introduction

Azo colorants are substances that have a coloring effect containing one or more azo groups (N=N double bonds) in their chemical structure. They are synthesized by coupling one or more diazotized aromatic amines with other aromatic compounds (Geisberger, 1997). They are largest group of synthetic colorants (60-70%) and can be used to color a large number of different substrates, such as synthetic and natural textile fibres, plastics, leather, paper, mineral oils, waxes, and even (with selected types) foodstuffs and cosmetics (Carliell et al., 1995). The color of azo dyes is due to azo bonds, the associated auxochromes and a system of conjugated double bonds. Azo dyes have become of concern in wastewater treatment because of following reasons;

- The color from the azo dye is unpleasant aesthetically. A very small amount of azo dye in water (10-50 mg/l) is highly visible (Wong &Yu., 1999),
- Azo dye reduces transparency of receiving water, the organisms in water are damaged,
- Aromatic amines that occurred from reducing of azo dyes in the sediment have toxic effect on the organisms,
- Azo dyes and its products may inhibit conventional biological treatment systems,
- Azo dye affects gas solubility of water bodies

A necessary criterion for the use of these dyes is that they must be highly stable in light and during washing. They must also be resistant to microbial attack. Therefore, they are not readily degradable under natural conditions and are typically not

removed from wastewater by conventional wastewater-treatment system (Pagga & Brown, 1986). They also exhibit structural variety and so as a group, they are not uniformly susceptible to microbial attack. As with other aromatic pollutants, microorganisms have only limited capacity to degrade azo dyes under natural conditions (Rajaguru et al., 2000).

In the microbial degradation of azo dyes, the initial process is their decolorization. The highly electrophilic azo bond must be cleaved for azo decolorization to take place. Under aerobic conditions neither the activated sludge (Shaul et al., 1991) nor aerobic bacterial isolates were able to degrade azo dyes. On the other hand, various azo dyes were shown to be decolorized by anaerobic sludge (Brown &Labeour, 1983; Brown & Hamburger, 1987), anaerobic sediments (Weber and Wolfe, 1987) and by pure cultures of bacteria (Haug et al., 1991; Chung & Stevens, 1993).

The addition to biological treatment, physical and chemical methods are also used for removal of colored dye substances in wastewater. In fact, the latter two have been more widely adopted for their effectiveness. Chemical methods often involve coagulation of dye substance followed by precipitation of the chemical sludge or oxidation process using ozone, Physical methods involve mainly adsorption by activated carbon or its alike (Robinson, 2001). In hybrid physical/chemical or physical/biological processes, ionizing irradiation and ultrafiltration are useful methods for pretreatment. Nevertheless, both physical and chemical methods have their shortcomings. Coagulation produces excess amount of chemical sludge and creates problem of its disposal. Oxidation employs costly ozone and is not effective for reductive or sulphur dye wastewater. Activated carbon also incurs high operating expenses and additional capital on activity regeneration. These are obvious opportunities and challenges for biological treatment method to offer viable alternatives in treating dye-containing wastewater (Mou, 1991). So far, the treatment of textile wastewater containing azo dyes has been based mainly on aerobic biological processes. The most commonly employed processes are conventional and extended activated sludge systems. The oxygen consumption and sludge yield are generally high and implement high operational costs. Moreover the aerobic processes

have been insufficient to degrade most azo dyes. Anaerobic processes have been investigated for the treatment of some toxic wastewater. However, direct application of anaerobic processes in textile wastewaters treatment has been limited. Previous studies have demonstrated that, under anaerobic conditions, azo dyes are easily converted to aromatic amines. However these aromatic amines resist and even inhibit further anaerobic degradation (Brown & Hamburger, 1987; Field et al., 1995; Chung and Stevens, 1993). Most such end products are further degraded by aerobes (Weber &Wolfe, 1987; Ekici et al., 2001). Some aromatic amines have been shown or suspected to be carcinogenic and therefore need to be degraded further before discharge to natural water bodies (Kuai et al., 1998). Recently researchers reported the extensive utilization of the sequential anaerobic/aerobic processes to treat the simulated dyeing wastewater or synthetic wastewater with pure dye (Zaoyan et al., 1992; An et al., 1996; Basibuyuk & Forster, 1997; Cruz & Bultron, 2000; Kalyuzhnyi & Sklyar, 2000a; Kudlich et al.,1996; Luangdilok & Pansvad 2000; O'Neill et al., 2000a; O'Neill et al., 2000b; Panswad et al. 2001; Rajaguru et al., 2000; Tan et al., 1999; Tan et al., 2000).

However, following subjects about removal and mineralization of the azo dye are not well documented in literature.

- Effect of inhibition of azo dyes on batch anaerobic cultures.
- Influence of dye concentration, substrate concentration, alkalinity effect, hydraulic retention time (HRT), on removal and mineralization of selected azo dyes by batch anaerobic and continuous sequential anaerobic/aerobic system.
- Treatment of the real wastewater, acid wool dyeing and cotton textile wastewaters, by continuous sequential anaerobic/aerobic system.
- Monitoring of toxicity reduction and mineralization products through azo dye mineralization by continuous sequential anaerobic/aerobic system.

Therefore, this dissertation will be focused on the subjects aforementioned.

1.2. Anaerobic/Aerobic Sequential Scenario for Mineralization of Azo Dyes

The azo dye in the treatment system is not biodegraded by the micro-organisms under aerobic conditions, instead the dye acts as an oxidizing agent for the reduced flavin nucleotides of the microbial electron transport chain and is reduced and decolourised concurrently with re-oxidation of the reduced flavin nucleotides (Gingell &Walker, 1971).

Anaerobic bioremediation allows azo and other water-soluble dyes to be decolorized. This decolorization involves an oxidation-reduction reaction with hydrogen rather than free molecular oxygen in aerobic systems. Typically, anaerobic breakdown yields methane and carbondioxide (Carliell et al., 1996).

Azo dye acts as an oxidising agent for the reduced flavin nucleotides of the microbial electron chain and is reduced and decolorized concurrently with reoxidation of the reduced flavin nucleotides (Gingell &Walker, 1971). In order to occur, additional carbon is required for decolorization to proceed at a viable rate. This additional carbon is converted to methane and carbon dioxide, releasing electrons. These electrons cascade down the electron transport chain to a final electron acceptor, in this case, the azo-reactive dye. The electrons react with the dye reducing the azo bonds, and ultimately causing decolorization (Carliell et al., 1996).

Anaerobic degradation of textile dyes yields only azo reduction. Mineralization does not occur. It has been shown that azo- and nitro-components are reduced in the sediments and in the intestinal environment, resulting in the regeneration of the parent toxic amines (Banat et al., 1996; Chung & Stevens, 1993; Tan et al., 1999). Therefore, careful monitoring is required before treated wastewater is released into receiving water. A major advantage of this anaerobic system, apart from the decolorization of soluble dyes, is the production of biogas. Biogas can be reused to provide heat and power, and will reduce the energy costs.

Azo dye reduction leads to the formation of aromatic amines. Aromatic amines are generally not degraded and accumulate under anaerobic conditions (Brown & Laboureur, 1983; Field et al. 1995), with the exception of a few aromatic amines characterized by the presence of hydroxyl and/or carboxyl groups (Razo-Flores et al. 1997; Kalyuzhnyi et al., 2000). Mineralization of the aromatic amines by aerobic bacteria and aerobic sludge in treatment plants is more common and, therefore, aerobic conditions are preferable to degrade the accumulated aromatic (Brown & Hamburger 1987; Weber, E.J., & Wolfe, N.L.; Zissi & Lyberatos, 1996; Ekici et al., 2001). However, it should be noted that some aromatic amines are readily autoxidized in the presence of oxygen to humic-like oligomeric and polymeric structures (Parris 1980). Equations1.1,1.2, and Figure 1.1. shows these explainations.

$$2e^- + 2H^+ + (R\text{-}N{=}N\text{-}R^{\wedge}) \rightarrow (R\text{-}NH - NH - R^{\wedge}) \qquad (1.1)$$

$$2e^- + 2H^+ + (R\text{-}NH\text{-}NH\text{-}R^{\wedge}) \rightarrow (R\text{-}NH_2) + (NH - R^{\wedge} - NH_2) \qquad (1.2)$$

Where; R and, $R^{\wedge}$ are phenyl and naphtol roots, respectively.

The product of Equation 1.1 is colorless and unstable, and azo bond (N=N) and color may be occurred with oxidizing.

The process of anaerobic azo reduction has been intensively studied (Carliell et al., 1995; Razo-Flores et al.; 1997; Beydilli et al., 1998). Most researchers have investigated the decolorization of azo dye in the anaerobic batch cultures. In a study performed by Işık and Sponza (2003) decolorization of Congo Red and Direct Black 38 was investigated using *Escherichia coli* and *Pseudomonas* sp. cultures under anaerobic, aerobic and microaerophilic conditions. They reported that anaerobic conditions were more favorable than other conditions for decolorization. Furthermore, in some previous batch studies, the following decolorization efficiencies were obtained for C.I. Reactive Black 5 azo dye: 67% in 24 h (Nigam et al., 1996), 67 and 73% color removal in 10.5 and 79 h, respectively (Setiadi and van Loosdrecht, 1997), 79% in 10 h (Beydilli et al., 1998), 95% in 48 h (Oxspring et al.,

1996), and 66% in 24h (Ganesh et al., 1994). Beydilli et al. (1998) noted that 50 and 300 mg/l concentrations of Red 2 resulted in gas production higher than the control, whereas 500, 1000 and 2000 mg/l dye concentrations inhibited anaerobic culture activity and decreased the total gas and methane production to 67% and 57% of control culture, respectively, at the concentration of 2000 mg /l. Batch decolorization studies performed by Brás et al., (2001) demonstrated that the decolorization rate constant decreased from 0.04 1/h to 0.016 1/h while the Acid Orange concentration increased to 300 mg /l.

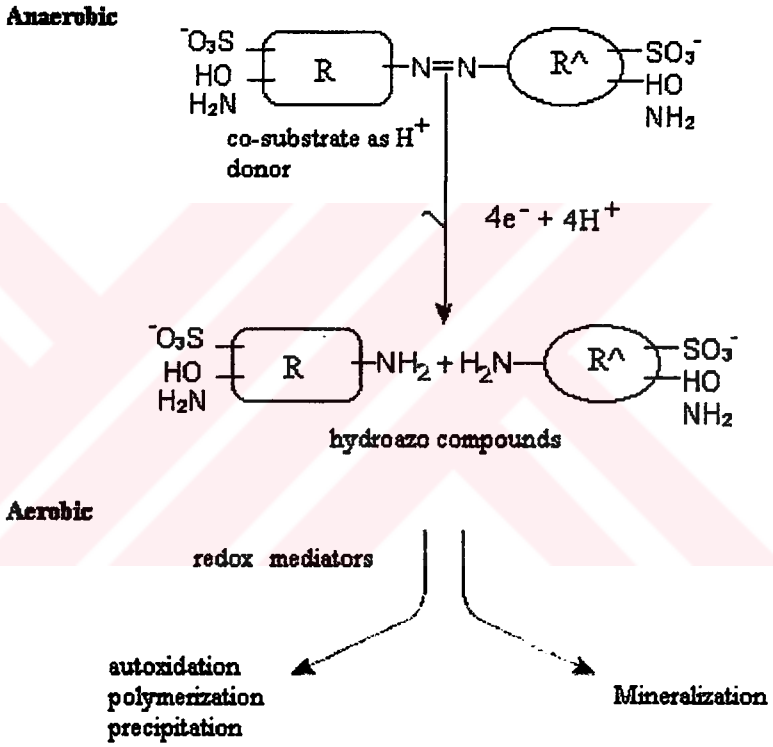


Figure 1.1. Degradation of an azo dye in anaerobic/aerobic sequential environments (Knackmuss, 1996)

Both zero order (Dubin and Wright, 1975; Brown, 1981) and first order (Kalyuzhnyi and Sklyar, 2000; Wuhrmann et al., 1980; Weber, 1991; Carliell et al., 1995; Beydilli et al., 1998) kinetics have been reported for the dye concentration through co-substrate degradation. Furthermore, the decolorization of 20 selected dyes

by granular sludge was studied by van deer Zee et al. (2001). They found that decolorization reactions were followed by first-order kinetic. 97 and 96 % decolorizations were obtained in azo dyes Direct Black 19 and Reactive Black 5, respectively. Yoo (2002) found that the decolorization of C.I. Reactive Orange 96 shows a first order kinetic (0.12 mM/min) with respect to both dye (0.1 M) and sulfide concentrations (0.01M). However, Chang et al. (2001) used the Michaelis-Menten kinetic to describe the apparent correlation between the decolorization rate and dye concentration in batch reactor containing immobilized *Pseudomonas luteola* and Reactive Red 22 dye. A first order reaction was obtained for both mesophilic and thermophilic decolorisation in azo dye Reactive Red 235 (Willetts and Ashbolt, 2000). The kinetics observed under thermophilic conditions (k= 0.0096 1/min) were three times faster than under mesophilic conditions when comparing the first order rate constants.

Toxic compounds can be tolerated by methanogens in continuous upflow anaerobic sludge-bed (UASB) reactors if they are degraded or undergo biotransformations to less toxic products. Toxic chloro-, nitro- and azo- substitutions of aromatics are subject to reductive biotransformations in anaerobic environments (Field et al. 1995).

Basibuyuk & Forster (1997) reported that the anaerobic filter converted some 80 % of incoming COD (2000 mg/l) to volatile acids and the overall process removed > 92% of the COD and 99 % of the Basic Red (200 mg/l) in the 22 h of total HRT by anaerobic/aerobic biofilter system.

For the sequential anaerobic/aerobic treatment of textile wastewaters, the process units consisted of an GAC (granule active carbon)-amended anaerobic UASB reactor and SCAS (Semi continuous activated sludge) allowed a stable performance, COD and color removal of 98 and 95%, respectively at about 2 day of HRT (Kuai et al, 1998).

Zaoyan et al. (1992) reported that the anaerobic/aerobic rotating biological contactor (RBC) system removed COD and color of 75-80 and 72-78%, respectively at total HRT varying between 11 and 13 hours.

To treat the wastewater from a dye manufacturing factory with COD concentration of 1200 mg/l and a color of 500 degree (dilution factor) in an UASB reactor (4.5 l) and an activated sludge tank (5 l), COD and color were removed more than 83 % and 90 % at HRT of 6-10 hours for the anaerobic stage and 6.5 h for the aerobic stage (An et al., 1996).

In a study performed by O'Neill et al. (2000b), The effluent from a 30 l UASB reactor at a HRT of 1 day and a temperature of 35°C, was passed to a 20 l aerobic stage which operated at HRT of 16 h. The feed was a simulated textile effluent containing 0.45 g/l hydrolyzed reactive azo dye, Procion Red H-E7B. COD and color removal of 85 and 75 %, respectively was obtained with the system.

A sequential anaerobic/aerobic treatment process was used to degrade the sulfonated azo dyes Orange G, Amido Black 10B, Direct red 4BS and Congo Red. Under anaerobic conditions the azo dyes were reduced and amines were released by bacterial biomass in a fixed bed column using glucose as co-substrate,. The amines were completely mineralized in a subsequent aerobic treatment (Rajaguru et al., 2000).

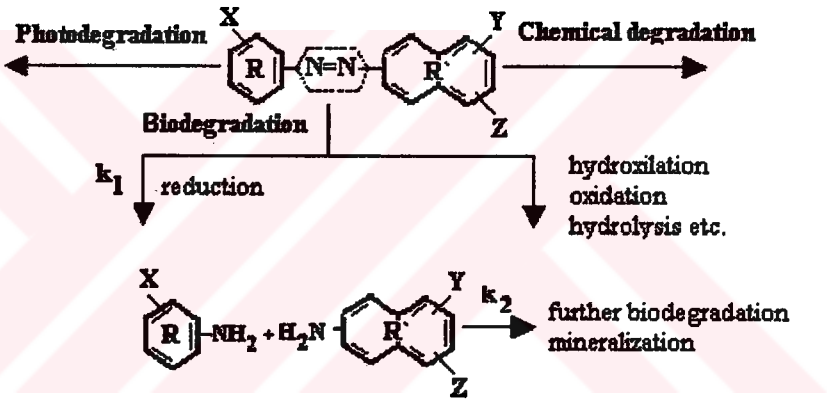
Razo Flores et al. (1997) reported that a pharmaceutical azo dye, Azodisalicylate, constructed from two 5-aminosalicylic acid (5ASA) molecules was mineralized in an acclimated methanogenic consortium to CH_4 and NH_3 with transient accumulation of 5ASA as a degradation of intermediate products in both anaerobic batch and continuous bioreactors.

1.3. Removal Processes of Azo Dyes from Wastewater

The process of the reduction of azo dyes with the cleavage of aromatic R–NH_2 amines is one of the ways of the degradation of those dyes. The others are: chemical

degradation, photodegradation and biodegradation by means of hydroxylation, oxidation, and hydrolysis as shown in Figure 1.2. However, in human organisms, it is the biological reduction of the azo dye that is responsible for the possible presence of the toxic amine in the organisms. Currently the main methods of textile dye treatment are physical and chemical means with concentrating on cheaper effective alternatives as shown in Table 1.1.

Physical and chemical methods of dye removal are effective only if the effluent volume is small. This limits the use of physio-chemical methods, such as membrane filtration and cucurbituril, which is a cyclic polymer of glycoluril and formaldehyde. and to small-scale in situ removal. A limiting factor of these methods is higher cost. This is true even in lab-scale studies, and therefore, are unable to be used these methods by in large-scale industries (Robinson et al, 2001).



where; R and, R' are phenyl and naphtol roots, respectively.
X, Y and Z are functional groups such as OH, NO_2, NH_2, SO_3H etc.
k_1 is reduction rate constant of azo dye
k_2 is mineralization rate constant of aromatic amines

Figure 1.2. Schematic model of degradation of an azo dye

1.4. Importance of the Toxicity of Azo Dyes

Several studies supported by American Dye Manufacturers Institute (ADMI) suggested that the toxicity of commercial dyes to fish and mammals was minimal (Anliker 1977; Little & Chillingworth, 1974) They concluded that commercial dyes, with the exception of benzidine dyes and the triphenylmethane type of cationic dyes, were not generally hazardous chemicals. One of the triphenylmethane dyes was

found to be toxic to freshwater fish (Meyer, 1981). Benzidine and some of its derivatives were found to be mammalian carcinogens (Cerniglia et al., 1982). Azo reduction of a large number of azo dyes results in the formation of benzidine and benzidine derivatives. As a further caution, it should be noted that toxicity data at chronic low-level exposures for most of the commercial dyes and their derivatives are lacking (Chung &Stevens, 1993).

Table 1.1. Advantages and disadvantages of the current methods of dye removal from industrial effluents (Robinson et al., 2001)

Physical/Chemical Method	**Advantages**	**Disadvantages**
Fenton Reagent	Effective for decolorization of both soluble and insoluble dyes	Sludge generation
Ozonation	Applied in gaseous state: no alteration of volume	Short-half life is 20 min
Photochemical	No sludge production	Formation of by-products
NaOCl	Initiates and accelerates azo bond cleavage	Release of aromatic amines
Cucirbutirul	Good sorption capacity of variety dyes	Very expensive
Electrochemical destruction	Breakdown compound are non hazardous	High cost of electricity
Activated carbon	Good removal of wide variety of dyes	Very expensive
Peat	Good adsorbent due to cellular structure	Specific surface areas for adsorption are lower than activated carbon
Wood chips	Good sorption capacity for acid dyes	Requires log retention times
Silica gel	Effective for basic dyes	Side reaction the prevent commercial application
Membrane filtration	Removal in all dye types	Concentrated sludge production
Ion Exchange	Regeneration: no adsorbent loss.	Not effective at all dyes
Irradiation	Effective oxidation at lab scale	Requires a lot of dissolved O_2
Eloctrokinetic coagulation	Economical feasible	High sludge production

Dyes in aquatic environments were reported to affect the microbial population and their activities. Azo dyes were inhibitory to microbial oxidation processes in both activated sludge and stream water. The activated sludge method is one of the most widely used biological processes in wastewater treatment plants. The toxicity of azo dyes to microorganisms was shown to result in a decline in the treatment function of the activated sludge process. Moreover, inhibitory effects on the activity and growth of microorganisms are more pronounced at high azo dye concentrations. Azo dyes were inhibitory to the respiration of microorganisms in activated sludge. The inhibition by basic dyes was stronger than the inhibition by acid dyes when the pH was above the isoelectric point for the microorganisms. The inhibition of microbial respiration was weakened by the introduction of functional groups such as $-CH_3$, NO_2, $-SO_3H$, and -COOH to the azo dye or by replacement of the benzene ring with a naphthalene ring. However, the introduction of -Cl or -Br strengthened the observed inhibition (Chung & Stevens, 1993).

Some of these dyes may cleave-off under reducing conditions emitting aromatic amines, which proved carcinogenic in animal experiments. The process of the reduction of azo dyes with the cleavage of aromatic $R–NH_2$ amines is one of the ways of the degradation of those dyes. Aromatic amines are specified in the groups III A1 or III A2 of the Maximum workplace concentration (Maximale Arbeintsplatz Konzentration -MAK) list as well as in IARC (International Agency for Research on Cancer) and ETAD (Ecological and Toxicological Association of the Dyestuffs Manufacturing) lists (Table 1.2). The capability to form any of the below listed carcinogenic amines through cleavage of one or more azo groups is the reason why they all fall under the category of banned azo dyes (Pielesz, 1999).

Approximately, it was determined that 130 of 3200 azo dyes in use have produced carcinogenic aromatic amines because of reductive degradation of these (TTGV, 1996). In Turkey, although Turkish government declared that the azo dyes are banned dyes since 1 March 1995 due to the relevant aromatic amines (see Table 1.2). However, they have been still used in textile dyeing processes due to efficiency of dyeing in Turkey (Official Gazette, 1995).

Direct Black 38 is typical direct dye used in dyeing textiles and cotton yarn. Yarn dyed with the above dye was subjected to a chemical reduction in order to obtain the process of reconstruction of the MAK amine, which in this case was benzidine, after the reduction of dye (Pielesz, 1999). This case is shown in Figure 1.3.

Azo dyes can be reduced by two electrons to produce the corresponding hydrazo compound or by four electrons to generate two aromatic amines. In both reactions, color is lost through reduction. The hydrazo product is sensitive to oxygen and could revert to the azo form with oxidation (Nam and Renganathan, 2000).

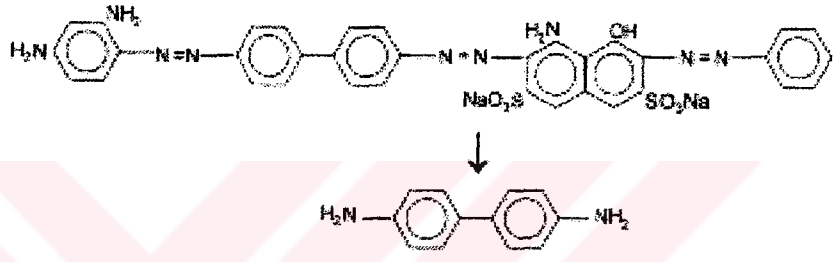


Figure 1.3. The azo dye Direct black 38 and the product of the reduction of the Direct Black 38 (benzidine).

1.5. Objectives and Scope of the Study

Generally, the inhibition of azo dyes is known, but limited investigations have been performed to detect the inhibition and the level of inhibition on microorganisms, particularly on partially granulated mixed anaerobic cultures. The anaerobic reduction studies with azo dyes performed until now showed that the methane productions and decolorization decreased and some intermediates such as aromatic amines and volatile fatty acids were accumulated through methanogenesis of azo dyes. Moreover, the inhibition effects of azo dyes on microbial activity, on methane gas productions and on substrate removal rates and their inhibition kinetics were not extensively investigated.

The first topic of this study was to examine the inhibition effect of selected azo dye, the level of inhibition, the effect of increasing dye concentrations on kinetic coefficients through simultaneous anaerobic substrate and color removals in batch studies. Furthermore, the kinetic of decolorization and substrate removals were studied, the methane gas productions, volatile fatty acids and aromatic amine concentrations were monitored throughout anaerobic batch experimental period at increasing dye concentrations.

Table 1.2. The list of MAK amines(Pielesz, 1999)

MAK groups	MAK amines	CAS* No
MAK III A1	Benzidine	92-87-5
	4-Aminodiphenyl	92-67-1
	4-Chloro-o-toluidine	95-69-2
	2-Naphtyloamine	91-59-8
MAK III A2	*o*-Aminoazotoluene	97-56-3
	2-Amino-4-nitrotoluene	99-55-8
	p-Chloroaniline	106-47-8
	2,4-Diaminoanisole	615-05-4
	4,4' -Diaminodiphenylomethane	101-77-9
	3,3' –Dichlorobenzidine	91-94-1
	3,3' -Dimethoxybenzidine *(o*-dianisidine)	119-90-4
	3,3' -Dimethylobenzidine *(o*-tolidine)	119-93-7
	3,3' -Dimethyl-4,40 –diaminodiphenylmethane	838-88-0
	p-crezidine	120-71-8
	4,4' -Methyleno-bis-(2-chloroaniline)	101-14-4
	4,4' -Oxydianiline	101-80-4
	4,4' -Tiodianiline	139-65-1
	2-methylaniline (o-toluidine)	95-53-4
	2,4-Toluylendiamine	95-80-7
	2,4,5-Trimethylaniline	137-17-7

*Chemical abstract number

Nowadays, azo dyes have not been removed effectively from industrial wastewater by conventional treatment methods such as activated sludge and physico-chemical process. Although several studies concerning the color removal efficiencies and the intermediate products of some dyes were carried out, little work has been

done to determine the effects of operational conditions on the performance of the COD, color removal and methane production efficiencies using anaerobic/aerobic sequential reactor systems. In this study the effect of operational conditions (shock organic loading, dye concentration, hydraulic retention times (HRT), sludge retention times (SRT) etc.) on the treatability of azo dyes which are in use in Turkey were investigated in an upflow anaerobic sludge blanket (UASB) and aerobic completely stirred tank reactor (CSTR) sequential reactor system. The UASB /CSTR sequential reactor system was used to demonstrate the formation, degradation, removal and recoveries of aromatic amines produced from the cleavage azo dyes through decolorization of the azo dyes. Furthermore, the effect of increasing dye loadings on methane gas, color, and COD removals Volatile Fatty Acids (VFA) and Bicarbonate Alkalinity (B.Alk.) concentrations were investigated under anaerobic and aerobic conditions.

On the other hand limited studies were performed to investigate the effect of co-substrate and alkalinity on the treatment efficiencies using continuous plug flow systems. The effect of different alkalinity levels and substrate concentrations on the treatment efficiencies were not reported in detail using continuous anaerobic reactors treating azo dyes in literature. The alkalinity required to buffer a wastewater containing the azo dyes at pH=7 and the supplementation of an external carbon source adversely affect the economical feasibility of the anaerobic treatment of the textile wastewaters. The cost for alkalinity and sometimes for additional co-substrate supplementation to wastewater can easily exceed the value of methane produced in UASB reactors. Therefore, the one of the objectives of this dissertation was designed to investigate the effect of glucose-COD concentrations and $NaHCO_3$ alkalinity levels on the reactor performances (COD, color, TAA removal efficiencies, VFA and methane gas productions) in a lab-scale UASB reactor treating Congo Red azo dye.

The efficiency in removing color and COD and the improvement of biodegradability of the dyes for further aerobic treatment using anaerobic stage were evaluated. In addition, the feasibility to use the anaerobic/aerobic sequential system

was also investigated for real wastewaters, which were taken from a acid dyeing units of wool, and textile industry.

The studies performed until now, showed that the kinetics of anaerobic/aerobic reactor treated simulated textile dyeing wastewater has not been investigated in detail. Different kinetic models such as Monod, Contois, Grau etc. were investigated to obtain the kinetic coefficients of UASB and UASB/CSTR total reactor system treating a simulated textile wastewater.

The other objective of this dissertation is to investigate whether azo dyes are biodegraded under integrated anaerobic/aerobic conditions by creating cocultures with anaerobic granular sludge and aerobic aromatic amine degrading bacterial enriched aerobic cultures. Furthermore, the overall mineralization of azo dye to CO_2, H_2O and NO_3 were performed using an anaerobic/aerobic sequential processes. The biodegradation and fate of aromatic amines under both aerobic and anaerobic conditions are the one of the main subject of this dissertation.

Finally, identification, and monitoring of toxicity in DR 28 and DB 38 azo dye metabolites was carried out, simultaneously, through the operation of anaerobic /aerobic sequential reactor system to explore the influence of the whole system on the reduction of toxicity produced from the azo dyes.

CHAPTER TWO
MATERIALS AND METHODS

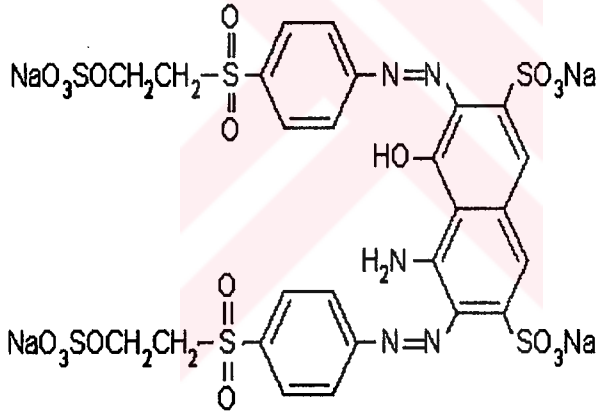
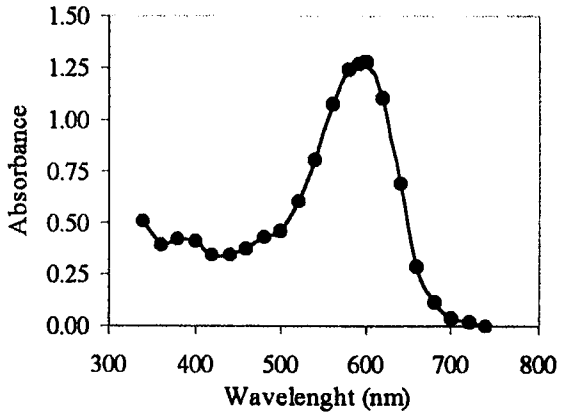
2.1 Azo Dyes

Table 2.1 summarizes the dyes selected for this study. All dyes are of commercial quality. Spectrophotometric scanning of the diluted dye solutions was performed to identify the absorption maxima for each dye. COD values were determined for 1000 mg/l of dye solution. Calibration curves were prepared for each dye at their respective absorbance maxima. Although dyes are ticked "*" were banned by the governments since they contain MAK (Maximum Work Place) III A1 amine (benzidine) has still used in textile dyeing in Turkey and other countries (Pielesz, 1999).

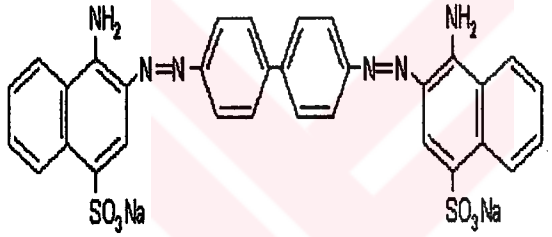
Table 2.1.a. Properties of azo dyes

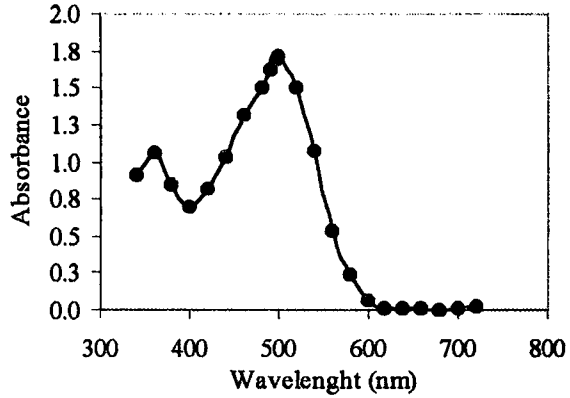
Color Index Name	Number of Color index	Formulas of dyes	Molecular weight (g/mol)	λ_{max} (nm)	1000 mg dye/l		
					COD (mg/l)	S.DEV.	n
Reactive Black 5 (Remazol Black 5)	20505	$C_{26}H_{25}N_5O_{19}S_6\ Na_4$	991.8	597	524	28	3
Direct Red 28 * (Congo red),	22120	$C_{32}H_{24}N_6O_6S_2\ Na_2$	696.7	497	746	22	3
Direct Black 38*,	30235	$C_{34}H_{25}N_9O_7S_2Na_2$	781.7	520	370	40	3
Direct Brown 2*	22311	$C_{29}H_{19}N_5Na_2O_7S$	627.5	400	1034	0	3
Direct Yellow 12	24895	$C_{30}H_{26}N_4O_8S_2Na_2$	680.7	400	497	7	3

Table 2.1.b. Open formulas and absorbance spectrums of azo dyes

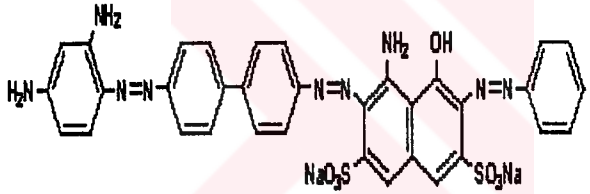
Dye name	Open formulas	Absorbance spectrums
Reactive Black 5 (RB 5)		

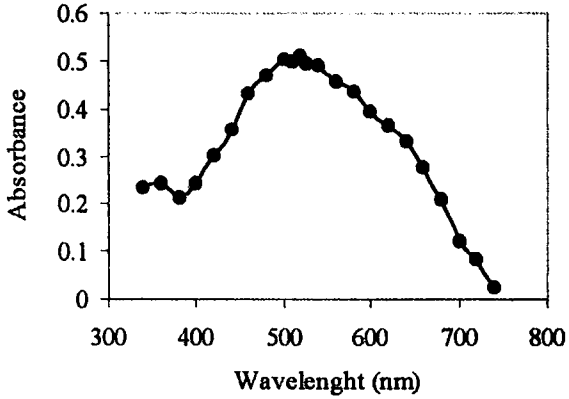
Direct Red 28 *
(CR 28)



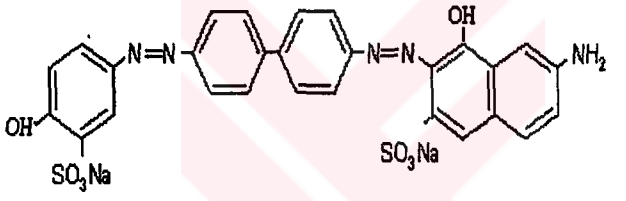


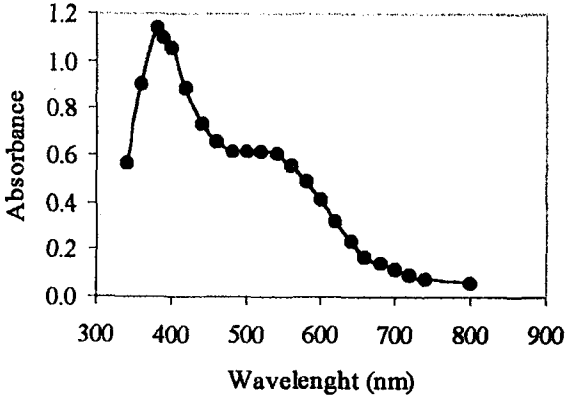
Direct Black 38
(DB 38)



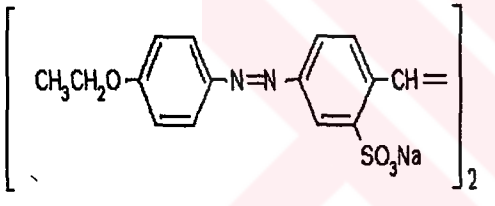


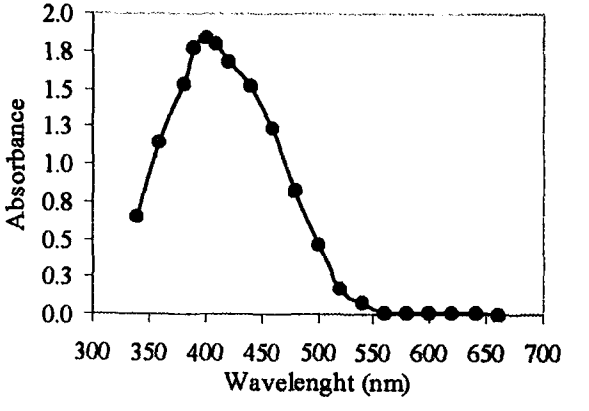
Direct Brown 2
(DB 2)





Direct Yellow 12
(DY 12)





*Banned azo dyes which contain benzidine (MAK III A1 amine)

2.2. Batch Studies

2.2.1. Lab-scale batch anaerobic reactors, synthetic media, and seed used throughout the experiments

The studies were performed in 115 ml dark glass serum bottles sealed with 5mm butyl rubber septum, kept in place, by a screw cap (Figure 2.1). Methanogenic granular sludge from a full-scale upward-flow anaerobic sludge blanket reactor (UASB) treating yeast industry wastewater of PAKMAYA in Izmir, Turkey, was used as the inoculum. The sludge was not previously acclimated to any of the azo dyes. Vanderbilt mineral medium was used as mineral feed. This mineral medium was used in all batch investigations and consisted of the following inorganic composition (in mg/l): NH_4Cl, 400; $MgSO_4.7H_2O$, 400; KCl, 400; $Na_2S.9H_2O$, 300; $(NH_4)_2HPO_4$, 80; $CaCl_2.2H_2O$, 50; $FeCl_3.4H_2O$, 40; $CoCl_2.6H_2O$, 10; KI, 10; $(NaPO_3)_6$, 10; L-cysteine, 10; $AlCl_3.6H_2O$, 0.5; $MnCl_2.4H_2O$, 0.5; $CuCl_2$, 0.5; $ZnCl_2$, 0.5; NH_4VO_3, 0.5; $NaMoO_4.2H_2O$, 0.5; H_3BO_3, 0.5; $NiCl_2.6H_2O$, 0.5; $NaWO_4.2H_2O$, 0.5; Na_2SeO_3, 0.5 (Speece, 1996).

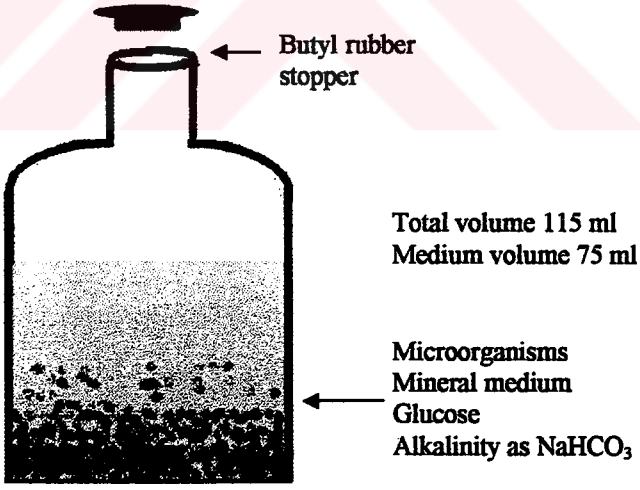


Figure 2.1. Anaerobic batch reactor used for decolorization and substrate kinetics test

The anaerobic conditions were maintained by adding 667 mg/l of Sodium Thioglycollate (0.067 %) that is proposed between 0.01-0.2 % (w/w) for maintaining

anaerobic conditions. The alkalinity and neutral pH were adjusted by addition of 5000 mg /l $NaHCO_3$. 3000 mg /l of glucose–COD was used as co-substrate providing the reducing equivalents with electron fission. All incubations were carried out in a temperature controlled incubator at 35°C. The bottles were vigorously shaken at certain intervals and the liquid samples were taken from the supernatants with a syringe for analysis. Table 2.2. shows the feeding protocol for anaerobic batch reactors.

Table 2.2. Protocol for simultaneous degradation, decolorization and kinetics throughout batch tests.

Stock	Seed control(ml)	Abiotic control (ml)	Dye containing bottles(ml)	Resulting conc (mg /l)
Sludge (75 g/l)	4	-	4	4000
Glucose (30 g COD /l)	-	7.5	7.5	3000
Vanderbilt min. med.	7.5	7.5	7.5	Desired composition
$NaHCO_3$ (50 g /l)	7.5	7.5	7.5	5000
Sodium Thioglycollate, (50 g /l)	1.0	1.0	1.0	(w/w) % 0.067
Dye (10 g /l)	-	1.5	0; 1.5; 3; 6; 12; 24	0; 200; 400; 800; 1600;3200
Total volume (ml)	75	75	75	

2.2.2. Experimental procedure

Throughout the batch tests, a control (without dye) and a seed blank sample were used to detect and compare the methane production in order to correct the methane production in batch serum bottles containing at all dye concentrations. Serum bottles containing dyes and controls were duplicated throughout the batch tests to detect the accuracy of the kinetic and the absorbance data through simultaneous substrate removal and degradation of azo dyes. Throughout these tests the data necessary for substrate kinetic was detected from both the COD concentrations and the methane gas productions. The COD conversion of methane gas was carried out as follows: 0.395 ml methane gas was produced through the removal of 1 mg COD /l.

2.3. Continuous Studies in Anaerobic/Aerobic Sequential Reactor

2.3.1 Experimental system

A continuously fed stainless steel anaerobic UASB and aerobic CSTR reactors were used in sequence for the experimentation. The UASB reactor had 2.5 l of effective volume with an internal diameter of 6 cm and a height of 100 cm. The CSTR reactor consisted of an aeration tank (effective volume = 9 l) and a settling compartment (effective volume= 1.32 l). A schematic of the lab-scale sequential UASB and CSTR reactor used in this study is presented in Figure 2.2. Treated effluent passed through a U-tube for level control and to trap biomass before running to the aerobic tank. Wastewater passage from the aeration tank to the sedimentation tank was through the holes on the inclined plate and the sludge recycle was through the gap under the plate. The effluent of the anaerobic UASB reactor was used as the influent of aerobic CSTR reactor.

In the aeration part, the water was aerated from the bottom with diffuser by an air pump, type HAS PRO 40. The dissolved oxygen concentration did not decrease below 2 mg/l, for the bacterial growth and the mixing liquor. Turbulence produced from the air pump in suspended liquid provided homogenous mixed liquor in one side of the inclined plate having three holes with a 5mm diameter. The sedimentation of the microorganisms was provided with the stability of the mixed liquor in the other side of the inclined plate. The feed was preserved in a refrigerator at 4°C in order to keep the substrate undecomposed particularly in summer.

Sludge retention time (SRT, θ_C) is the total quantity of active biomass in the reactor divided by the total quantity of active biomass withdrawn daily. Since no sludge wasting was applied for granule formation in the UASB reactor, θ_C in this reactor was determined using equations (2.1) and (2.2).

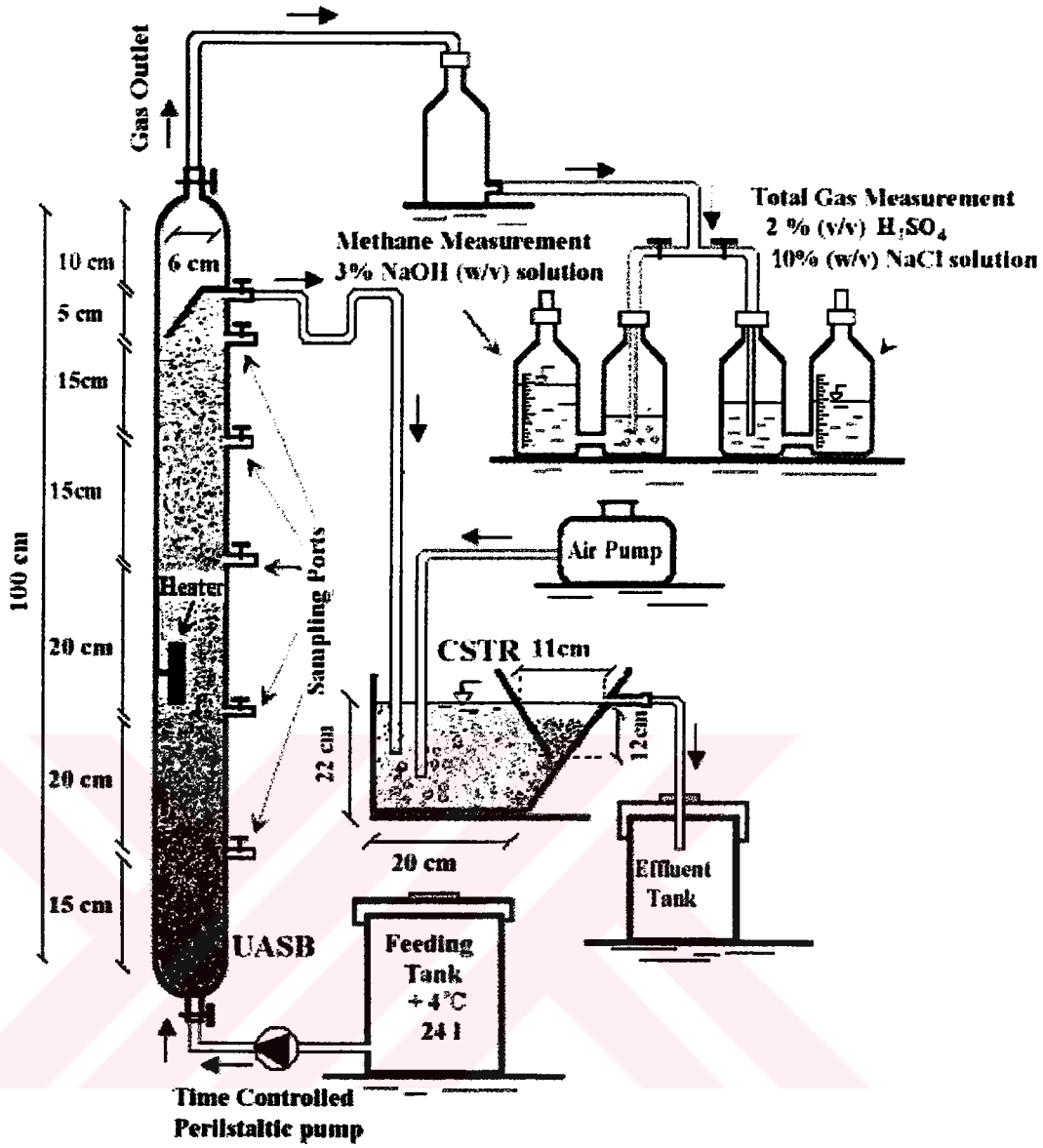


Figure 2.2. Schematic configuration of lab-scale anaerobic/aerobic sequential reactor system

$$\theta_{CU} = \frac{V_U * X_U}{Q_{UW} * X_{UW} + Q_{UE} * X_{UE}} \qquad (2.1)$$

The term $Q_{UW}*X_{UW}$ only makes sense if there is a waste sludge stream. Since there is no a sludge wastage in the UASB reactor, θ_{CU} can be expressed as follows:

$$\theta_{CU} = \frac{V_U * X_U}{Q_{UE} * X_{UE}} \qquad (2.2)$$

Stepwise increases in organic loading cause increases in both flow rates (Q_{UE}) and microorganism concentrations in the effluent (X_{UE}) of the UASB reactor. Therefore, θ_C in the UASB reactor was calculated on the basis of equation (2. 2) and varied depending to shock organic loading applied.

The sludge wasting in the CSTR reactor occurred from the aeration tank and the solids in the effluent (X_{CE}) were taken into consideration. Therefore θ_C in this reactor was adjusted by using equations (2. 3) and (2.4).

$$\theta_{CC} = \frac{V_C * X_C}{Q_{CW} * X_{CW} + Q_{CE} * X_{CE}} \qquad (2.3)$$

Since the activated sludge was withdrawn from the inside of the aeration stage, the microorganism concentration in the reactor (X_C) was equal to the wasted microorganism concentration (X_{CW}). If X_{CE} is negligible, equation (2.4) was used to calculate the θ_C in CSTR reactor.

$$\theta_{CC} = \frac{V_C}{Q_{CW}} \qquad (2.4)$$

2.3.2. Seed

Partially granulated anaerobic sludge was used as seed in UASB reactor and was taken from the methanogenic reactor of Pakmaya Yeast Baker Factory in Izmir. Activated sludge culture obtained from DYO Dye Industry in Izmir was used as seed for the aerobic CSTR reactor.

2.3.3. Simulation of cotton textile wastewater for kinetic studies

A simulated textile wastewater was prepared to give the reproducibility for continuous kinetic studies. The processes which generate wastewater in a cotton textile mill are desizing, scouring, bleaching, dyeing, and printing sources. The simulated wastewater based these processes are similar to the composition of wastewater reported by Shaw et al., (2002) and O'Neill et al., (2000a). The composition and characteristics of simulated wastewater used in this study are given in Table 2.3.

Table 2.3. Characteristics of simulated wastewater

Materials used	Conc. mg/l	The functions of material
Carboxymethyl cellulose (CMC)	150	sizing
Starch	1500	sizing
Acetic acid	500	suitable pH
Dyes	50	dyeing
NaOH	660	hydrolysis
H_2SO_4	357	pH neutralization
NaCl	500	for fixing
Na_2CO_3	1000	for fixing
$NaHCO_3$	2000	pH tampon in UASB
Glucose	2062.5	substrate for bacterial growth
Vanderbilt	1/1	for bacterial growth
Parameters		
Total COD	4214±241 (n= 6)	
Soluble COD	3743±154 (n=4)	
TOC	1914	
BOD_5	3120	
pH	8.83±0.11 (n=9)	
Total Alkalinity as mg $CaCO_3$/ l	3090±260 (n=9)	

2.3.4. Operating conditions

In the start up, period reactor was fed with synthetic wastewater containing 3000 mg/l of Glucose-COD, 5000 mg/l of $NaHCO_3$, and defined Vanderbilt mineral

medium. In 43 days following the start up period, while approximately 60% COD removal and 65% methane production efficiencies were obtained at a flow rate of 4 l/day, the UASB reactor was brought into operation with 100 mg/l of Reactive Black 5 dye. The COD concentration was kept constant at 3000 mg/l. The operating parameters throughout continuous feed studies are summarized in Table 2.4.a, b, c, d, e, f, g, and h.

Once Steady-state conditions achieved at each run (for different feed flow-rates, loading conditions) the daily volume of methane produced, and COD, pH, total volatile fatty acids of the different effluents were determined. Steady state was defined by constant daily biogas production, effluent COD concentrations, effluent volatile fatty acid (VFA) concentrations and operating pH values within 5% variation for consecutive days in each reactor. The samples were collected and analyzed for at least 5 consecutive days. The steady state value of a given parameter was taken as the average of these consecutive measurements for that parameter when the deviations between the observed values were less than 5% in all cases. Each experiment had a duration of two to three times the corresponding loading condition.

Reactor was operated with synthetic wastewater containing 100 mg/l of RB 5 azo dye during 152 day in order to investigate the effect of hydraulic retention time (HRT) on color and COD removals. HRT decreased from 30 h to 3 h organic loading rate (OLR) increased from 2.5 to 24.6 kg/m^3.day for UASB reactor.

After this operation, the effect of HRT was investigated for synthetic wastewater containing 100 mg/l of Direct Red 28 azo dye in UASB/CSTR system. OLR of UASB reactor was changed from 4.5 to 30.62 kg COD/m^3.day with decreasing HRT from 18 to 2.5 h during 112 days of the operation period. Secondly in Direct Red 28 operation, the effect of increasing DR 28 concentration on performance of reactors was investigated at approximately constant HRT during operation time between 113 and 187days. Finally the effect of glucose–COD and $NaHCO_3$ alkalinity on performance of reactors was investigated for synthetic wastewater containing 100

mg/l of DR 28 azo dye. 3000 mg/l of glucose-COD and 1500 mg/l of $NaHCO_3$ concentrations in feed decreased stepwise to 0 and 250 mg/l, respectively.

Reactor system was operated during 146 days of operation time in order to investigate treatability of real colored wastewaters obtained from wool and cotton textile mill.

Finally, reactor system was operated with simulated wastewater containing the mixture of used azo dyes, starch, CMC, etc. in order to obtain kinetic coefficients for reactor system

Table 2.4. Operating parameters and conditions in anaerobic and aerobic reactors for Reactive Black 5 (a), Direct Red 28 (b), Direct Black 38 (c), Acid dyeing (d) Real textile wastewater (e), and (f) Kinetic studies of simulated wastewater

(a) Reactive Black 5

Stage	Period (days)	Anaerobic (UASB) reactor						Aerobic (CSTR) reactor			
		HRT (θ_H)*,	O. load. (kg COD/m^3d)	F/M ratio (kg COD/kg SS. d)	SRT (θ_C) (d)	Dye loading rate (g dye/ m^3 h)	RB 5 conc. (mg/l)	HRT (θ_H)*,	Org. load. (kg COD/m^3d)	F/M ratio (kg COD/kg MLSS.d)	SRT (θ_C) (d)
Start up	0-43	15.4	4.8	0.38	30	-		2.54	0.430	0.147	10
Effect of HRT on color and COD removals											
Run 1	1-26	15.7	4.8	0.156	30	6.66	100	2.27	0.57	0.17	28
Run 2	27-89	19.9	4.9	0.159	31	6.46	100	3.20	0.56	0.27	10
Run 3	90-103	30.0	2.5	0.085	59	3.38	100	4.49	0.40	0.049	11
Run 4	115-120	10.4	7.1	0.23	21	9.58	100	1.56	1.24	0.38	7
Run 5	121-134	8.1	9.3	0.30	16	12.94	100	1.22	1.78	0.39	8
Run 6	135-138	5.0	14.8	0.48	10	20.00	100	0.75	2.95	0.74	6
Run 7	142-145	3.8	19.4	0.63	8	26.33	100	0.56	3.94	1.40	2
Run 8	149-152	3.0	24.6	0.80	6	33.33	100	0.45	5.01	4.48	3

(b) Direct Red 28

Stage	Period (days)	Anaerobic (UASB) reactor						Aerobic (CSTR) reactor			
		HRT (θ_H)*,	O. load. (kg COD/m^3d)	F/M ratio (kg COD/kg SS. d)	SRT (θ_C) (d)	Dye loading rate (g dye/ m^3 h)	DR 28 conc (mg/l)	HRT (θ_H)*,	Org. load. (kg COD/m^3d)	F/M ratio (kg COD/kg MLSS.d)	SRT (θ_C) (d)
Effect of HRT on color and COD removals											
Run 1	0-30	18.6	4.53	0.110	73	0	0	2.8	0.625	0.443	9
Run 2	31-87	18.5	4.74	0.153	78	6.12	100	2.77	0.575	0.197	8
Run 3	88-95	8.3	9.1	0.280	34	12	100	1.25	1.274	0.402	3
Run 4	96-101	5.72	13.31	0.408	24	17.50	100	0.86	1.88	0.778	3
Run 5	102-108	4.23	18.29	0.56	18	23.70	100	0.64	2.56	1.729	2
Run 6	109-112	2.55	30.62	0.938	10	39.5	100	0.38	4.91	2.586	2

Effect of increasing dye concentration on color and COD removals											
Run 7	113-119	26.01	2.76	0.085	219	0	0	3.9	0.23	0.08	8
Run 8	120-133	21.05	3.67	0.112	176	3.57	75	3.15	0.255	0.165	12
Run 9	134-140	26.24	2.95	0.090	220	5.78	150	3.94	0.170	0.074	14
Run 10	141-165	22.55	3.61	0.111	189	13.67	300	3.83	0.159	0.077	14
Run 11	166-171	19.83	4.14	0.127	166	25.65	500	2.98	0.176	0.079	16
Run 12	172-175	28.13	3.58	0.109	236	32.19	750	4.23	0.218	0.071	20
Run 13	176-182	18.69	5.25	0.161	156	107.00	2000	2.80	0.404	0.080	9
Run 14	183-187	18.75	8.33	0.255	157	213.33	4000	2.81	0.793	0.881	8
Effect of glucose on color and COD removals											
Run 15	188-203	15.7	4.54	0.143	136	0	0	2.35	0.15	0.029	13
Run 16	204-210	19	0.845	0.027	162	5.3	100	2.85	0.06	0.018	11
Run 17	211-217	18	0.437	0.014	231	5.6	100	2.75	0.03	0.012	22
Run 18	218-224	18.3	0.286	0.009	384	5.5	100	2.74	0.24	0.108	13
Run 19	225-237	18.9	0.195	0.006	1603	5.4	100	2.84	0.43	0.12	12
Effect of alkalinity on color and COD removals											
Run 20	238-243	20.4	2.3	0.073	326	0	100	3.06	0.79	0.034	8
Run 21	244-250	15.54	3.27	0.103	248	6.43	100	2.33	1.27	0.10	10
Run 22	251-257	15.54	3.27	0.103	248	6.43	100	2.33	1.31	0.09	8
Run 23	258-264	18.19	2.86	0.09	290	5.62	100	2.73	0.76	0.05	10
Run 24	265-278	21.83	2.42	0.53	348	4.76	100	3.27	0.86	0.05	10
Run 25	279-290	17.6	2.99	0.65	272	5.88	100	2.56	2.30	0.09	11

(c) Direct Black 38

Stage	**Period (days)**	**Anaerobic (UASB) reactor**						**Aerobic (CSTR) reactor**			
		**HRT (θ_H)*, **	**O. load. (kg COD/m^{3}d)**	**F/M ratio (kg COD/kg SS. d)**	**SRT (θ_C) (d)**	**Dye loading rate (g dye/ m^3 h)**	**DB 38 conc (mg/l)**	**HRT (θ_H)*, **	**Org. load. (kg COD/m^{3}d)**	**F/M ratio (kg COD/kg MLSS.d)**	**SRT (θ_C) (d)**
Effect of increasing dye concentration on color and COD removals											
Run 1	0-8	16.5	2.91	0.09	187	6.1	100	2.5	0.2	0.113	14
Run 2	9-17	15.4	3.32	0.1	170	13.0	200	2.3	0.30	0.222	16
Run 3	18-28	16.0	3.90	0.11	193	25.4	400	2.4	0.23	0.104	17
Run 4	29-38	15.4	3.61	0.11	173	52.0	800	2.3	0.27	0.078	16
Run 5	39-45	15.0	4.53	0.16	166	106.7	1600	2.3	0.39	0189	12
Run 6	46-53	15	5.37	0.17	166	213	3200	2.3	0.77	0.229	8

(d) Acid dyeing

Stage	Period (days)	Anaerobic (UASB) reactor						Aerobic (CSTR) reactor			
		HRT (θ_H)*,	O. load. (kg COD/m^3d)	F/M ratio (kg COD/kg SS. d)	SRT (θ_C) (d)	Dye loading rate (g dye/ m^3 h)	Upflow vel. (m/h)	HRT (θ_H)*,	Org. load. (kg COD/m^3d)	F/M ratio (kg COD/kg MLSS.d)	SRT (θ_C) (d)
Effect of acid dyeing wastewater on color and COD removals											
Run 1	0-7	11.9	3.94	0.12	253	-	0.085	1.8	0.221	0.114	23
Run 2	8-17	18.18	2.13	0.067	388	-	0.056	2.73	0.092	0.034	12
Run 3	18-33	17.1	1.74	0.055	365		0.062	2.6	0.125	0.100	10
Run 4	34-49	16.10	2.16	0.068	344	-	0.063	2.42	0.292	0.165	15

(g) Real textile wastewater

Stage	Period (days)	Anaerobic (UASB) reactor						Aerobic (CSTR) reactor			
		HRT (θ_H)*,	O. load. (kg COD/m^3d)	F/M ratio (kg COD/kg SS. d)	SRT (θ_C) (d)	Dye loading rate (g dye/ m^3 h)	Upflow vel. (m/h)	HRT (θ_H)*,	Org. load. (kg COD/m^3d)	F/M ratio (kg COD/kg MLSS.d)	SRT (θ_C) (d)
Effect of textile wastewater on color and COD removals											
Run 1	1-17	30	1.80	0.06	869	-	0.033	4.50	0.14	0.12	10
Run 2	18-24	30	1.03	0.03	869		0.033	4.5	0.13	0.09	15
Run 3	25-31	30	0.89	0.03	869	-	0.033	4.50	0.15	0.148	-
Run 4	32-38	30	0.39	0.01	869	-	0.033	4.5	0.08	0.04	25
Run 5	39-48	30	0.39	0.01	869	-	0.033	4.50	0.08	0.04	20
Run 6	49-59	30	0.80	0.03	869	-	0.033	4.5	0.139	0.136	16
Run 7	60-73	30	0.80	0.76	869		0.033	4.50	0.102	0.100	16
Run 8	74-80	30	0.41	0.013	869	-	0.033	4.50	0.103	0.099	15
Run 9	81-87	30	0.90	0.028	869	-	0.033	4.5	0.172	0.164	15

(e) Kinetic studies

Stage	Period (days)	Anaerobic (UASB) reactor						Aerobic (CSTR) reactor			
		HRT (θ_H)*,	O. load. (kg COD/m^3d)	F/M ratio (kg COD/kg SS. d)	SRT (θ_C) (d)	Dye loading rate (g dye/ m^3 h)	Upflow vel. (m/h)	HRT (θ_H)*,	Org. load. (kg COD/m^3d)	F/M ratio (kg COD/kg MLSS.d)	SRT (θ_C) (d)
Effect of hydraulic retention time on color and COD removals											
Run 1	0-9	100	1.01	0.032	736	2.5	0.010	15	0.06	0.04	25
Run 2	10-17	46	2.19	0.069	339	5.42	0.022	6.92	0.16	0.11	25
Run 3	18-27	32	3.20	0.101	232	7.92	0.032	4.74	0.23	0.15	25
Run 4	28-32	14	7.16	0.225	104	17.71	0.071	2.12	1.05	0.55	25
Run 5	33-38	9	11.88	0.374	63	29.38	0.12	1.28	2.18	0.58	25
Run 6	39-46	6	15.84	0.50	47	39.17	0.16	0.96	3.11	0.70	25

* Unit for HRT: hours for anaerobic reactor but days for aerobic reactor

2.4. Analytical Procedures

2.4.1. Gas measurements

Gas productions were measured with liquid displacement method. Total gas was measured by passing it through a liquid containing 2% (v/v) H_2SO_4 and 10% (w/v) NaCl (Beydilli et al., 1998). Methane gas was detected by using a liquid containing 3% NaOH to scrub out the carbon dioxide from the biogas (Razo-Flores et al., 1997). The methane percentage in biogas was also determined by Dräger Pac®Ex methane gas analyzer.

2.4.2 Color measurements

In batch studies, color measurements were carried out in 5 ml samples removed from the supernatants of the serum bottles to detect the decolorization efficiencies once 2-3 hours. The color was measured by an UV-VIS spectrophotometer (Pharmacia LKB-NovaPec II) at wavelength in which maximum absorbance spectra were obtained for dyes. The samples were centrifuged at 7000 rpm for 10 min and the absorbance values of supernatants were recorded for color measurements. Color removal ratio was determined according to equation (2.5).

$$\text{Color removal (\%)} = \frac{(A_0 - A_t)}{A_0} * 100 \qquad (2.5)$$

The decolorization of the azo dyes with partially granulated anaerobic mixed cultures was measured as the decrease of light absorbance at their maximum wavelengths. Concentrations of dyes were determined from the measured light absorbance at maximum wavelength after determining the best fit with higher correlation coefficient (R^2>0.95) for absorbance versus concentration during batch incubation.

In the continuous studies in relation to pure dye, color in the influent and effluent samples of the anaerobic and aerobic reactors was measured at maximum wavelengths. The samples were centrifuged at 7000 rpm for 10 min and the absorbance values of supernatants were recorded for color measurements. Since the effluents of the reactors contained not only the undegraded substances but also some of the colloidal organic matters such as metabolic excretes and dead cells these substances interfered the color measurements. Therefore, in order to measure the real color removals the absorbance values of the reactor effluents at λ_{max} was calibrated with the control samples containing no dye.

In the studies with real colored wastewater, the measurement of color was carried out following approaches described by Olthof et al. (1976) and Eckenfelder (1989) since various dyes could be used in dyeing process. According these methods, the color content was determined by measuring the absorbance at three wavelengths (445, 540 and 660 nm), and taking the sum of the absorbances at these wavelengths.

To convert the absorbance into m^{-1} the equation given below was used:

$$\alpha = \frac{A}{d} * f \qquad (2.6)$$

where:

α: color in m^{-1} unit

A: measured absorbance value from spectrophotometer

d: sample length (cell width, 10mm)

f: factor (1000)

Abiotic tests performed with autoclaved anaerobic partially granulated sludge and mineral medium showed that the microbial decolorization was preceded primarily by biological degradation. Since acclimatization was not carried out in the studies performed in batch mode, it may suggested that the dye removal would occur because of abiotic removal such as adsorption of dye onto anaerobic biomass,

chemical removal of dye with Na_2S and sodiumthioglycollate in mineral medium as well as biotic removal. However, in the continuous studies the dye removal would occur only due to the biodegradation while the adsorption would occur only during the first few days of reactor start up and once biomass is in equilibrium with aqueous phase concentration. Therefore, it can be suggested that more adsorption could not occurred. Furthermore, as sodiumthioglycollate had never used in feed, this chemical substance had not been considered the effect on the color removal through continuous feed studies.

2.4.3. Determination of aromatic amines and their recoveries

The total aromatic amines were determined colorimetrically at 440 nm after reacting with 4-dimethylamino benzaldehyde-HCl according to the method described by Oren et al. (2001). Total aromatic amines (TAA) released from anaerobic and chemical reduction were quantified using benzidine as standard at absorbance maxima of 440 nm. The chemical reduction of the azo dyes was carried out with sodium dithionite. The conditions of reduction process were as follows: 0.06 g of dye sample was heated at boiling point with 1 M NaOH for 1 hr and after 30 min, 0.6 g sodium dithionite was added. 1000 mg /l of dye was reduced according to the method described by Pielesz (1999). The method described by Oren et al. (1991) was applied to diluted samples of reduced dye. Calibration lines are depicted in Figure 2.3. The aromatic amine recoveries were calculated from the ratio of TAA values of effluents to expected TAA resulting from chemical reduction of the equivalent dye.

The determination of TAA could not be carried out in samples containing Reactive Black 5 (RB 5) because the intermediates of RB 5 re-colored immediately through auto-oxidation during sampling and analyzing.

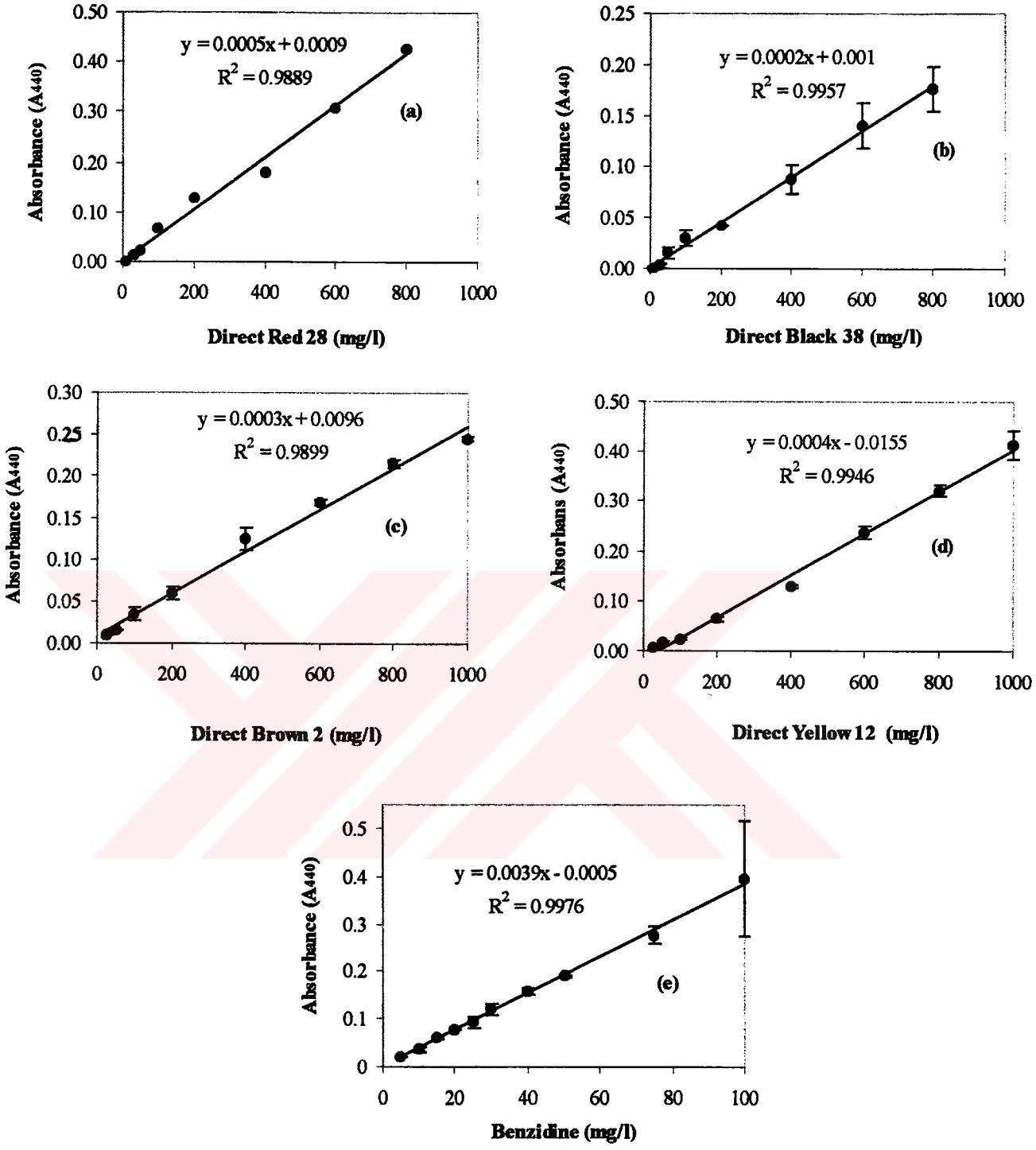


Figure 2.3. The calibration curves of benzidine, and reduced dyes versus absorbance at 440 nm for TAA determination

2.4.4. Chemical oxygen demand (COD)

The soluble COD was measured colorimetrically by using closed reflux methods (APHA AWWA. 1992). First the samples were centrifuged 10.0 min at 7000 rpm. Secondly, 2.5 ml volume samples were treated with 1.5 ml 10216 mg/l $K_2Cr_2O_7$ with 33.3 g/l $HgSO_4$ and 3.5 ml 18 M H_2SO_4 which contains 0.55% (w/w) Ag_2SO_4. Thirdly the closed sample tubes were stored in a 148°C heater for two hours. Finally, after cooling the samples were measured at 600 nm with the Pharmacia LKB-NovaPec II spectrophotometer

2.4.5. Inert COD

The effluent soluble COD (S_T) of a biological rector includes apart from the remaining portion of the readily biodegradable influent COD (S_S), initially inert COD, (S_I), that by passes the treatment system without any change, and COD associated with inert soluble microbial products (S_P) as illustrated below;

$$S_T = S_S + S_I + S_P \qquad (2.7.)$$

Inert COD content of real textile wastewater was determined with glucose comparison method developed previously by Germirli et al. (1990) in all soluble form COD. In this method, two batch reactor were operated in parallel and started with the same initial COD, one being fed with the soluble part of the wastewater to be tested, and the other with glucose. The soluble COD in each reactor was measured periodically until a plateau value was reached, at which point the biodegradable substrate was almost entirely depleted (variations in COD within ± 5% were taken as an indication that the minimum COD was attained). This final residual COD, S_R, was assumed to be equal to sum of S_I and S_P. The COD of the reactor fed on the glucose reached a lower value, which was taken to represent S_P alone, as glucose itself would have contained no inert organic material. The S_I content of the wastewater was calculated from the equation 2.8 with assumption that $(S_P)_{wastewater} \cong (S_P)_{glucose}$;

$$S_I = (S_R)_{wastewater} - (S_P)_{glucose} \quad (2.8)$$

2.4.6. Biochemical oxygen demand (BOD_5), total organic carbon (TOC), amonium (NH_4-N), and nitrate (NO_3-N)

BOD 5 was measured using the WTW Oxi Top IS 12 system. TOC was determined with DOHRMANN DC-190 Model High-Temperature TOC analyzer. Ammonium and nitrate nitrogen were quantified using specific chemical analysis kits (Merck-Spectroquant), and spectrometric methods were used for analysis.

2.4.7. Total alkalinity (T.Alk), bicarbonate alkalinity (B.Alk.), and volatile fatty acid (VFA)

Total alkalinity of samples was determined by titrating a sample with 0.1 N of standard sulphuric acid solution to a pH of 4.3. Bicarbonate alkalinity (B.Alk.) and total volatile fatty acid (VFA) concentrations were measured simultaneously by Anderson &Yang, (1992) titrimetric method. The test was carried out as follows: the pH of the sample was measured , the sample was titrated with standard sulphuric acid (0.1 N) through two stages (first to pH 5.1, then from 5.1 to 3.5), and the VFA and B.Alk concentrations were calculated with a computer program solved equation 2.9 and 2.10.

$$A1 = \frac{[HCO_3^-]*([H]_2 - [H]_1)}{[H]_1 + K_C} + \frac{[VA]*([H]_2 - [H]_1)}{[H]_2 + K_{VA}} \quad (2.9)$$

$$A2 = \frac{[HCO_3^-]*([H]_3 - [H]_1)}{[H]_3 + K_C} + \frac{[VA]*([H]_3 - [H]_1)}{[H]_3 + K_{VA}} \quad (2.10)$$

where; A1 and A2 are the molar equivalent of the standard acid consumed to the firs and second endpoints; $[HCO_3^-]$ is the bicarbonate concentration; [VA] is the volatile fatty acid ion concentration; $[H]_{1,2,3}$ are the hydrogen ion concentrations of the original sample and at the first and the second endpoints; K_C is a conditional

dissociation constant of carbonic acid; and K_{VA} is a combined dissociation constant of the volatile fatty acids (C_2 to C_6), it was assumed that this pair of constants, being 6.6×10^{-7} for bicarbonate and 2.4×10^{-5} for volatile acids.

2.4.8. pH, oxidation reduction potential (ORP) , dissolved oxygen (DO), and T

The pH was determined immediately after sampling to avoid any change due to CO_2 evolution, using a pH meter, type NEL pH 890. ORP was measured using WTW Sentix ORP meter (Germany) with a $Ag/AgCl_2$ reference electrode in saturated KCl solution and Pt electrode. Dissolved oxygen (DO) and temperature of aeration tank of CSTR were measured with an oxygen meter (model Oxi 330/SET) which was manufactured by WTW(Germany).

2.4.9. Mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids, (MLVSS), suspended solids,(SS) and volatile suspended solids (VSS)

Biomass was measured as total suspended solids and volatile suspended solids (VSS) in UASB reactor, and mixed liquor suspended solids (MLSS), and mixed liquor volatile suspended solids (MLVSS), in aeration tank of CSTR. Assays were performed according to Standard Methods for Examination of Water and Wastewater (APHA AWWA, 1992).

2.4.10. Anaerobic toxicity assay (ATA) and specific methanogenic activity (SMA)

ATA test was performed at 37°C using serum bottles with a capacity of 115 ml as described by Owen et al. (1979) and Donlon et al. (1995). Serum bottles were filled with stock solutions to give suitable Vanderbilt mineral medium, 3000 mg /l of glucose-COD, 667 mg /l of sodiumthioglycollate for providing reducing conditions. Furthermore, 5000 mg /l of $NaHCO_3$ for maintaining the neutral pH, 4000 mg MLSS /l providing anaerobic granules enriched in anaerobic treatment plant of yeast industry. The glucose COD in the serum bottles was stoichiometrically replenished

to 3000 mg /l with stock solution after 3 days of exposure to dye containing synthetic wastewater. The glucose COD level of 3000 mg /l ensured the presence of non-limiting substrate conditions in the serum bottles. Duplicate controls were performed on the assay containing no-dye. Methane production of each assay bottle was determined during subsequent 6 h of incubation period. Maximum specific methanogenic activity was calculated from the methane production through this period. Methanogenic activities of samples containing inhibitory dyes were compared to the control samples to determine the degree of azo dyes inhibition effect on glucose utilization. Inhibition was defined as a decrease in cumulative methane compared to the control sample.

2.4.11. Respiration-inhibition Test

This test was based on BOD measurements and the simulation to ATA test. Respiration-Inhibition tests were carried out into bottles of WTW Oxi Top IS 12 system. Aerobic sludge from the lab scale CSTR reactor was fed with molasses, mineral medium, glucose-COD of 1500 mg/l. Furthermore, wastewater samples were added to bottles in certain volumes. The final volume was adjusted to 97 ml with distilled water. The glucose COD in the bottles was stoichiometrically replenished to 1500 mg /l with stock solution after 3-4 days of exposure to synthetic wastewater. Duplicate controls were performed in the bottles containing no toxic substances (dye free wastewater). Oxygen consumption was monitored every day and compared to the control samples. Inhibition was defined as a decrease in oxygen consumption compared to the control sample.

2.4.12. LUMIStox toxicity assay

Toxicity to the bioluminescent organism *Vibrio fischeri* was assayed using the LUMIStox measuring system according to DIN 38412 L34, L341, (1993). LUMISmini type photometer (Dr. LANGE Campany) was used for the lumistox assay. All samples were serially diluted in 2% NaCl (w/v), and each assay was performed at pH 7.0 and a temperature of 15°C. Sodium chloride (2%) was used as

the control. Inhibition percentage (I %) values refer to decreasing activity in samples causing inhibitory effect of a test substances and/or a toxic wastewater during the light emission. Color correction was performed according to the DIN 38412 instructions.

2.4.13. *Daphnia magna* toxicity test

Toxicity was tested using 24 h born *Daphnia magna*. Test animals were obtained from the Zoology Department of the Engineering Faculty at Aegean University in Izmir. After a cloning periods in our laboratory, toxicity experiments were performed. Experiments were carried out using 5 daphnids in test beakers with 100 ml of effective volume at 7-8 pH, proving minimum 6 mg/l of dissolved oxygen at ambient temperature (20-30°C). A blank was also carried on. Results were expressed as mortality percentage of the daphnids after 24 h. The immobile animals which were not able to move were determined as death of daphnids.

2.4.14. High performance liquid chromatography–diode array detection (HPLC-DAD) and gas chromatography- mass spectra (GC-MS)

HPLC analyses were carried out using LC 10 model (Shimadzu) HPLC-DAD equipped with C 18 (Russia) chromatographic column (i.d. 4mm, length 25 cm, stationary phase particle size 7μ).In the operation mobile phase: methanol/water (55:45) was used at a flowrate of 1 ml/min. All samples were diluted at a ratio of 1:10 with distilled water.

For GC-MS analysis, the samples were extracted by liquid/liquid extraction as follows: The aqueous sample first adjusted with sodium hydroxide to a pH greater than 10. Sodium chloride was added until reached saturation. The sample then was extracted twice with 25 ml of methyl-*tert*-butyl-ether. The ether fraction are combined and evaporated to dryness. Finally, the extract is reconstituted in 2 ml of methanol for GC/MS analysis. The analyses were done using a Shimadzu QP5050A model GC-MS device. The mass spectrometer was operated in the

electron impact mode with electron current of 70 EV. Aliquots of 1µl were injected automatically by a auto sampler (AUC20i) in splitless mode via an GC inlet (injector temperature 250 °C). A Optima -5-MS capillary column, 30 m long, 0.25 mm i.d., 0.25 µm film thickness was connected directly to the ion source of the mass spectrometer. The oven temperature was isothermal for 10 min at 60°C initially kept, then increased to 300°C at a rate of 10°C/min, and finally 10-min hold step was applied at 300°C.

GC-MS and HPLC-DAD analyses were performed in TÜBITAK Bursa Test and Analysis laboratories.

2.5. Kinetic Models for Batch Studies

2.5.1. Kinetic model based on Monod equation

Monod type kinetic models have been widely used to describe the apparent decolorization/biodegradation rate and co-substrate/dye removal (COD originated from glucose or dye) through simultaneous dye degradation and decolorization (Chang et al., 2001). The substrate removal rate in the Monod kinetic is commonly expressed by a deterministic model in a batch reactor as follows (Grady et al., 1999):

$$\frac{dS}{dt} = R = \frac{R_{max} * S}{K_S + S} \qquad (2.11)$$

with integration and partial fractionation of Eq.(2.11), and assumptions of X is constant, R_{max} is equal to $k_{max}.X$ and $S=S_0$ at $t_0=0$ (Equation 2.12):

$$\ln\frac{S_0}{S_i} * \frac{1}{t_i} = -\frac{1}{K_S} * \frac{S_0 - S_i}{t_i} + \frac{R_{max}}{K_S} \qquad (2.12)$$

where;

R, substrate utilization rate (mg/l.h)

R_{max}, (k_{max} .X) maximum substrate removal rate (mg/l.h)

k_{max}, maximum specific substrate utilization rate (1/h)

X, biomass concentration (mg/l)

S, co-substrate concentration (mg/l)

K_S, half saturation concentration (mg/l)

2.5.2. Kinetic model for co-substrate degradation through decolorization of azo dye

Although there has been some success in applying Monod type kinetics to the dye decolorization process, some investigations showed that it is difficult to apply them for the anaerobic systems. Therefore, the removal of co-substrate throughout decolorization of the azo dyes can follow zero, first and second order reaction kinetic which can be expressed by equations (2.13), (2.14) and (2.15).

$$S_t = S_0 - k_0 * t \qquad (2.13)$$

$$S_t = S_0.e^{-k_1 * t} \qquad (2.14)$$

$$\frac{1}{S_t} = \frac{1}{S_0} + k_2 * t \qquad (2.15)$$

where;

S_t, residual glucose-COD concentration at selected time (t) through co-substrate removal (mg/l)

S_0, glucose-COD concentration at the beginning of the batch incubation through co-substrate removal (mg/l)

k_0, zero order rate constant through co-substrate removal (mg/l.h)

k_1, first order rate constant through co-substrate removal (1/h)

k_2, second order rate constant through co-substrate removal (l /mg .h)

t, time (h)

Similarly, zero, first and second order kinetics were confirmed for azo dye decolorization in batch anaerobic mixed cultures and were expressed in equations (2.16), (2.17) and (2.18):

$$C_t = C_0 - K_0 * t \qquad (2.16)$$

$$C_t = C_0 * e^{K_1 * t} \qquad (2.17)$$

$$\frac{1}{C_t} = \frac{1}{C_0} + K_2 * t \qquad (2.18)$$

where;

C_t, residual dye concentration at selected time (t) of batch test through decolorization (mg dye /l)

C_0,dye concentration at the beginning of the incubation through decolorization (mg/l)

K_0, zero order rate constant through decolorization (mg/l.h)

K_1, first order rate constant through decolorization (1/h)

K_2, second order rate constant through decolorization (l /mg.h)

2.5.3. Determination of kinetic coefficients

0^{th}, 1^{st} and 2^{nd} order reaction kinetic and Monod kinetic were applied to the residual COD values obtained from the removal of co-substrate throughout the batch incubation period in order to detect the substrate degradation kinetic. Experimental data obtained from the batch tests were plotted in form In (S_0/S) / t versus (S_0-S) / t, S versus time, ln S versus time, and 1/S versus time using equations (2.12), (2.13), (2.14) and (2.15), respectively.

The residual dye concentrations remained in the batch fed anaerobic reactors were plotted in form C versus time, ln C versus time, and ln C/C_o versus time using equations (2.16), (2.17) and (2.18), respectively in order to determine the decolorization kinetic.

2.6. Kinetic Approaches in Anaerobic/Aerobic Continuous Studies

Process modeling is a useful tool for describing and predicting the performance of anaerobic digestion systems. Monod type kinetic models have been widely used to describe the process kinetics of anaerobic digesters (Anderson et al., 1996). Although there has been some success in applying Monod type kinetics to the anaerobic process, some research workers found it difficult to apply them for their systems. For instance, Grady et al. (1972) have shown that the effluent substrate concentration, expressed as COD, was not dependent to the substrate concentration entering the reactor when pure or heterogeneous cultures were used.

In the equation proposed by Contois (1959), the specific growth rate was considered as a function of the growth limiting nutrient in both influent and effluent substrate concentration by using an empirical constant, which was related to microbial concentrations. On this basis, Chen and Hashimoto (1980) developed a kinetic model for substrate utilization and methane production and suggested that the Contois type kinetic models would be more suitable than the Monod type kinetic models to predict the performance of a digester.

2.6.1. Application of Monod kinetic

For a UASB reactor without biomass recycle, the rate of change of biomass in the system can be expressed as Equation (2.19):

$$\frac{dX}{dt} = \frac{Q}{V} * X_0 - \frac{Q}{V} * X_E + \mu * X - K_d * X \qquad (2.19)$$

where;

X_0, X, X_E concentrations of biomass in the feed, reactor and reactor effluent.

μ, K_d, specific growth rate and death rate constant, respectively.

The ratio of the total biomass in the reactor to biomass wasted per given time represent the average time that microorganisms spend in the reactor. This parameter

called as mean cell-residence time (θ_C) and calculated from the Eq. 2.20 for UASB reactor.

$$\theta_C = \frac{V * X}{Q * X_E} \tag{2.20}$$

If it is assumed that the concentration of biomass in the influent can be neglected, at steady-state dX/dt= 0, and the HRT(θ_H) is defined as the volume of the reactor divided by the flow rate of the influent, the relationship between the specific growth rate and the rate limiting substrate concentration can be expressed by the Monod equation (2.21):

$$\mu = \frac{\mu_{max} * S}{K_S + S} \tag{2.21}$$

Equation (2.19) reduced to as follows:

$$\frac{Q}{V} * X_E = X * (\mu - K_d) \tag{2.22}$$

$$\frac{Q * X_E}{V * X} + K_d = \mu \tag{2.23}$$

$$\mu = \frac{1}{\theta_C} + K_d \tag{2.24}$$

$$\frac{\mu_{max} * S}{K_S + S} = \frac{1}{\theta_C} + K_d \tag{2.25}$$

This equation can be rearranged to estimate the effluent substrate concentration at the steady state condition as follows:

$$S = \frac{K_S * (K_d + \frac{1}{\theta_C})}{\mu_{max} - K_d - \frac{1}{\theta_C}} \tag{2.26}$$

The rate of change in substrate concentration in the system could be expressed as the equation (2.27):

$$-\frac{dS}{dt} = \frac{Q}{V} * S_0 - \frac{Q}{V} * S - \frac{\mu * X}{Y} \qquad (2.27)$$

where;

Y, is the yield coefficient (g VSS / g COD), S_0, the substrate concentration in the feed (g COD / l).

Under steady-state conditions, the rate of change in substrate concentration (-dS/dt) is negligible and by a similar technique to that used for the substrate concentration, the above equation with substituting Eq. (2.24) can be reduced to equation 2.28

$$\frac{(S_o - S)}{\theta_H} = \frac{X}{Y} * (\frac{1}{\theta_C} + K_d) \qquad (2.28)$$

The above equation can then be rearranged to estimate the effluent biomass concentration under steady-state condition as follows:

$$X = \frac{\theta_C * Y * (S_0 - S)}{\theta_H * (1 + K_d * \theta_C)} \qquad (2.29)$$

The kinetic parameters Y, K_d can be obtained by rearranging Eq. (2.28) as shown below:

$$\frac{(S_0 - S)}{\theta_H * X} = \frac{1}{Y} * (\frac{1}{\theta_C}) + \frac{1}{Y} * K_d \qquad (2.30)$$

By plotting Eq. (2.30), the values of Y and K_d can calculated from the slope and intercept of the line. The value of μ_{max} and K_S could be determined plotting Eq. (2.31), which was derived by rearranging Eq. (2.25). The value of μ_{max} can then be

calculated from intercept of the straight line while K_S can be obtained from the slope of the line.

$$\frac{\theta_C}{1+\theta_C * K_d} = \frac{K_S}{\mu_{max}} * \frac{1}{S} + \frac{1}{\mu_{max}} \qquad (2.31)$$

2.6.2. Contois kinetic model

A similar technique was used to develop a kinetic model based on the Contois equation. The relationship between the specific growth rate and the rate limiting substrate concentration can be expressed by the Contois equation as follows:

$$\mu = \frac{\mu_{max} * S}{\beta * X + S} \qquad (2.32)$$

where;
β is the kinetic parameter (g COD/g biomass).

By substituting Eq. (2.32) instead of the Monod equation into Eq. (2.19) can be obtained Eq. (2.33):

$$\frac{\mu_{max} * S}{\beta * X + S} = \frac{1}{\theta_C} + K_d \qquad (2.33)$$

Substituting Eq. (2.29) into Eq. (2.33) and then rearranging it, the effluent substrate concentration at steady state condition can be expressed using Eq. (2.34):

$$S = \left(\frac{\beta * Y}{(\mu_{max} - K_d) * \theta_C + \beta * Y - 1}\right) * S_0 \qquad (2.34)$$

In this model, the equation for the effluent biomass concentration has the same expression as Eq. (2.29) due to a similar technique being used.

Eq. (2.26) and Eq. (2.29) form the basis of the Monod type model while Eq. (2.34) and Eq. (2.29) form the basis of the contois type model. If the kinetic parameters are

known Eq. (2.21) Eq (2.29) and Eq. (2.26) can be used to predict the effluent subsrate concentration and the microbial biomass concentration under steady state.

Similarly, the values of $\mu_{max,}$ and β can be obtained by plotting Eq. (2.35), which is obtained by rearranging Eq. (2.33). The value of μ_{max} can be calculated from the intercept of the straight line and finally, β could be obtained from the slope of the line.

$$\frac{\theta_C}{1+\theta_C * K_d} = \frac{\beta}{\mu_{max}} * \frac{X}{S} + \frac{1}{\mu_{max}} \qquad (2.35)$$

2.6.3. Chen-Hashimoto kinetic model

A reduction of 1 g COD is equivalent to production of 0.35 l of methane at standard conditions of temperature and pressure (STP). Knowing the COD loading applied to the reactor and the volume of methane produced, the remaining COD in the digester can be calculated.

If B denotes the volume (in litre) of methane produced under normal conditions of pressure and temperature per gram of substrate (COD) added into the anaerobic reactor, and B_0 is the volume of methane produced under normal conditions of pressure and temperature per gram of substrate added at infinite retention time or for complete utilization of substrate, the biodegradable COD in the reactor will be directly proportional to (B_0-B), and B_0 will be directly proportional to the biodegradable COD loading (Chen &Hashimoto, 1980). Therefore from Eq (2.34) obtained from the Contois kinetic model could be equalized as follows:

$$\frac{(B_0 - B)}{B_0} = \left(\frac{\beta * Y}{(\mu_{max} - K_d) * \theta_C + \beta * Y - 1}\right) \qquad (2.36)$$

Eq. (2.36) can rearranged as Eq. (2.37):

$$\theta_C = \frac{1}{\mu_{max} - K_d} + \frac{K}{\mu_{max} - K_d} * \frac{B}{B_0 - B} \qquad (2.37)$$

where; K is a dimensionless kinetic parameter equal to Y.β.

Minumum sludge retention time indicating the washout of microorganisms is equal to the reciprocal of the maximum growth rate minus death rate constant.

$$\theta_{C_{min}} = \frac{1}{\mu_{max} - K_d} \qquad (2.38)$$

In order to obtain the parameter B_0 Eq. (2.39) could be used, which is derived from Eq. (2.36):

$$B = B_0 * (1 - \frac{K}{(\mu_{max} - K_d) * \theta_C - 1 + K}) \qquad (2.39)$$

When $\frac{\theta_C}{\theta_{C_{min}}} = (\mu_{max} - K_d).\theta_C >> (1 - K)$, the graphs of B versus $\frac{1}{\theta_C}$ are straight line in which B coincides with B_0 when the retention time is infinite, therefore the intercept of the lines coincides with B_0 as in Eq.(2.40):

$$B = -\frac{B_0 * K}{(\mu_{max} - K_d)} * \frac{1}{\theta_C} + B_0 \qquad (2.40)$$

Thus, by first calculating the value of B_0, the graph of θ_C versus $\frac{B}{B_0 - B}$ produces a straight line with intercept of $\frac{1}{\mu_{max} - K_d}$ and with a slope of $\frac{K}{\mu_{max} - K_d}$.

2.6.4. Grau first-order multicomponent substrate removal model

The general equation of a first-order kinetic model is illustrated in Eq. (2.41) (Grau et al., 1975)

$$-\frac{dS}{dt} = k_f' * X * \frac{S}{S_0} \qquad (2.41)$$

Taking into account that the value of X was constant during the experiment and, hence, $k_f' * X$ is a constant (k_f). If Eq. (2.41) is integrated and then linearilized, Eq. (2.42) will be obtained:

$$-\ln(S/S_0) = \frac{k_f * \theta_H}{S_0} \qquad (2.42)$$

if p is equal to k_f / S_0 in order to calculate the value of kinetic constant of the first order model the equation (2.42) transformed as follows:

$$-\ln(S/S_0) = p * \theta_H \qquad (2.43).$$

As p and S_0 are constants for each loading conditions studied plotting $-\text{Ln}(S/S_0)$ against θ_H, will yield a straight line. By fitting the data to a linear function, using the least-squares method, the slopes (p) of the lines can be calculated. Finally k_f can be determined by the equation:

$$k_f = p * S_0 \qquad (2.44)$$

where;

S is the concentration of the organic matter at any time (g COD/l),

S_0 is the initial organic matter concentration (g COD/l),

k_f' is a first order multicompenant substrate removal kinetic constant (1/day)

X is the initial concentration of microorganisms expressed as g VSS /l,

θ_H, hydraulic retention time (day)

2.6.5. Grau second-order multicomponent substrate removal model

The general equation of a second-order kinetic model is illustrated in Eq. (2.45) (Grau et al., 1975., Öztürk et al., 1998)

$$-\frac{dS}{dt} = k_s * X * (\frac{S}{S_0})^2 \qquad (2.45)$$

If Eq. (2.41) is integrated and then linearilized, Eq. (2.46) will be obtained:

$$\frac{S_0 * \theta_H}{S_0 - S} = \theta_H + \frac{S_0}{k_s * X} \qquad (2.46)$$

If the second term of the right part of Eq. (2.46) is accepted as a constant, the Eq. (2.47) will be obtained,

$$\frac{S_0 * \theta_H}{S_0 - S} = b * \theta_H + a \qquad (2.47)$$

where;

a, So/(k_s.X) (day)

b, constant

(So-S)/So expresses the substrate removal efficiency and is symbolized as E. Therefore, the last equation can be written as follows:

$$\frac{\theta_H}{E} = a + b * \theta_H \qquad (2.48)$$

where;

S and So are effluent and influent substrate concentrations (mg COD/l)

X, the average biomass concentration in the reactor (mg VSS/l)

θ, hydraulic retention time (day)

k_s, second-order substrate removal rate constant (1/day).

2.6.6. Modified Stover-Kicannon model

Since the early 1970s, Stover and Kincannon have proposed a design concept of total organic loading rate and established a kinetic model for biofilm reactors. In this model the substrate utilization rate is expressed as functions of the organic loading rate by monomolecular kinetics for biofilm reactors such as rotating biological contactors and biological filters. A special feature of Modified Stover/Kincannon model is the utilization of the concept of total organic loading rate as the major parameter to describe the kinetic of an anaerobic filter (AF) in terms of organic matter removal and methane production (Yu et al., 1998). The removal of organic substrate in the AF process can be determined on the basis of the substrate removal rate as a function of the substrate concentration.

Equation of the modified Stover/Kincannon model are as follows (Yu et al., 1998):

$$\frac{dS}{dt} = \frac{Q}{V} * (S_0 - S) \qquad (2.49)$$

where dS/dt is defined in Eq. (2.50):

$$\frac{dS}{dt} = \frac{R_{max} * (Q * S_0 / V)}{K_B + (Q * S_0 / V)} \qquad (2.50)$$

Eq. (2.51) obtained from linearization of Eq. 2.50 as follows:

$$(dS/dt)^{-1} = \frac{V}{Q * (S_0 - S)} = \frac{K_B}{R_{max}} \frac{V}{Q * S_0} + \frac{1}{R_{max}} \qquad (2.51)$$

where;

dS/dt is substrate removal rate (g/l.day)

R_{max} is maximum utilization rate (g/l.day)

Q is inflow rate (l/day), V is volume of the UASB reactor (l)
K_B is saturation value constant (g/l.day).

2.6.7. Zero order substrate removal model

The rate of change in substrate concentration in the system with assuming zero order model for substrate removal could be expressed in Eq. (2.52):

$$-\frac{dS}{dt} = \frac{Q}{V}S_o - \frac{Q}{V}S - k_0 \qquad (2.52)$$

Under steady-state conditions, the rate of change in substrate concentration (-dS/dt) is negligible and the Eq. (2.52) can be reduced to Eq. (2.53) as follows:

$$S_0 - S = k_0.\theta_H \qquad (2.53)$$

where;
k_0 is zero order kinetic constant, (mg/l.day)

The value of k_0 can be obtained from the slope of the line by plotting Eq. (53).

2.6.8. First order substrate removal model

The rate of change in substrate concentration in the system with assuming the first order model for substrate removal could be expressed as follows:

$$-\frac{dS}{dt} = \frac{Q}{V}S_o - \frac{Q}{V}S - k_1.S \qquad (2.54)$$

where;
k_1 is first order kinetic constant, (1/day)
Under steady-state conditions, the rate of change in substrate concentration (-dS/dt) is negligible and the equation given above can be reduced to the Eq. (2.55):

$$\frac{S_0 - S}{\theta_H} = k_1.S \qquad (2.55)$$

The value of k_1 can be obtained by plotting $\frac{S_0 - S}{\theta_H}$ versus S in Eq. (2.55), which is obtained by rearranging Eq. (2.54). The value of k_1 can be obtained from the slope of the line.

2.6.9. Second order substrate removal model

The rate of change in substrate concentration in the system with assuming the second order model for substrate removal could be expressed as follows:

$$-\frac{dS}{dt} = \frac{Q}{V}S_o - \frac{Q}{V}S - k_2.S^2 \qquad (2.56)$$

where;
k_2 is second order kinetic constant, (l/mg.day)

Under steady-state conditions, the rate of change in substrate concentration (-dS/dt) is negligible and the equation given above can be reduced to Eq. (2.57):

$$\frac{S_0 - S}{\theta_H} = k_2.S^2 \qquad (2.57)$$

The value of k_2 can be obtained by plotting $\frac{S_0 - S}{\theta_H}$ versus S^2 in Eq. (2.57), which is obtained by rearranging Eq. (2.56). The value of k_1 can be obtained from the slope of the line.

Six data set obtained from the continuous operation of UASB reactor at steady state conditions were used to determine the kinetic parameters given in section 2.6 for these kinetic models.

CHAPTER THREE
RESULTS AND DISCUSSION

3.1. Batch Studies

3.1.1. Co-metabolism of azo dyes and degradation kinetic of co-substrate

The co-substrate (glucose) used in this study is defined as the carbon and energy source for microbial growth and maintenance and release of the electrons for cleavage of dyes under reducing environments. The co-metabolism of azo dyes is defined as the removal of non-growth substrates (azo dyes) by microorganisms in the presence of a growth substrate (co-substrate) (3000 mg mg/l of COD as glucose equivalent). The anaerobic bacteria utilized the electrons generated from the glucose degradation for the reductive cleavage of azo dyes. In other words, the azo dyes were removed by fortuitous metabolism in which the anaerobic bacteria do not derive energy from these dyes and could remove azo dyes after the addition of a primary substrate.

Figure 3.1. demonstrates typical residual COD (S_i) levels throughout 300 hours of incubation periods of azo dyes concentrations varying between 200 and 3200 mg/l. At lower dye concentrations the COD concentrations dropped almost linearly until degradation was completed while the profile for high dye concentrations exhibited extra phases (indicated by different slopes) during the early stage of degradation.

The remaining COD concentrations were 685 and 1673 mg/l in batch reactors containing 200 mg/l and 3200 mg/l of RB 5 azo dyes, respectively, after 100 hours of the incubation period. Similarly, the residual COD concentrations were 39 and 1562

mg /l in samples containing 200 mg/l and 3200 mg/l of RB 5 azo dye, respectively, at the end of 300 hours of the incubation period.

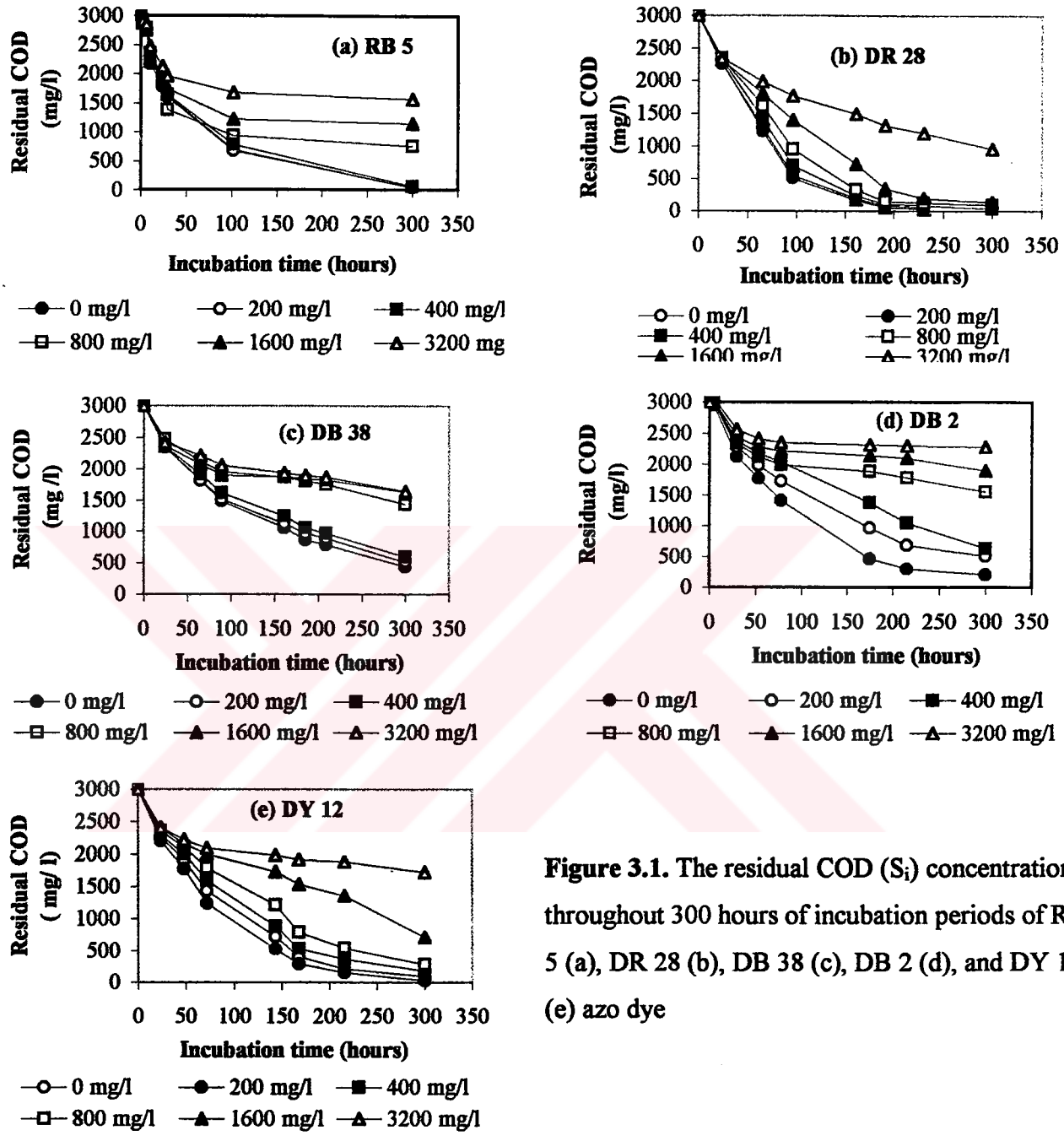


Figure 3.1. The residual COD (S_i) concentrations throughout 300 hours of incubation periods of RB 5 (a), DR 28 (b), DB 38 (c), DB 2 (d), and DY 12 (e) azo dye

The remaining COD concentration was 500 mg/l in batch reactors containing 200 mg/l of DR 28 azo dye, after 100 hours of the incubation period. Similarly, the residual COD concentrations were 0 and 954 mg/l in dye-free and in reactors containing 3200 mg/l of DR 28 dye, respectively at the end of 300 hours of the incubation period. The remaining COD concentrations were 1514 and 2054 mg/l in

batch reactors containing 200 mg/l and 3200 mg/l of DB 38 azo dye, respectively, after 89 hours of the incubation period. Similarly, the residual COD concentrations were 510 and 1645 mg /l in samples containing 200 mg/l and 3200 mg/l of DB 38 dyes, respectively, at the end of 300 hours of the incubation period.

The remaining COD concentrations were 1725 and 2350 mg/l in batch reactors containing 200 mg/l and 3200 mg/l of DB 2 azo dye, respectively, after 78 hours of the incubation period. Similarly, the residual COD concentrations were 510 and 2283 mg /l in samples containing 200 mg/l and 3200 mg/l of DB 2 dyes, respectively, at the end of 300 hours of the incubation period.

The remaining COD concentration was 1236 mg/l in 200 mg/l of DY 12 azo dye, after 72 hours of the incubation period. Similarly, the residual COD concentrations were 97 and 1717 mg /l in dye free and 3200 mg/l of DY 12 dye containing samples, respectively at the end of 300 hours of the incubation period.

The results given above indicates that the COD was biodegraded faster and better in batch reactors containing low concentrations of dyes and dye-free samples compared to higher concentrations of dyes containing reactors. Furthermore, the COD was biodegraded faster and better in batch reactors containing DR 28 azo dye than in the samples containing other dyes (RB 5, DB 38, DB 2, and DY 12). The high residual COD concentrations could be attributed to the presence of residual dye and dye metabolites that were not completely mineralized. The dye contribution to the feed COD was 524, 746, 370, 1034 and 497 mg /l in 1000 mg /l of RB 5, DR 28, DB 38, DB 2 and DY 12 solution, respectively, so the measured levels of residual COD indicate that the metabolites seem to introduce a significant level of COD in the batch reactors. This result suggests that degradation of intermediates did not took place, contrarily to the data obtained by Brás et al. (2001) and Razo Flores et al. (1997).

Figures 3.2 a, b, c, and d show the plots between In $(S_0/S_i)/t_i$ versus $(S_0-S_i)/t_i$, and S_i, ln S_i and $1/S_i$ versus time using equations (2.13), (2.14), (2.15) and (2.16), respectively in order to determine the kinetic constants through the degradation of

co-substrate at DR 28-free samples. The kinetic and correlations coefficients relevant to the Monod, the zero, first and second orders are summarized in Table 3.1 for all azo dyes in batch reactors. As shown in this table, the comparison of regression coefficients (R^2) relevant to Monod, zero, first, second order rate constants (K_S, R_{max}, k_0, k_1 and k_2 values) showed that COD was removed according to the first order reaction kinetic. Increases in dye concentrations from 0 to 3200 mg /l reduced the k_1 values from 0.0141 to 0.0019 1/h in batch studies performed with RB 5 azo dye. Similarly, the k_1 values decreased from 0.0096 to 0.0008 1/h as the dye concentrations increased from 0 to 3200 mg /l in batch reactors containing DB 2 azo dye. Increases in dye concentrations from 0 to 3200 mg /l reduced the k_1 values from 0.0187, 0.0062, and 0.012 to 0.0035, 0.0017, 0.0015 1/h in batch studies for DR 28, DB 38 and DY 12 azo dyes, respectively.

Similar batch studies performed by Brás et al. (2001) demonstrated that the COD degradation rate constant (first order kinetics) decreased from 0.04 1/h to 0.016 1/h while the Acid Orange concentration increased to 300 mg /l.

3.1.2. Decolorization kinetics of azo dyes

In order to test the effect of pH on the dye spectra solutions of dyes were scanned at pH values of around 4.0, 7.0, and 10.0, represent acidic, neutral, and alkaline conditions. This case is shown in Figure 3.3. A significant change was observed for Direct Red 28 with converting to blue from red color around pH 5. It is assumed that the spectrum of all dyes was not affected by pH changing at experiment conditions during incubation.

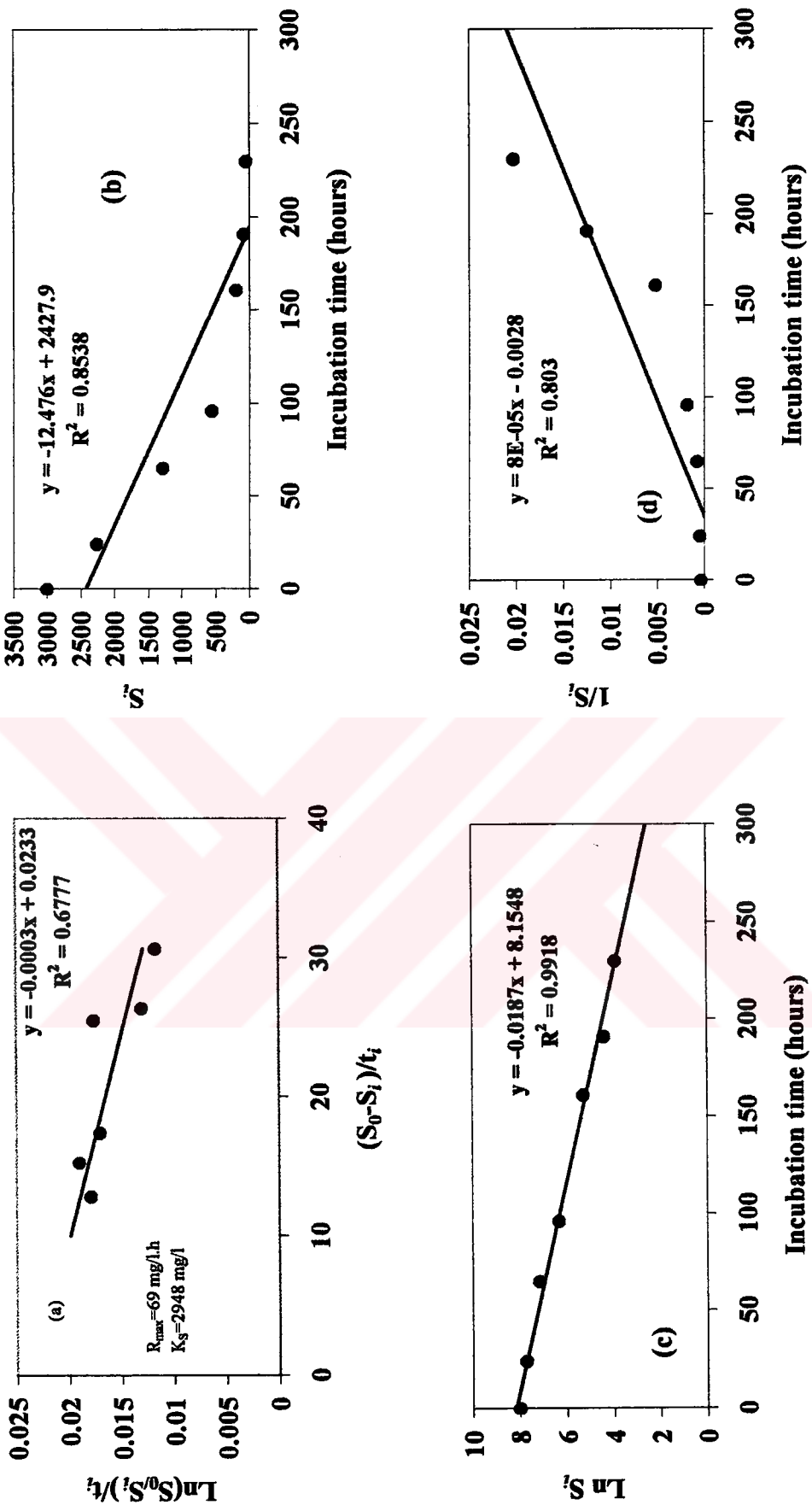


Figure 3.2. Monod (a), zero (b), first(c), and second (d) order reaction kinetic for 0 mg/l of DR 28 azo dye

Table 3.1. Zero, first, second orders and Monod kinetic constants obtained in anaerobic batch tests during COD degradation

Dye		Constants	0 mg/l	200 mg/l	400 mg/l	800 mg/l	1600 mg/l	3200 mg/l
RB 5	0.	k_0 (mg/l.h)	9.1	9.0	9.1	6.6	5.3	4.1
		R^2	0.763	0.749	0.772	0.589	0.548	0.541
	1.	k_1 (1/h)	0.0141	0.0142	0.0130	0.0044	0.0029	0.0019
		R^2	0.997	0.996	0.996	0.716	0.641	0.606
	2.	k_2 (l /mg.h)	0.0000806	0.0000836	0.0000580	0.0000033	0.0000017	0.0000009
		R^2	0.920	0.920	0.925	0.834	0.723	0.673
	Monod	R_{max} (mg/l.h)	69	57	74	72	375	-
		K_S (mg/l)	2948	2064	3799	4832	36400	-
		R^2	0.678	0.760	0.570	0.332	0.010	
DR 28	0.	k_0 (mg/l.h)	12.5	12.6	12.6	12.6	11.9	6.9
		R^2	0.854	0.847	0.800	0.837	0.911	0.894
	1.	k_1 (1/h)	0.0187	0.0213	0.0159	0.0132	0.0111	0.0035
		R^2	0.992	0.987	0.990	0.963	0.974	0.973
	2.	k_2 (l /mg.h)	0.0000793	0.0001429	0.0000505	0.0000344	0.0000180	0.0000021
		R^2	0.803	0.697	0.781	0.917	0.848	0.989
DB 38	0.	k_0 (mg/l.h)	8.1	7.7	7.4	4.3	3.5	3.7
		R^2	0.901	0.896	0.909	0.826	0.687	0.786
	1.	k_1 (1/h)	0.0062	0.0056	0.0051	0.0021	0.0016	0.0017
		R^2	0.995	0.991	0.991	0.895	0.756	0.856
	2.	k_2 (l /mg.h)	0.0000060	0.0000049	0.0000041	0.0000010	0.0000008	0.0000008
		R^2	0.920	0.933	0.947	0.937	0.820	0.912
DB 2	0.	k_0 (mg/l.h)	9.6	8.4	7.7	4.2	3.1	2.1
		R^2	0.875	0.908	0.955	0.742	0.691	0.551
	1.	k_1 (1/h)	0.0096	0.0061	0.0050	0.0019	0.0013	0.0008
		R^2	0.986	0.988	0.989	0.815	0.743	0.574
	2.	k_2 (l /mg.h)	0.0000155	0.0000054	0.0000037	0.0000009	0.0000006	0.0000003
		R^2	0.955	0.978	0.920	0.877	0.793	0.597
DY 12	Monod	R_{max} (mg/l.h)	226	131	-	-	-	-
		K_S (mg/l)	>3000	>3000	-	-	-	-
		R^2	0.123	0.450	-	-	-	-
	0.	k_0 (mg/l.h)	9.6	9.6	9.3	8.9	6.5	3.3
		R^2	0.871	0.839	0.893	0.930	0.939	0.718
	1.	k_1 (1/h)	0.012	0.01507	0.01	0.0078	0.0041	0.0015
		R^2	0.992	0.988	0.993	0.989	0.945	0.791
	2.	k_2 (l /mg.h)	0.0000300	0.0000884	0.0000159	0.0000098	0.0000030	0.0000007
		R^2	0.823	0.643	0.872	0.870	0.838	0.856

Azoreductase enzyme systems help bacteria to decolorize high concentrations of azo dyes with a co-substrate under anaerobic conditions (Carliell et al., 1995; Yoo, 2002; Wuhrmann et al., 1980). Taking these characteristics into consideration, the effect of increasing azo dyes concentrations on decolorization of dyes was determined. To determine the decolorization kinetic of dyes, experiments with

constant initial substrate (3000 mg /l of glucose COD) and different initial dye concentrations varying between 200-3200 mg /l were performed in order to detect the residual dye concentrations and percentages throughout 25-50 hours of the incubation period. The residual dye percentages for different initial dye concentrations are depicted in Figure 3.4 for azo dyes.

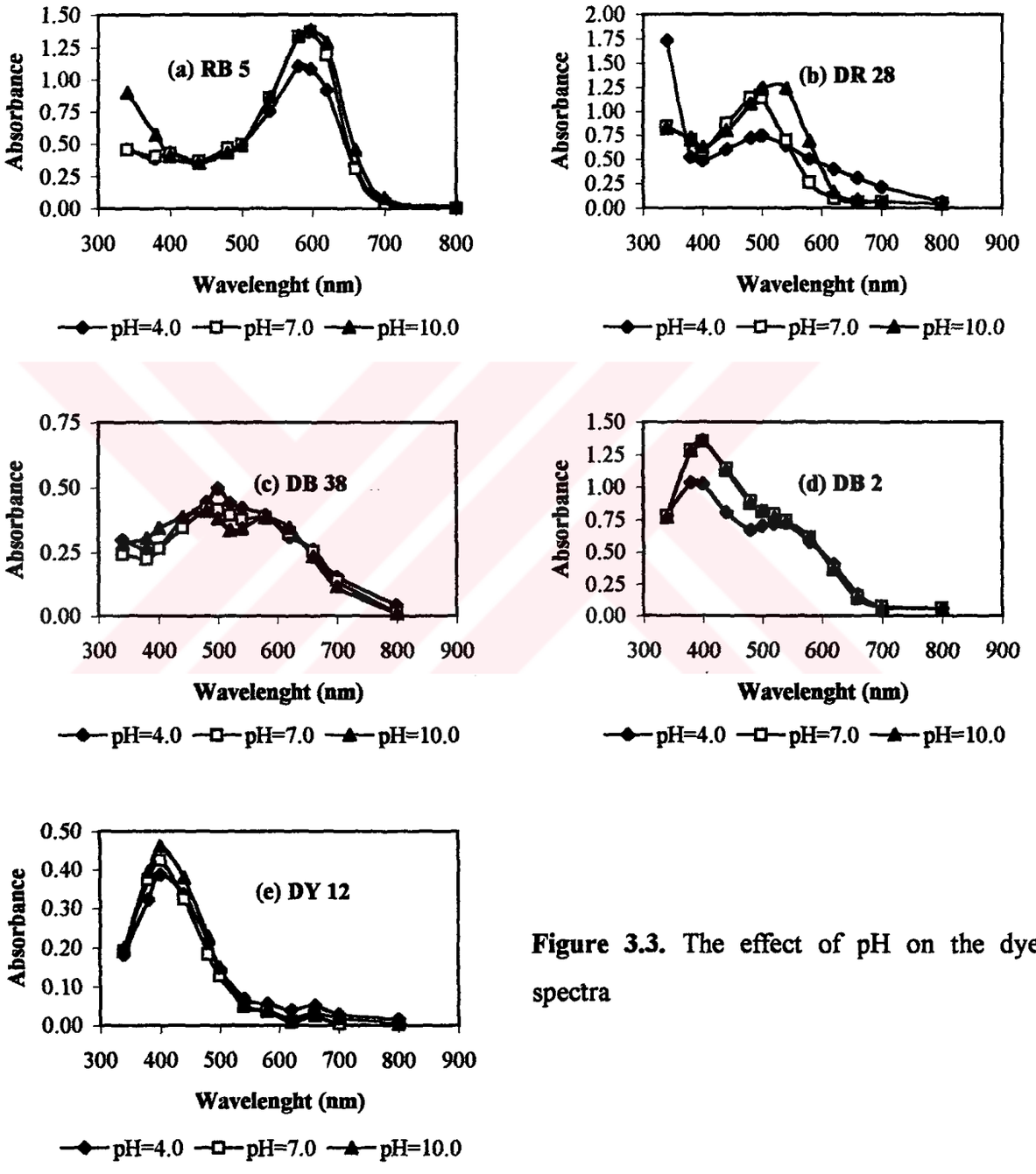


Figure 3.3. The effect of pH on the dyes spectra

Abiotic tests performed with autoclaved anaerobic partially granulated sludge showed that the microbial decolorization was preceded primarily by biological degradation. As shown in Figure 3.4, the color remained almost completely in abiotic samples, and only 10 % of the color was removed in this way in both tests carried out with the RB 5, DR 28, DB 38 and DB 2 azo dyes. In other words, more than 90 % color removal was obtained in biotic conditions incubation compared to abiotic conditions since last one led to only 10% color removal, which was probably due to physical adsorption of dye molecules by the cells dying or by auto-oxidation. (Bras et al, 2001). This fact also implies that the physical adsorption of dye molecules on cell mass was a negligible mechanism in color removal.

Figure 3.4 shows the residual color percentages for azo dyes, RB 5, DR 28, DB 38 and DB 2, respectively, during the 30 and 55 hours of incubation period. The average residual color was 51% in batch reactors containing 200, 400, 800, 1600 and 3200 mg /l RB 5 azo dye in the first 4 hours followed by significant decolorization and the next 10 hours resulting in 25 % residual color. After 30 hours of the incubation period the residual color percentages were almost zero, indicating the color was not completely removed (nearly 90-95 % for RB 5) in all RB 5 concentration (Figure 3.4a). The average residual color (%) was between 78 % and 95 % in batch reactors containing 200, 400, 800, 1600 and 3200 mg /l DR 28 azo dye, in the first 10 hours. After 54 hours of the incubation period the residual color percentages were almost 10, indicating the color was not completely removed for 200 mg /l of DR 28 (Figure 3.4b). Residual colors were 41%, and 55% in batch reactors containing 200 and 400 mg /l of DB 38 azo dyes, respectively, with the first 10 hours followed by significant decolorization. After 30 hours of the incubation period the residual color percentages were 20 and 10% for 200 and 400 mg /l of DB 38, respectively, indicating the color was not completely removed (Figure 3.4c). The average residual color was 89% in batch reactors containing 200, 400, 800, 1600 and 3200 mg /l DB 2 azo dye, respectively, with the first 4 hours followed by significant decolorization and the next 10 hours resulting in 56 % residual color. After 30 hours of the incubation period the residual color percentages were almost zero, indicating the color was not

completely removed (nearly 70 % for DB 2 azo dye) in all concentration of DB 2 azo dye.

As seen in Figure 3.4, all the color removal reactions proceeded without a lag phase. The decolorization was complete or nearly complete in dyes for most of the dye concentrations. An important exception was observed for DB 2 azo dye. Compared to the azo dye RB 5, the color removal efficiency of this dye was lower, resulting in relatively high residual dye concentration. DB 2 azo dye has a slow rate of decolorization, which was not complete even after 30 hours of the incubation (See Figure 3.4d).

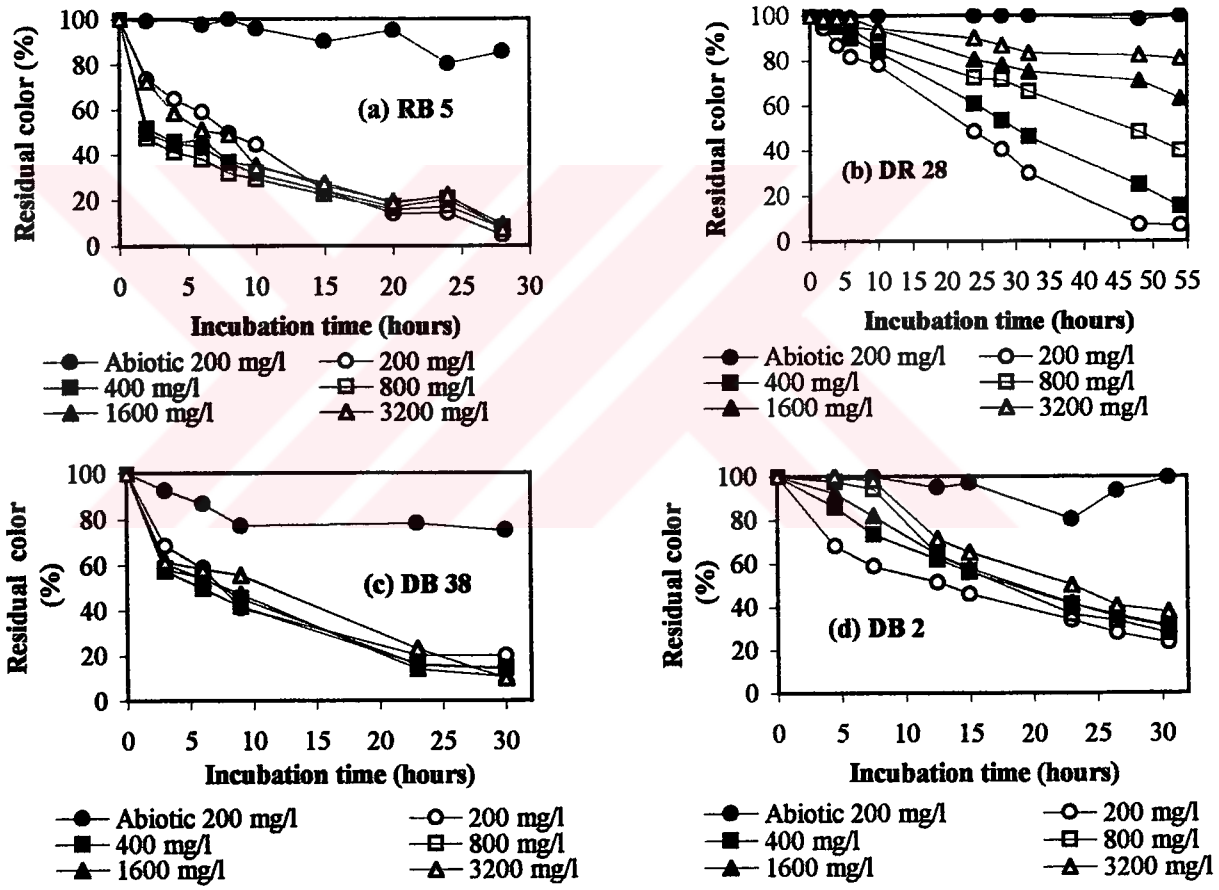


Figure 3.4. Percentages of residual color at different azo dyes concentrations throughout the incubation period

Experimental data obtained from the batch tests were plotted in form C versus time, ln C versus time, and 1/C versus time using equations (2.16), (2.17) and (2.18), respectively. In the following step, the color removal rate constants were found for

zero, first and second order reaction kinetic from the slopes of the best fit lines, respectively. Among the data obtained, the rate constants with the highest regression coefficients and relevant reaction kinetic were accepted as the most suitable kinetic and kinetic coefficients. The zero, first, and second order rate constants (K_0, K_1 and K_2), through color removals, resulting from the fitting equation (2.16), (2.17) and (2.18) to the whole curves in all dyes are listed in Table 3.2.

Table 3.2. The rate constants through decolorization of all azo dyes

Dye		Constants	200 mg/l	400 mg/l	800 mg/l	1600 mg/l	3200 mg/l
RB 5	0.	K_0 (mg/l.h)	5.8	8.7	16.6	33.4	81.6
		R^2	0.915	0.654	0.591	0.642	0.815
	1.	K_1 (1/h)	0.098	0.070	0.069	0.056	0.076
		R^2	0.954	0.880	0.885	0.878	0.917
	2.	K_2 (l /mg.h)	0.00302	0.00087	0.00042	0.00017	0.00011
		R^2	0.733	0.744	0.814	0.793	0.735
DR 28	0.	K_0 (mg/l.h)	3.6	6.4	8.7	10.4	11.8
		R^2	0.981	0.998	0.996	0.973	0.967
	1.	K_1 (1/h)	0.04996	0.0322	0.0159	0.008	0.0041
		R^2	0.949	0.957	0.976	0.975	0.974
	2.	K_2 (l /mg.h)	0.001165	0.000206	0.0000309	0.0000062	0.0000014
		R^2	0.789	0.806	0.924	0.970	0.980
DB 38	0.	K_0 (mg/l.h)	4.6	9.2	19.1	40.5	79.9
		R^2	0.805	0.771	0.812	0.846	0.883
	1.	K_1 (1/h)	0.0532	0.061	0.06335	0.07264	0.06884
		R^2	0.929	0.950	0.959	0.980	0.963
	2.	K_2 (l /mg.h)	0.00073	0.00051	0.00027	0.00018	0.00008
		R^2	0.962	0.983	0.975	0.977	0.846
DB 2	0.	k_0 (mg/l.h)	5.1	9.3	22.9	39.5	77.3
		R^2	0.850	0.980	0.965	0.997	0.951
	1.	k_1 (1/h)	0.044	0.038	0.047	0.041	0.036
		R^2	0.954	0.999	0.960	0.989	0.944
	2.	k_2 (l /mg.h)	0.000418	0.000172	0.000105	0.000046	0.000017
		R^2	0.998	0.992	0.957	0.973	0.925

If a high degree linear relationship (R^2 >0.95) were obtained between the dye concentration (C_i), ln of dye concentration (In C_i), reciprocal of dye concentration ($1/C_i$) and time can be assumed that the color was removed according to zero, first and second order kinetic in all batch decolorization tests, respectively. As seen in Table 3.2, the decolorization rate constants of RB 5 dye are significantly higher than the rate constants of other dyes when first order kinetic constants considered for all dyes. In other words, the RB5 azo dye decolorized much faster than the other dyes.

This could be attributed to the groups in the composition of azo dyes, and intermetabolites produced could not be completely degraded and exhibited inhibition as reported by Chung and Stevens (1993). The decolorization rates decreased slightly with the increases in dye concentrations for RB 5 (Table 3.2). For instance, the decolorization constants were found to be 0.098 1/h - 0.076 1/h at RB 5 azo dye concentrations of 200 mg /l and 3200 mg /l, respectively, while the same rate constants were 0.050-0.041, 0.0532-0.069, and 0.044-0.036 1/h at the same DR 28, DB 38 and DB 2 azo dye concentrations, respectively.

Among the data obtained for DR 28, the rate constants with the highest regression coefficients and relevant reaction kinetic were accepted as the most suitable kinetic and kinetic coefficients. A high degree linear relationship (R^2 >0.97) between the dye concentration (C) and time showed that the color was removed according to zero order kinetic in all batch decolorization tests. As seen in Table 3.2., the decolorization rate constants of samples containing higher concentration of DR 28 azo dye are significantly higher than the rate constants of samples containing lower concentration of DR 28 azo dye. The decolorization rate constants were found between 3.6 and 11.8 mg/l.h at DR 28 azo dye concentrations of 200 mg /l and 3200 mg /l, respectively. This result contradicts with the study performed by Weber (1991) who reported that the degradation of benzidine based azo dye in an anaerobic pond sediment followed pseudo first order kinetic with half lives ranging from 2 to 6 days.

The kinetic data obtained in this study showed that first order rate constant depend upon the dye concentration for DB 38 concentrations varying between 1600 and 3200 mg/l. However, the decolorization rate approached to second order kinetic for DB 38 concentrations varying between 200 mg/l and 800 mg/l. In this study, the decolorization constants were found to be 0.0532 1/h-0.0688 1/h at DB 38 concentrations of 200 mg/l and 3200 mg/l for first order kinetic, respectively while the same rate constants were found to be between 0.044 1/h and 0.036 1/h at DB 2 concentrations of 200 mg /l and 3200 mg /l, for first order kinetic, respectively.

First order kinetics with respect to dye concentration have also been reported by several researchers (Carliell et al., 1995; van deer Zee et al., 2001; Wuhrmann et al., 1980; Weber and Wolfe, 1987; Weber, 1991), whereas others found zero order kinetics (Dubin and Wright, 1975; Brown, 1981). In the study performed by Van der Zee et al. (2001), 297.5 mg /l (0.3 mM) of RB 5 azo dye decolorized approximately two times faster than our study with 0.208 1/h of rate constant in anaerobic conditions with granular sludge. This could be due to the extremely dense and compact structure of granules providing very high metabolic activity under anaerobic reduced conditions resulting in very high decolorization rates.

3.1.3. The effect of increasing dye concentrations on methane production during simultaneous biodegradation and decolorization

Figure 3.5. shows the cumulative methane gas productions during the incubation period in batch reactors containing 3000 mg /l glucose COD, and dyes separately at concentrations varying between 0 and 3200 mg /l. The quantities of cumulative methane gas showed that a quick initial methane production was observed due to hydrolysis and degradation of glucose together with azo dyes. Afterwards, the methane production became slower because of the accumulation of aromatic amine and volatile fatty acids, which would progress the methanogenic stage less readily, was taking place (Beydilli et al., 1998; Donlon et al., 1995). In all assays, the cumulative methane decreased as the azo dyes concentrations increased. This could have occurred during the solubilization of azo dye degradation products or intermetabolites, and, these products could inhibit the activity of anaerobic bacteria. 3000 mg /l of glucose–COD was completely converted to methane gas in dye-free batch reactors. As shown in Figure 3.5 a, b, c, d and e, the cumulative gas measured at azo dye concentrations of 3200 mg /l are about 49, 70, 50, 30 and 45% of the dye-free and 200 mg /l of dye containing reactors for RB 5, DR 28, DB 38, DB 2 and DY 12 azo dyes, respectively.

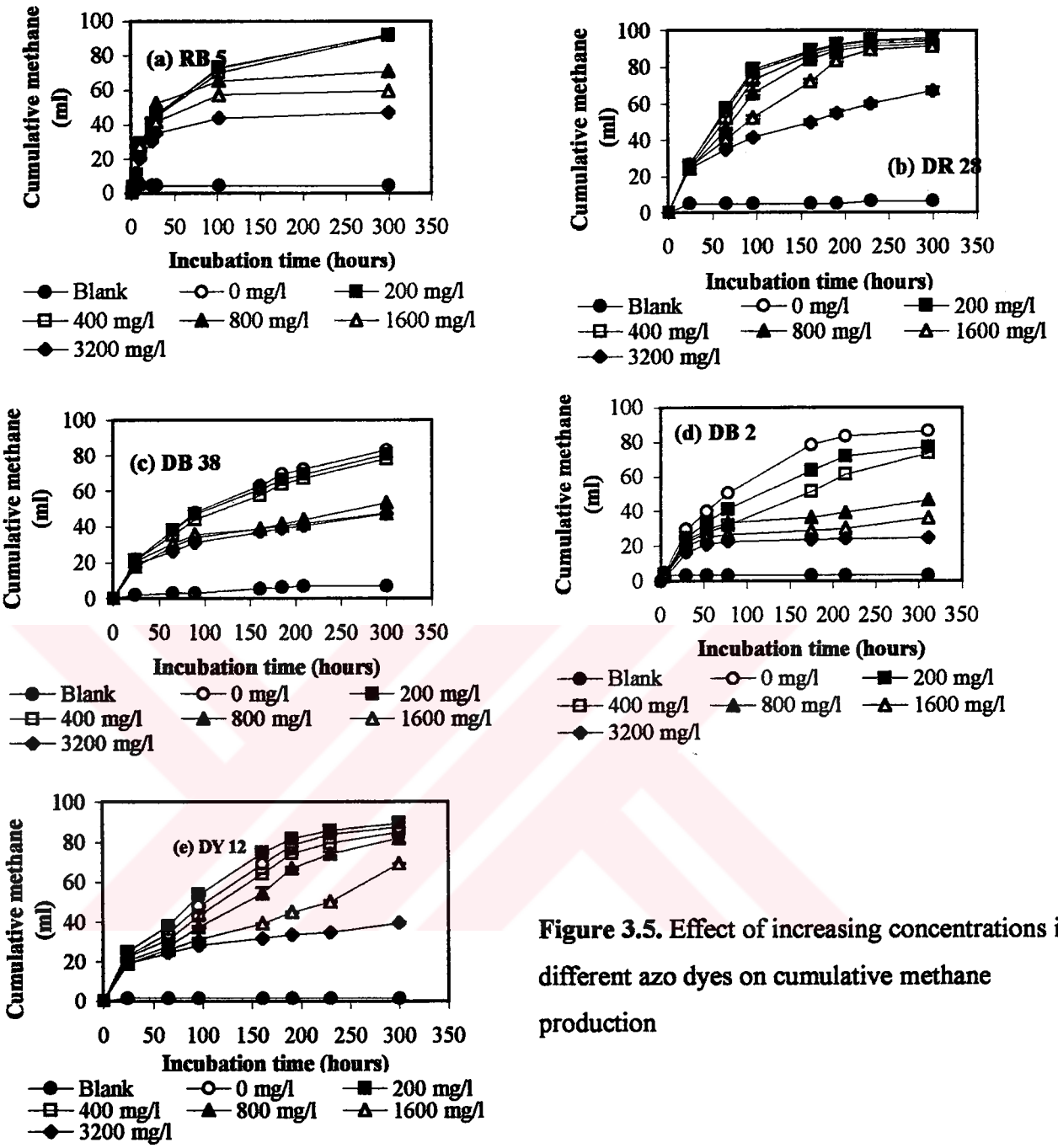


Figure 3.5. Effect of increasing concentrations in different azo dyes on cumulative methane production

It is clear that high concentrations of azo dyes caused inhibition of anaerobic degradation of glucose, resulting in lowering of methane gas during incubation. 50, 30; 50, 70 and 55% lower methane gas productions were achieved in 3200 mg /l of

dye amended cultures at the end of 300 hours of the incubation periods in RB 5, DR 28, DB 38, DB 2 and DY 12 azo dyes, respectively (Figure 3.5a, b, c, d, and e)

Carliell et al. (1995) Razo-Flores et al. (1997), and Chinwetkitvanich et al. (2000) reported that the decolorization took place under the methane production step. The bacteria could be using the azo bonds in the chromophores of the dye as an electron acceptor together with electrons coming from a H-donor co-substrate, resulting in decolorization. As a result, the level of methane formation should reflect the degree of the color removal. In our studies the methane gas production is in agreement with the residual dye concentrations measured in the batch reactors seeded with partially granulated mixed (methanogenic+acidogenic bacteria) sludge. This statement is also in agreement with those of Carliell et al. (1995) and Razo-Flores et al. (1997) but contradicts with Panswad et al. (2000) who found that decolorization with some facultative bacteria versus anaerobes. The study performed by Işık and Sponza (2003) also showed that non methanogenic bacteria are responsible for the color reduction in these processes.

3.1.4. Variations of total aromatic amine (TAA), volatile fatty acids (VFA), Bicarbonate alkalinity (B. Alkalinity) and pH in batch samples

TAA, VFA, B. Alkalinity levels and pH values were measured in all batch reactors containing azo dye concentrations ranging from 0-3200 mg/l at the end of the incubation period. The concentrations of total aromatic amines released from the azo dyes increased as the azo dye concentrations were increased. Figure 3.6 shows the variations of TAA (a) and VFA, B.Alkalinity concentrations, and pH values (b) for increasing concentrations of azo dyes. At higher azo dye concentrations, with the exception of 3200 mg/l of DB 2 azo dye, while the pH and the B. Alkalinity levels decreased, while the VFA concentrations increased. This could be due to the inhibition of methanogenesis and accumulation of inter-metabolites through simultaneous degradation and decolorization of azo dyes (Beydilli et al., 1998; Brás et al., 2001; Donlon et al., 1995). Inhibition in both acetogenesis and methanogenesis was considered at the samples containing 3200 mg/l of DB 2 azo dye. At this high

azo dye concentration no significant VFA accumulation (1067 mg/l) was observed. This may be attributed to lower acidogenic activity of acetogenic bacteria due to the inhibition of high DB 2 azo dye concentrations. The inhibition of acidogenesis was reported in a continuous fed system by Kuai et al. (1998) and Willetts & Ashbolt (2000). Figures 3.6a clearly shows the accumulation of aromatic amines (as benzidine) during the degradation of azo dyes having high concentrations. Higher azo dye concentrations cause lower methane production because of the accumulation of aromatic amines and VFA, which are known to be toxic to methanogenic bacteria (Beydilli et al., 1998; Speece, 1996).

As a result, increases in TAA, VFA concentrations and decreases in pH were observed as increases in azo dyes concentrations while the B. Alkalinity decreased as it was consumed to neutralize the synthetic medium through VFA production.

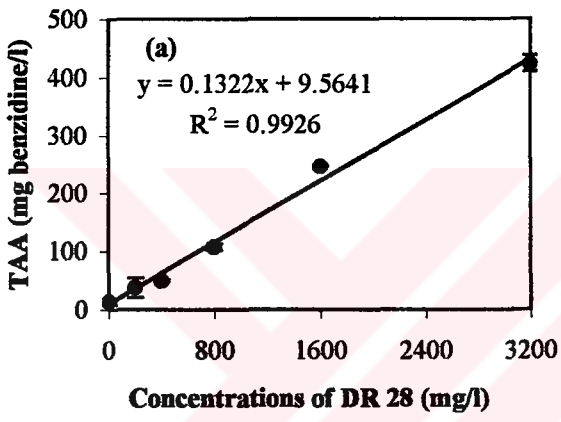
The determination of TAA could not be carried out in batch reactors containing RB 5 because the intermediates of RB 5 re-colored immediately through auto-oxidation during sampling and analyzing.

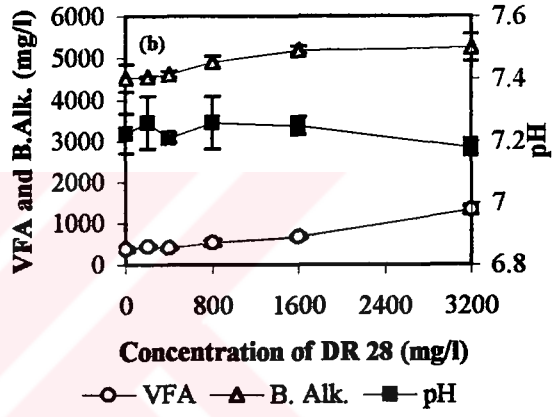
The accumulation of some intermediates, which could be explained by structural modifications of the azo dye molecule due to the azo bond reduction, producing metabolites such as aromatic amines was reported by many researchers (Knapp and Newby, 1995; Brás et al., 2001; Wuhrmann et al., 1980). The high toxicity and the carcinogenicity of these compounds were reached with the toxicity provided by VFA and TAA accumulation, resulting in the high toxicity of the aforementioned azo dye. The presence of these intermediates in decolorized samples was confirmed by some researchers (Rajaguru et al, 2000; Donlon et al., 1997; Razo-Flores et al., 1997 & Tan et al., 1999).

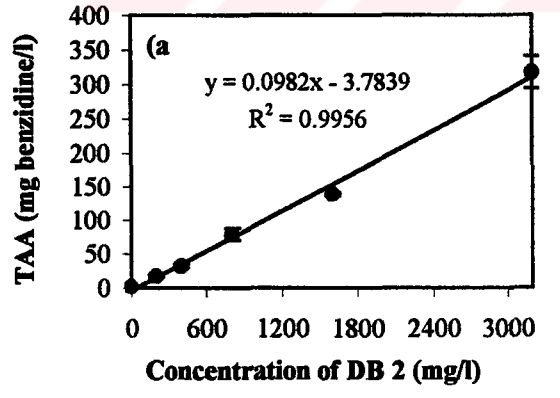
The high residual COD concentrations could be attributed to the presence of residual dye and dye metabolites that were not completely mineralized. The contribution of dyes to the fed COD was important levels, so the measured levels of

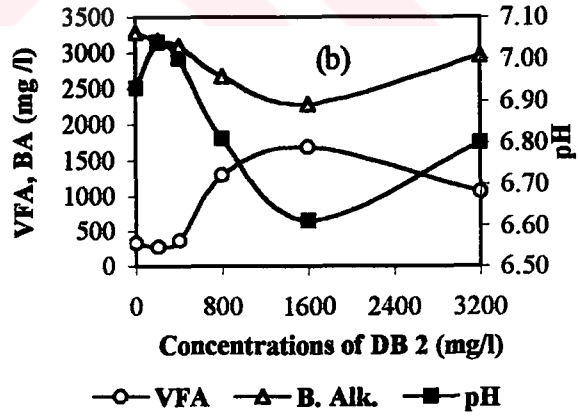
residual COD (data not shown) and TAA in supernatants indicate that the metabolites seem to introduce a significant level of COD in the batch reactors.

The accumulation of some intermediates, which could be explained by structural modifications of the azo dye molecule due to the azo bond reduction, producing metabolites such as aromatic amines was reported by many researchers (Knapp and Newby, 1995; Wuhrmann et al., 1980). Contrarily, the data obtained by Razo-Flores et al. (1997) and Brás et al. (2001). showed that degradation of intermediates took place under anaerobic conditions.









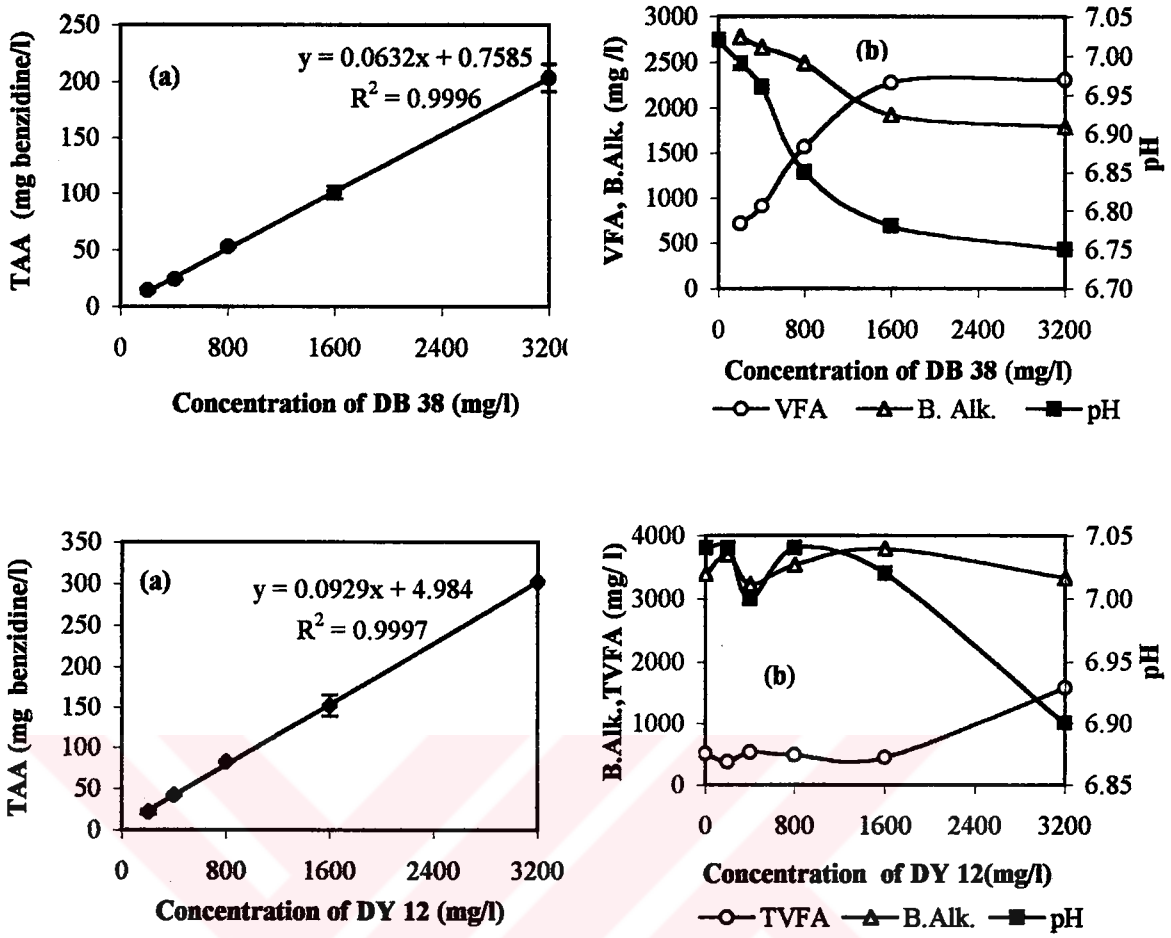


Figure 3.6. Variations in TAA (a),VFA, B.Alk. concentrations and pH values (b) after reduction of azo dyes concentrations ranging from 0-3200 mg/l

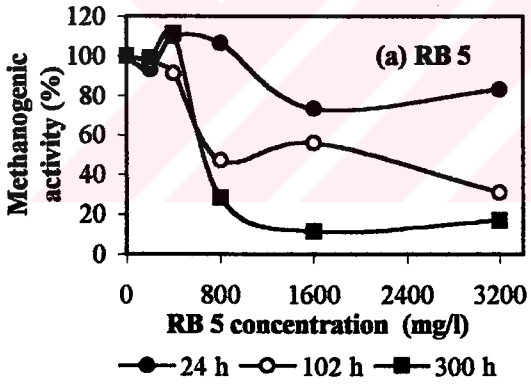
3.1.5. Assessment of anaerobic toxicity assay (ATA) results

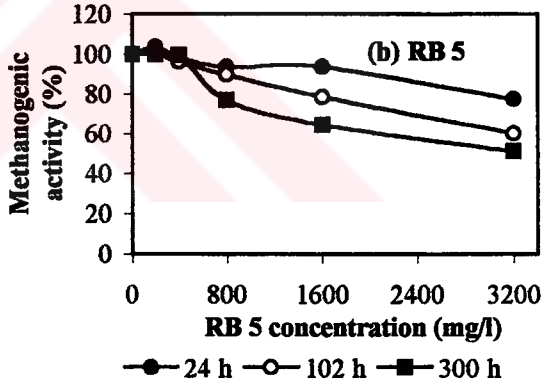
Relative methanogenic activities from the screening tests for the five dye chemicals are presented in Figure 3.6a and 3.6b which represents daily and cumulative methane activities, respectively. The screening test indicated that the relative activity varied with time. This implies that toxicity data must be interpreted carefully. At the beginning, the toxicity is less than last time. Dyes converted to aromatic amines anaerobically and produced aromatic amines thus inhibited the methanogenic activity through decolorization of azo dyes. This indicates that aromatic amines produced in reducing environments dye are more toxic than the

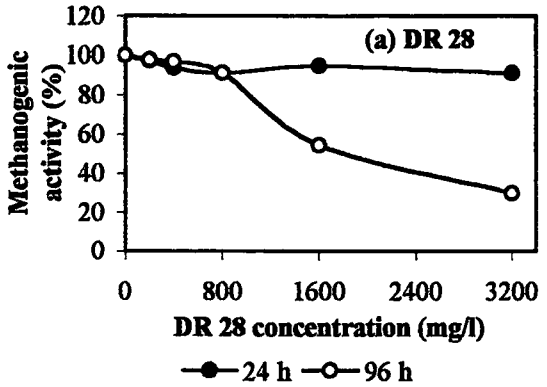
parent dye as reported by Chung & Stevens, 1993 and Hu et al, 2001. All the figures confirmed this result. These results are summarized in Table 3.3 based on IC_{25} and IC_{50} values when taking into consideration the initial gas productions. IC values given in the aforementioned Table were calculated from the gas produced in samples containing dye with comparing the gas of control samples without dyes during incubation times.

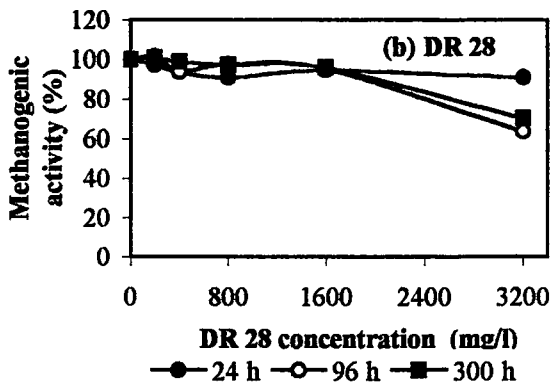
IC_{25} values are also given in Table 3.3 because the azo dyes did not cause 50% inhibition at the concentrations as high as 3200 mg/l. In that case, IC_{25} and IC_{50} values were greater than 3200 mg/l of dye concentration. These values was calculated from the slope of line on the graph given in Figure 3.7. by plotting percent methanogenic activity versus dye concentration in the beginning of incubation period. Generally, the order of toxicity on methanogenic activity increased as follows (Table 3.3):

DB 2>DB 38>RB 5> DY 12>DR 28









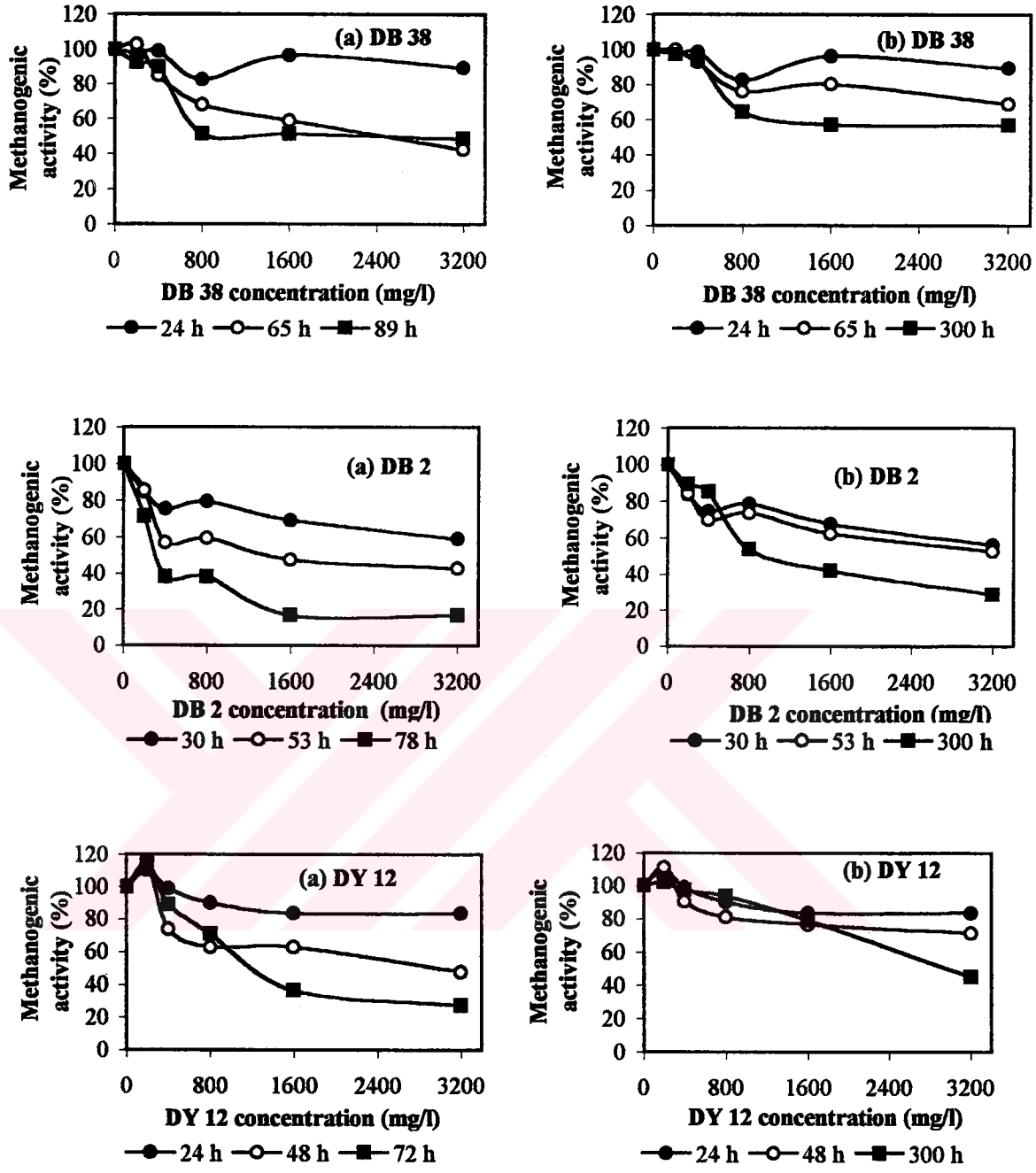


Figure 3.7. Relative activities for the various dyes (a) interval time activities (b) cumulative time activities

Table 3.3. The 25 % and 50 % of IC values observed in this study for azo dyes

Dye	Inhibiting concentration, (IC), mg/l				Incubation period (hour)
	IC_{25}		IC_{50}		
	mg/l	mM	mg/l	mM	
Reactive Black 5	3705*	3.74	7130*	7.18	24
Direct Red 28	10814*	15.52	23314*	33.46	24
Direct Black 38	2247	2.87	4907*	6.28	65
Direct Brown 2	1196	1.91	3469*	5.53	30
Direct Yellow 12	3787*	5.56	7358*	10.81	24

*values were calculated from line in graph given the decreases in activity.

In a study performed by Bell et al, (2000) reported that toxicity of dyes (IC_{50} values at varying concentrations (0.05-50 g/l) in batch anaerobic sludge were found as follows:

19.6 g/l for C.I. Yellow 3; 0.25g/l C.I. Red 3; 2.48 g/l for C.I. Brown 1; 20g/l for C.I. Red 117; >20g/l for C.I. Red 10; 14.3g/l for C.I. Yellow 4; >20 g/l for C.I. Red 7, and >20 g/l for C.I. Black 1 azo dyes .

Beydilli et al. (1998) noted that 50 and 300 mg/l concentrations of Red 2 resulted in gas production higher than the control, whereas 500, 1000 and 2000 mg/l dye concentrations inhibited the anaerobic culture activity and decreased total gas and methane gas production to 67% and 57% of the control culture, respectively, at dye concentration of 2000 mg/l. Batch decolorization studies performed by Brás et al., (2001) demonstrated that the decolorization rate constant decreased from 0.04 1/h to 0.016 1/h while the Acid Orange concentration increased to 300 mg /l.

The IC_{50} values of azo dyes given in this study and the studies presented above were higher than the IC_{50} values of other toxic aromatic compounds were reported by Speece (1996), and Donlon et al. (1995). For example, the IC_{50} values of toxic aromatic compouds were 0.081, 0.014, 0.030, 13.83, 18.81, 14.22 mM for Nitrobenzen , 2-Nitroanilin, 3-Nitroanilin, fenol, 3-aminofenol and 4-Aminofenol, respectively (Donlon et al., 1995). The IC_{50} values given in Table 3.3 show that toxicity of azo dyes are less than Nitroaromatic compounds including NO_2, and were

near to toxicity levels of aromatic amines. These results agree with the findings of Donlon et al. (1995) who reported the toxicity of the monosubstituted benzenes which they follow the order given below:

$COOH<H<OH<NH_2<NO_2$

These results are expected since aromatic amines were released with the cleavage of azo bond through batch anaerobic condition.

However, in the sample containing 200 mg l of DR 28 and DY 12, no inhibition was observed. This result is in agreement with the study performed by Kalyuzhnyi & Sklyar (2000a). They reported that low concentrations of azo dyes have stimulatory effect on degradation of substrate.

3.1.6. General discussion for batch studies

In this study glucose COD is the electron donor and consumed to provide the reducing equivalents throughout azo dye cleavage by azoreductase catalyzed reduction as reported by Zimmermann et al. (1982). The azo bonds are cleaved under anaerobic conditions to form corresponding intermediate aromatic amines while glucose-COD was converted to VFA, which was accomplished with methane conversion as reported by Carliell et al. (1995). Similarly, the experimental data found in this study showed that the glucose-COD transformed to VFA resulting in methane production while the azo bonds of dyes cleaved and transformed to aromatic amines. At high dye concentrations when TAA and VFA accumulated were accumulated; dyes are competing for the active sites of azoreductase enzyme together with substrate (glucose COD). Dyes and the intermediate substances may block the active sites of this enzyme, which inhibit the cleavage of the azo bond. In other words, at higher azo dye concentrations, the accumulation both in VFA and TAA resulting low methane productions was observed corresponding with lower rate of azo dye co-metabolism. A first order kinetic model gives the dependence of co-substrate degradation rate on initial concentration of dye which similar results was

obtained by Brás et al. (2001), van der Zee et al. (2001), and Willets and Ashbolt (2000).

Many metabolic enzymes and cofactors are induced by utilization of growth substrate through decolorization of azo dyes. A non-growth substrate (dye) is biotransformed by the specific enzymes, but it can not be utilized by microorganism to support their growth during color removal period. As the growth substrate, glucose is first converted to volatile fatty acid by acidogen bacteria while the color of the dye was removed by the cleavage of azo bond with electrons transferring from glucose under reductive environments. The kinetics of decolorization were shown to be first order with respect to dye concentration and this confirms the findings of Wuhrmann et al. (1980), Weber &Wolfe (1987), and Carliell et al. (1995) whereas other researchers found zero order kinetics (Dubin &Wright, 1975; Brown, 1981; Harmer & Bishop, 1992). A probable explanation of these contradictory observations is that the rate limiting step in the reduction of azo dyes may differ between the different co-substrate and dyes with different reactive groups studied.

Partial reduction of azo bonds (-N=N-) to form a single bond (-NH-NH) resulting from incomplete breakdown of the azo bond. The inhibitor (dye) ceased the complete breakdown of azo bond to form aromatic amines as well as partial reduction of azo bond. As the inhibition increased the activity of microorganism or the azoreductase enzyme was severely inhibited (Chung & Stevens, 1993).

The dye/substrate accumulation shows that the dye was not utilized and the azo bond in the dye was not cleaved, since the electrons releasing from glucose and NADH utilization stopped as reported by Kudlich et al., (1997).

As seen in Table 3.1. the first order kinetic constant (k_1) decreased from 0.0096 to 0.0008 1/h in samples containing DB 2 azo dye, depending on the accumulation of glucose-COD, dye and corresponding intermetabolites such as volatile fatty acids and aromatic amines. This could be due to dyes, co-substrates, microorganisms used and anaerobic operation conditions applied in the studies are different. On the other

hand the rate of azo reduction was affected by the structure of, and the substituents on the aromatic ring of dyes such as OH^-, $-NH_2$, and $-SO_3H$ resulting in inhibition (Donlon et al., 1995; Hu, 2001). As reported by Urushigawa and Yonezawa (1977) and Zimmermann et al. (1982) azo group with hydroxy and amino group are more likely to be reliably degraded than those with a $-CH_3$, $-NO_2$, $-SO_3H$, $-OCH_3$ group. As azo dye used in this study are diazo and three azo dyes and these structures are complicated. Therefore they caused inhibition on anaerobic biomass activity. The benzidine released as anaerobic intermetabolite caused increases in toxicity of DB 2, DB 38 and DR 28 due to carcinogenic properties of this organic substance as reported by Chung and Steven (1993). They observed that the toxicity of parental dye on methanogens increased with the time as aromatic amines (benzidine) were produced.

The anaerobic conditions clearly provide an unexplored area potential advantage for dye decolorization even if the dye concentrations are as high as 800 and 3200 mg/l. In practice, the observed inhibition may not be important because real textile wastewater contains only 100-500 mg/l of these azo dyes. Thus, it may not inhibit the decolorization of textile wastewaters containing these azo dyes but it is important in degradation of anaerobic substrates since continuous exposure of anaerobic biomass to potentially toxic and variable loadings of these dyes could have adverse effects on the anaerobic COD removals, and cause to accumulation of aromatic amines.

3.2. Continuous Studies with Pure Azo Dyes

3.2.1. The removal of Reactive Black 5 dye

3.2.1.1. Start-up period

The reactor was filled with the sludge taken from the Pakmaya UASB reactor up to 1/2 of its volume. Seeds sludge had 232 g TSS/l. For aerobic reactor, the seed was taken from the aeration tank of wastewater treatment plants in DYO dye industry. After 29 days of operation with UASB reactor, aerobic Continuous Stirred Tank reactor was connected to UASB reactor in order to form the anerobic/aerobic sequential system through treatment of azo dyes. Only glucose was used as carbon and energy source in the start-up period. Vanderbilt mineral salts medium (Speece, 1996) was used in the feed to supply micro and macro elements. Desired alkalinity and neutral pH was obtained by the addition of 5000 mg/l $NaHCO_3$ into the feed media in the start up period.

At a HRT of 2.5±0.7 day, and an organic loading rate of 1.00±0.2 kg COD/m^3.day in UASB/CSTR sequential system, 56±10%, 87±3%, and 95±2 % of COD removal efficiencies were achieved at UASB, CSTR and UASB/CSTR reactor systems, respectively in the start up period of the operation. This corresponded to 1322±286 mg/l of COD and 155±59 mg/l of COD in the effluents of UASB and CSTR, respectively. UASB reactor and total system performances were given in Figures 3.8. and 3.9. The performances of total system was only illustrated for 2 weeks when the CSTR reactor was combined to UASB reactor through start-up period.

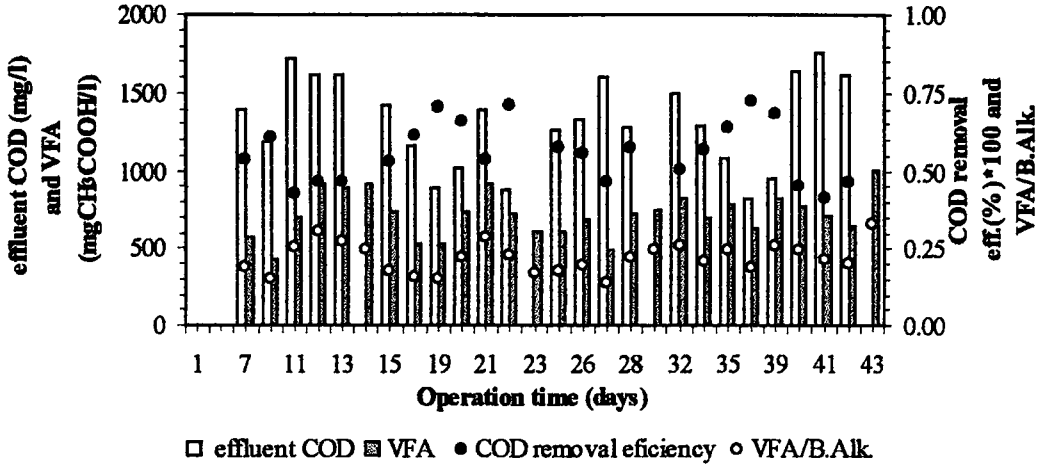


Figure 3.8. The variations of COD, COD removal efficiency, VFA and VFA/B.Alk. ratios versus operation times in the UASB reactor.

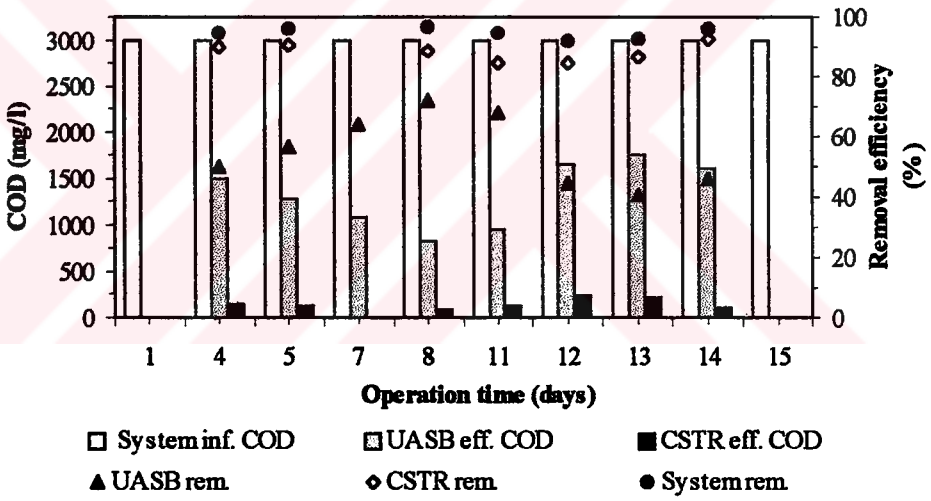


Figure 3.9. CODs and COD removal efficiencies versus operation times in Start-up period

43 days following the start up period, while approximately 60% COD removal and 65% methane production efficiencies were obtained at a flow rate of 4 l/day, the UASB reactor was brought into operation with 100 mg/l of Reactive Black 5 dye. The glucose-COD concentration was kept constant at 3000 mg/l.

The UASB reactor was operated at loading rates varying between 4 and 5 kg COD/m^3.day between days 0 and 90. The organic loadings were raised before reaching steady-state conditions to observe the effect of shock organic loading to COD, color removals and methane production rates in the aforementioned reactor. The organic loadings were increased from 2.5 kg COD kg COD/m^3 to 24.6 kg COD kg COD/m^3 between days 90 and 152 while HRT was decreased from 30 to 3 hours in UASB reactor.

3.2.1.2. COD and color removals in anaerobic UASB reactor

The effects of organic loading on the color and COD removal efficiencies are shown in Figure 3.10.In this study, since shock organic loading rates were applied before the UASB reactor reached steady state conditions, low COD removal efficiencies were obtained at high organic loadings. On the other hand, high color removal efficiencies varying between 91-100 % were observed even at organic loading rates as high as 25 kg COD/m^3.day. 41%, 100% COD and color and 26%, 91% COD and color removal efficiencies were observed at loading rates varying between 2.5 COD/m^3.day and 25 kg COD/m^3.day, respectively. As mentioned above, low COD removals at high organic loadings could be attributed to the accumulation of intermediate degradation products such as VFA and breakdown products.

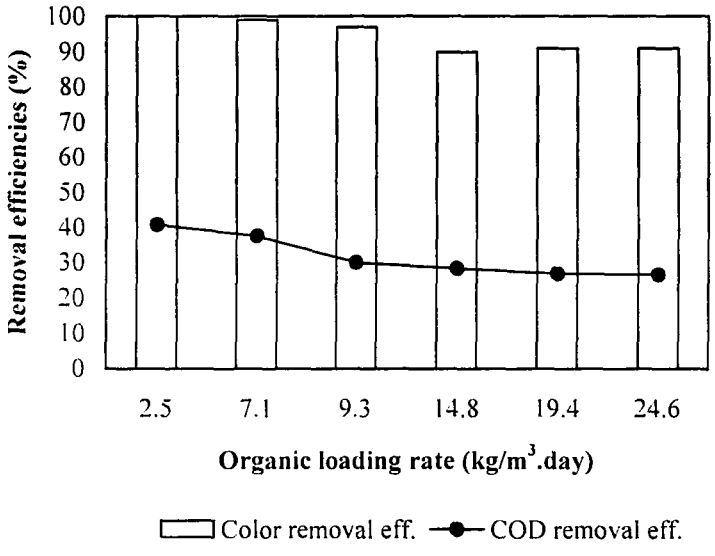


Figure 3.10. The effect of organic loading rate on the color and COD removal efficiencies in anaerobic UASB reactor

3.2.1.3. Effect of organic loading on gas production and composition in anaerobic UASB reactor

Figure 3.11 shows the effect of organic loading on the gas production and composition. It can be suggested that the acidogenesis is dominant at high organic loading rates while the rate of methanogenesis decreased. This results in low methane gas production rates. 76, 66, and 20% methane percentages were observed at loading rates of 2.5, 9.3, and 25 kg COD/m^3.day. A decrease in methane gas production was accompanied by increases in Volatile fatty acids/Bicarbonate alkalinity (VFA/B.Alk.) ratio (See Figure 3.11). Increases in VFA/B.Alk. ratio in UASB effluent samples indicate the activity of acid producing bacteria and VFA accumulation. The reactor is stable when the VFA/B.Alk ratio is lower than 0.4. If the VFA/B.Alk. ratio is lower than 0.8 the reactor system is moderately stable or unstable as reported by Behling et al. (1997). As shown in Figure 3.11 this ratio varied between 0.5 and 0.8, indicating the high VFA concentrations at high organic loading because of high COD and dye concentrations in the reactor. As a result, the intermediates and anaerobic degradation products could not be converted to methane and the reactor was unstable at shock organic loading as high as 20-25 kg

COD/m^3.day. In other words, although shock organic loads cause decreases in COD removal efficiencies, no negative effect was observed in color removal. This can be explained by color removal due to acidogenic bacteria together with methanogens as reported by Chinwekitvanich et al. (2000).

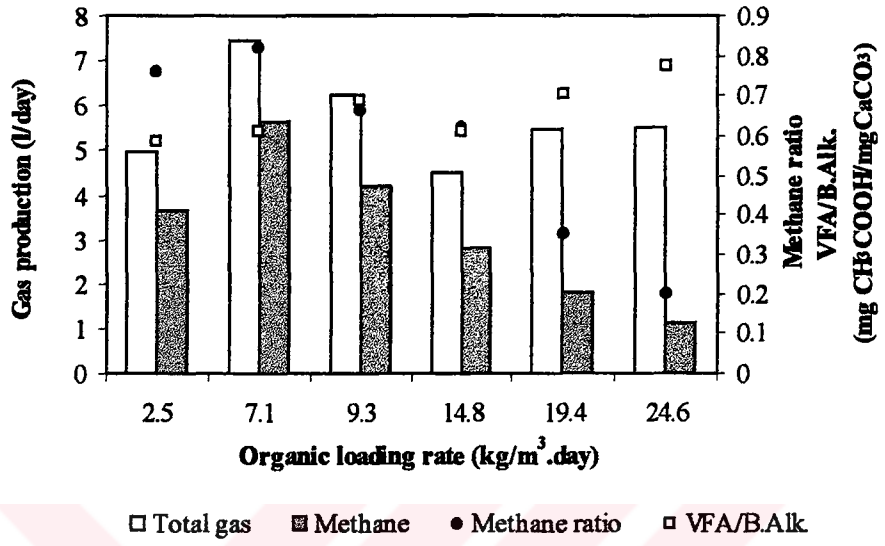


Figure 3.11. The effect of organic loading rate on the gas production, methane gas percentage, and VFA/B.Alk. ratio in anaerobic UASB reactor

3.2.1.4. Effect of F/M ratio on aerobic reactor performance

Since the anaerobic reactor effluent is used as influent of aerobic reactor, UASB reactor performance affects the aerobic reactor treatment efficiencies. Figure 3.12 shows the relationship between F/M ratio applied to the aerobic reactor and variations in the COD removal performances. COD removal efficiencies are lower (< 70%) for F/M ratios higher than 0.40 kg COD/kg MLSS.day. 90% COD removal efficiency was achieved at F/M ratios of 0.05 kg COD/kg MLSS.day. COD removal performances varying between 28% and 67% were obtained at F/M ratios of 0.4-1.4 kg COD/kg MLSS.day. Similarly lower COD removal efficiencies were obtained at high organic loading rates in aerobic reactor. 90%, 67% and 26% COD removal efficiencies were obtained at organic loading rates of 0.4, 1.25 and 5 kg COD/ m^3.day, respectively in aerobic reactor (See Figure 3.12).

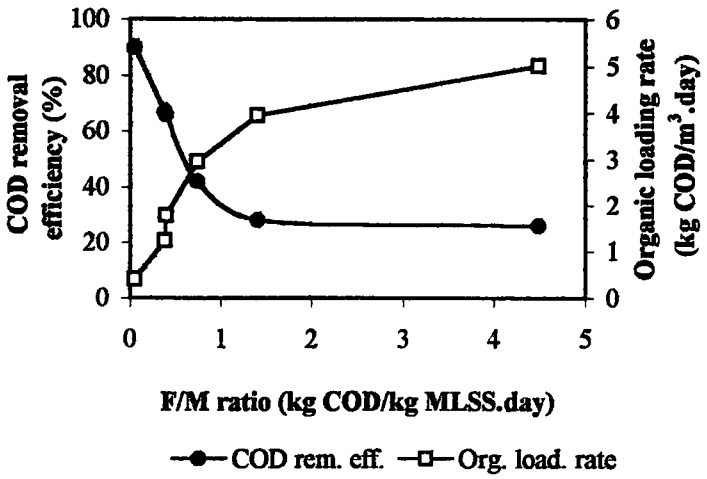


Figure 3.12. The effect of F/M ratios and organic loading rates on COD removal efficiencies in aerobic reactor

3.2.1.5. Effect of SRT on COD performance in aerobic reactor

COD removal efficiencies of 28%, 42% and 90% were obtained at STRs of 1.7, 5.7 and 11 days. Although increases in SRT values significantly affect the COD removal efficiencies in aerobic reactors only slight decreases were obtained in color removal. The suitable SRT value for COD was determined to be 11 days in aerobic reactor (Figure 3.13). At higher loading rates and low SRT values both MLSS and SVI values decreased because of washout of microorganisms from the effluent of the aerobic reactor (MLSS = 2000-2300 mg/l and SVI = 100-120 ml/g). Increases in MLSS and SVI values were observed at high SRT values (MLSS = 2900-4000 mg/l and SVI = 200-220 ml/g). A decrease in MLSS concentrations was observed at high organic loadings even were studied at high SRT values due to washout of biomass in effluent. This caused poor settling properties in CSTR reactor, which is associated with low COD removal efficiencies in aerobic reactor. In general, high COD removal efficiencies in aerobic reactor can be accomplished by low F/M ratios at low organic loadings resulting with active microorganisms at high concentration (See Figures 3.12 and 3.13).

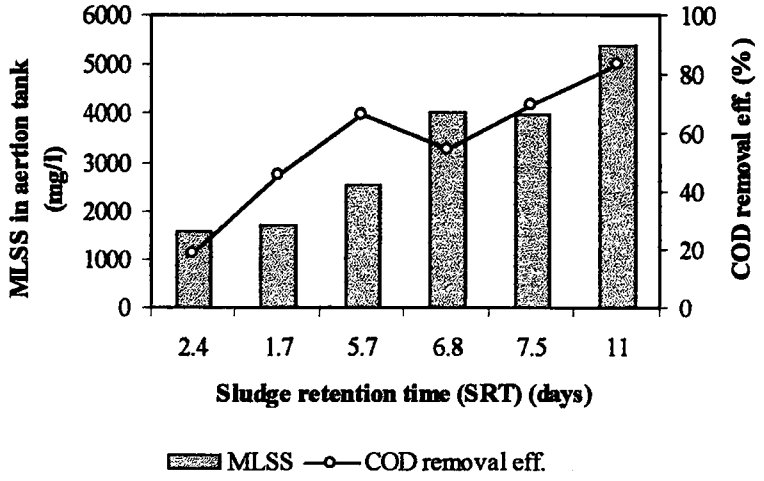


Figure 3.13. The variation in MLSS concentrations and COD removal efficiencies versus SRT in aerobic reactor

3.2.1.6. Absorbance measurements at different wavelengths

The absorbance maximal spectra measured in the influent and effluent wastewater of UASB and activated sludge reactor at an upflow rate of 2l/day are illustrated in Figure 3.14. The absorbance measurements showed that the aerobic effluent had a higher color than UASB reactor effluent. This could be due to increase in pH in the aerobic stage that occurs when the VFAs evaporate and are metabolized or due to the oxidation or polymerization of intermediate compounds formed in the anaerobic stage as reported O'Neill et al. (1999). Partially recoloration occurs in the response to the presence of oxygen and due to either a spontaneous on microbially catalyzed oxidation of some reduction products or intermediates of UASB effluents. Increases in color were simply the result of reformation of the azo bond since the formation of new oxidized intermediate products in the aerobic stage. The activated sludge and UASB effluent samples have no peak at 597 nm and show much higher absorbance at 325 nm. Second peak was observed at 600 nm for aerobic effluent. The same results were obtained by O'Neill et al. (1999) and Knapp and Newby (1995).

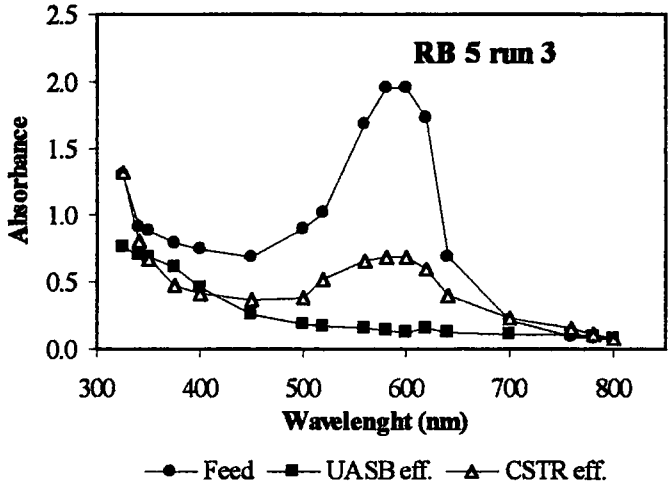


Figure 3.14. Absorbance spectra in influent and effluent samples at an upflow rate of 2 l/day

3.2.1.7. Performance of overall anaerobic/aerobic sequential system

88-75% color removal efficiencies were obtained for Reactive Black 5 at loading rates varying between 0.73 $g/m^3.h$ and 7.24 $g/m^3.h$ in the anaerobic/aerobic sequential reactor system. Increases in dye loading rates, did not significantly affect the color removal efficiencies. The color removal efficiencies were recorded between 75% and 88% (Figure 3.15). The total COD and color removal were 94.1 % and 80% in sequential UASB/CSTR reactor systems respectively at a HRT of 5.74 days. Above 80% COD removals were determined for HRT higher than 2 days (Figure 3.15). The optimum HRT was detected as 2 days for the total sequential reactor system.

The Reactive Black 5 removal efficiencies obtained in this study are comparable with the following studies; Nigam et al. (1996) found 67% color removal for 500 mg/l of RB 5 dye within 24 h in a batch reactor containing yeast extract as the carbon source. 68% color removal efficiency was obtained after 48 h of incubation period when glucose is used as carbon source (Beydilli et al., 1998). At the a incubation time of 48 h, > 95 color removal efficiency was obtained for a RB 5 concentration of

500 mg/l in a laboratory scale upflow anaerobic filter (volume: 0.125 l) with immobilized microbial consortium (Oxpring et al., 1996). 79% color removal efficiency was observed after 10 h of incubation at a Reactive Black 5 concentration of 200 mg/l (Luangdilok & Pansvad, 2000). 70-75 % color removal efficiency was achieved in a batch reactor within 4.5 h. In this study, medium consisted of 1000 mg/l glucose in a phosphate buffer at 52°C, inoculum was digester sludge from wastewater works receiving textile effluents (Carliell et al., 1994). 94% color removal was achieved at a HRT of 1 day in lab-scale a SBR (sequencing batch reactor) treating simulated textile wastewater included 500 mg/l RB 5, and other simulating materials such as starch, polyvinyl alcohol, carboxymethyl cellulose etc. (Shaw et al., 2002).

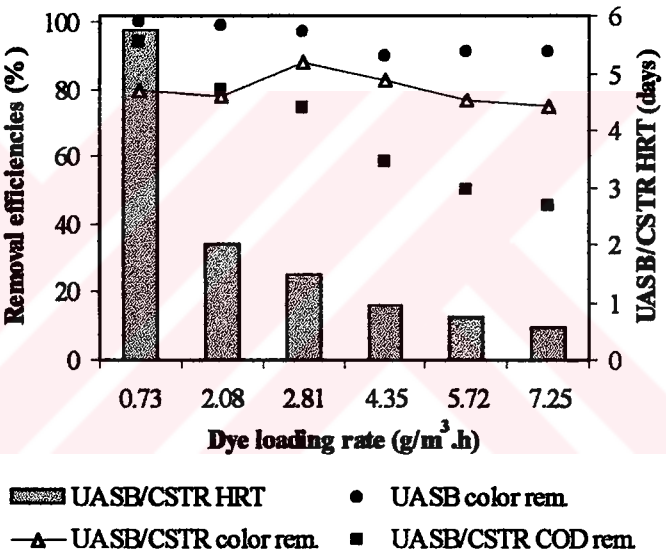


Figure 3.15. The effects of dye loading rates, and total HRT on color and COD removal efficiencies in anaerobic anaerobic/aerobic sequential system

The findings of this study showed that the decolorization of azo dye "Reactive Black 5" was not significantly affected by the shock organic loadings. Although the granulated sludge was not acclimated to the Reactive Black 5; the results of this run showed that this dye can decolorize with 88% color removal efficiencies in an UASB/CSTR sequential reactor system.

3.2.2. The removal of Direct Red 28 dye

The UASB reactor was initially started up after a month of adaptation period to glucose as the carbon source since the aforementioned reactor had previously been used to treat Reactive Black-5 dye at an organic loading rate as high as 24.6 kg COD /m^3.day. When the COD removal efficiency reached 60% the UASB reactor was started to operate with 100 mg/l of DR 28 dye. The UASB reactor was continuously operated for a period of 187 days in two different operation regimes (Run 1-Run 6 and Run 7-Run 14) to achieve the target objectives. In the first step the glucose-COD and the DR 28 azo dye concentrations were kept constant at 3000 mg/l and 100 mg/l, respectively, and the organic loading was raised with increasing upflow rates resulting in decreases in HRT from 18.5 h to 2.55 h in UASB reactor. In the second step the HRTs were approximately constant between 18-22 hours and the DR 28 concentrations were raised from 100 to 4000 mg/l. For each run, pseudo steady-state conditions were assumed to have been achieved if the effluent COD concentration and gas production value did not differ by more than ±5 % on at least three consecutive days.

3.2.2.1. UASB reactor performance

On day 60, 72% COD and 99% color removal efficiencies were achieved at an organic loading rate of 4.74 kg COD/m^3.day in the UASB reactor fed with 100 mg/l of DR 28 (Run 2). After this run, the COD removal efficiency decreased to 40 % as decreases in HRT to 2.55 from 18.5 hours as shown in Figure 3.16. In the second step, 85 and 65% COD removal efficiencies were achieved in the UASB reactor containing 750 and 4000 mg/l of DR 28 at dye loading rates of 13.6 and 213.3 g DR 28 dye/m^3.h on days between 141 and 187, respectively (See Figure 3.17). This corresponds to color removal efficiencies varying between 99% and 97% for the aforementioned days, respectively. Low COD removal efficiencies, particularly at high dye concentrations and dye loading rates indicated that the metabolites released through the decolorization of DR 28 dye were recalcitrant and could not be

ultimately mineralized under anaerobic conditions as reported by Rajaguru et al. (2000).

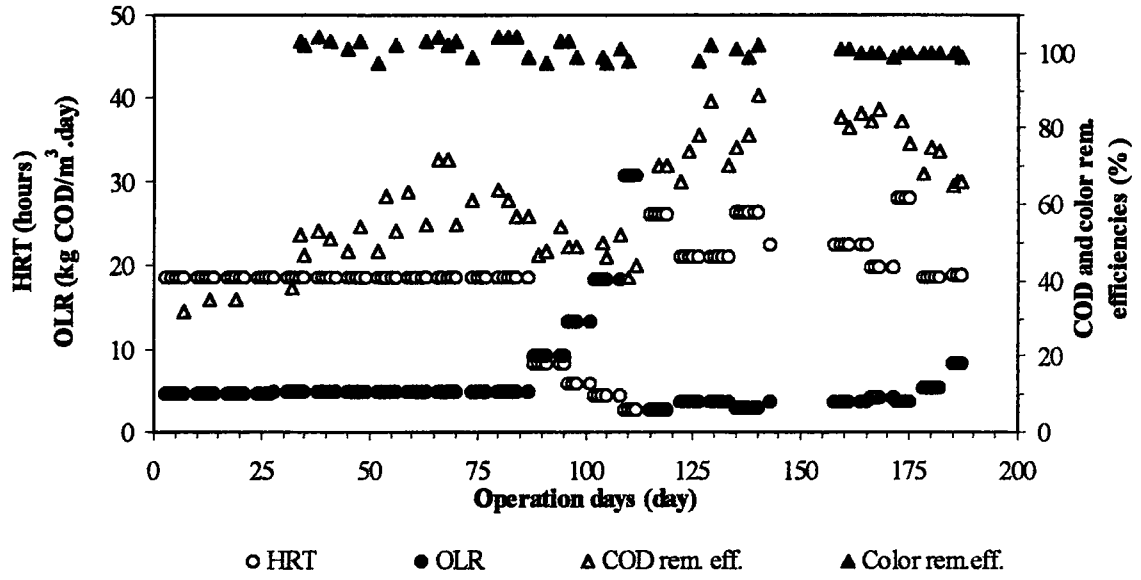


Figure 3.16. Effect of HRT and organic loading rate on COD and color removals in UASB reactor

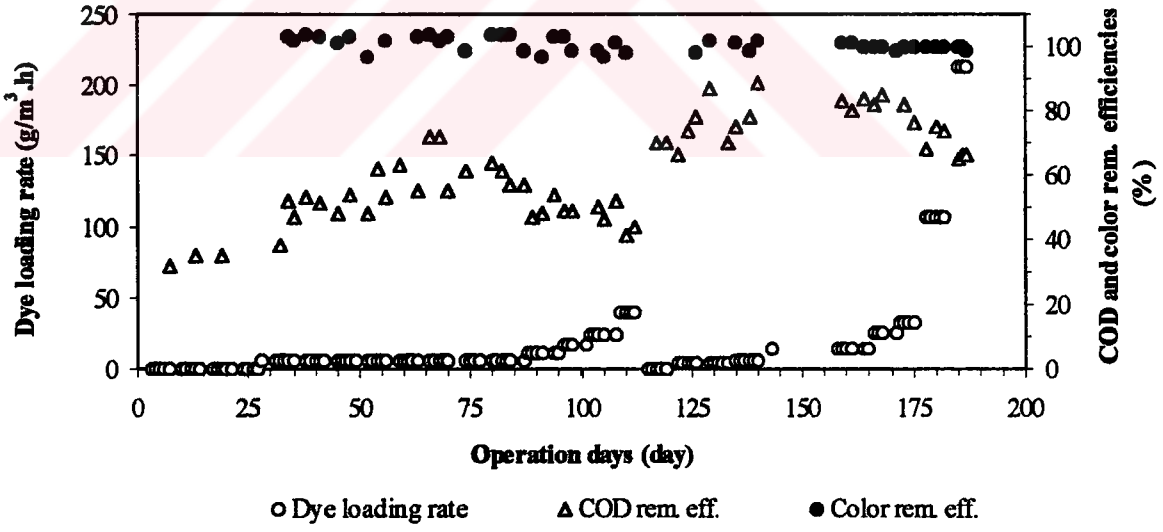


Figure 3.17. Effect of dye loading rate on COD and color removals in UASB reactor

3.2.2.2. Variations VFA, B.Alk. and methane gas production through increasing organic and dye loadings

In the first step of the studies it was observed that the methane production rates were 2000 and 6000 ml/day at loading rates of 4.7 kg/m^3.day (HRT= 18.5 hours) and 30.6 kg/m^3.day (HRT= 2.55 hours), respectively. In the same step, while the DR 28 dye was kept constant at 100 mg/l and the organic loading rate was increased to 30.6 kg/m^3.day, the VFA concentrations were at levels of 1150 mg CH_3COOH/l (Figure 18). This is extremely high for a stable operation of UASB reactor indicating the accumulation of VFAs and resulting in low COD removal efficiencies, such as 60%. The VFA/B.Alk. ratio varied between 0.4 and 0.5, indicating the moderate instability of the UASB reactor (Behling et al., 1997).

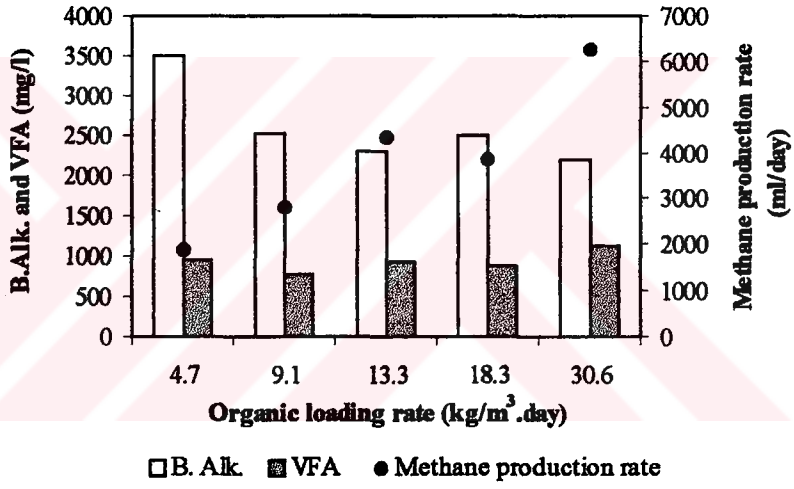


Figure 3.18. Variations in VFA and B.Alkalinity through increasing in organic loading rates

The results obtained in the second step of the study showed that the methane production rates increased with increases in dye-loading rates, indicating that a significant part of the DR 28 dye was converted to methane and used as a carbon and energy source by methanogens, as reported by O'Neill et al. (2000b). It also was found that the VFA concentrations were lower than the studies performed with low DR 28 concentrations (Figure 3.19). These results showed that the VFA accumulation was not observed at higher dye concentrations and dye loading rates,

such as 4000 mg/l of DR 28 concentration and a dye loading rate of 213 g DR 28 dye/m^3.h, respectively. In other words, the VFA production under high organic loadings was significantly higher than that produced at high dye loading rates (VFA/B. Alk. ratio was between 0.2 and 0.3, indicating the steady-state conditions).

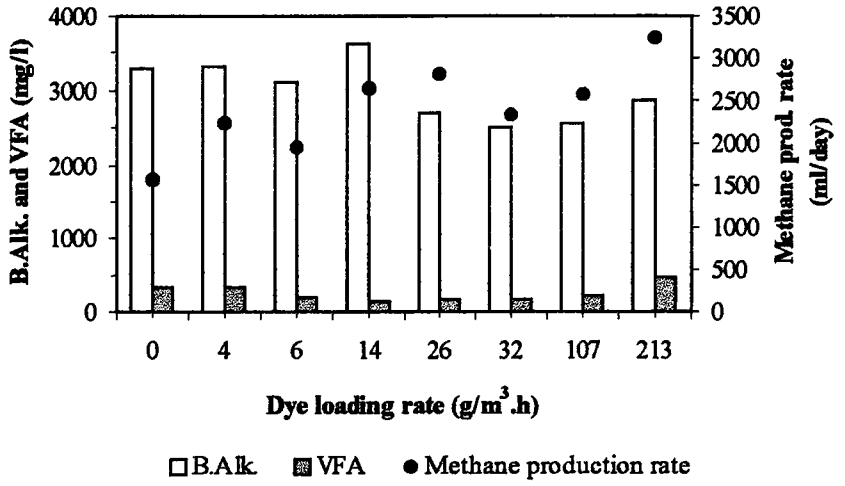


Figure 3.19. Variations in VFA and B.Alkalinity through increasing in dye loadings rates

Since the accumulation of VFA was higher and COD removal efficiencies were lower in very short HRTs (2.55 hours) compared to the condition in which high dye loading rates applied (18-22 hours), it can be assumed that short HRT values significantly affect the methanogenesis (See Figures 3.18 and 3.19). Therefore, HRT is a much more important parameter than the high dye loadings in the inhibition of methane bacteria, although dyes are known to cause toxicity and decrease the activity of the methanogenesis (Beydilli et al., 1998)

3.2.2.3. The removal of remaining COD from anaerobic stage through aerobic period

Since the effluent of the UASB reactor was used as the feed of the aerobic reactor, the COD coming from the anaerobic stage was significantly removed in the aerobic CSTR reactor (E= 60-93%) providing the treatment of organic compounds remaining from the anaerobic stage. As seen in Figure 3.20, it was observed that the COD

removal efficiencies decreased at low HRTs and high dye loading rates on days 112 and 185, respectively, in the aerobic reactor. Since 100 mg/l of DR 28 caused 74.6 mg/l of COD, the main reason for the lowered COD removal efficiencies were the low HRTs at high organic loading rates in the first step of the studies on days between 30 and 112. However, the reason for the accumulation of COD in the aerobic reactor in the second part of the study (on days 140 and 185) was the higher dye loading rates coming from the anaerobic reactor (at approximately constant HRTs). Probably significant part of the aromatic amines were not mineralized or were slowly degraded to other cleavage products, as mentioned by Tan et al. (2000).

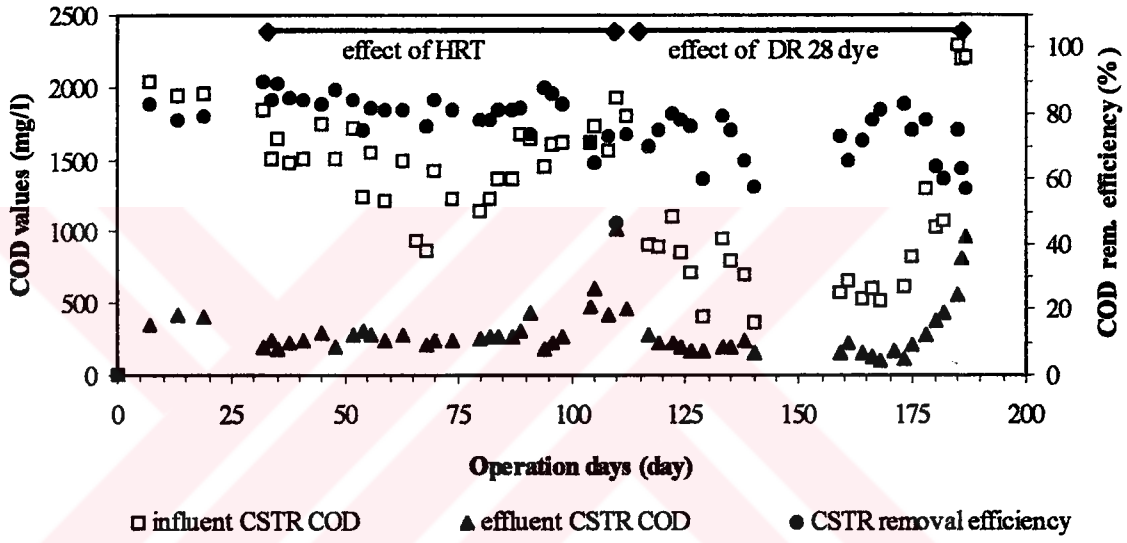


Figure 3.20. Removal of remaining COD from the anaerobic stage through aerobic period

3.2.2.4. Total system performance for COD and color removals

The performance of the complete UASB/CSTR reactor system is shown in Figure 3.21 in terms of COD and color removals during the 187 days of the operation period. 97% COD and 100% color; 88% COD and 100% color removal efficiencies were obtained at DR 28 dye concentrations of 500 and 4000 mg/l.

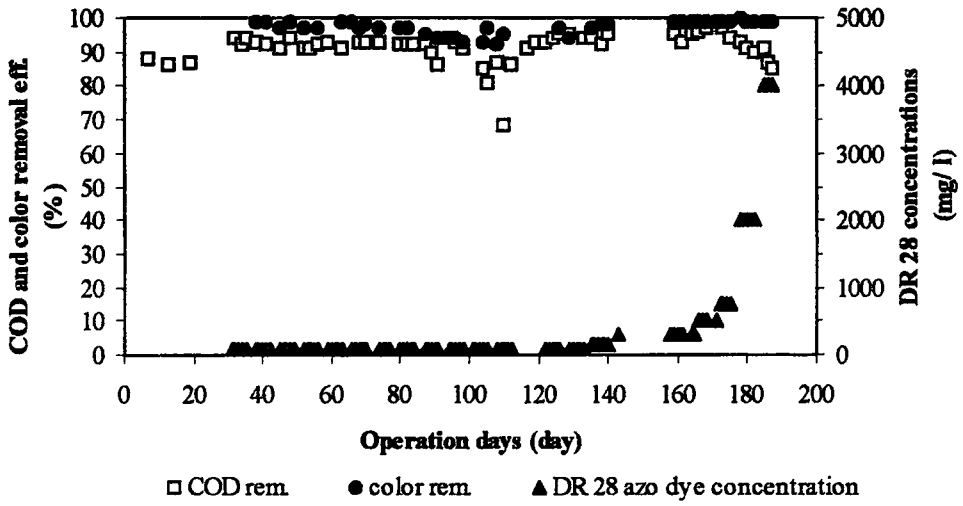


Figure 3.21. Performance of the system in terms of COD and color removal efficiencies

3.2.2.5. Total aromatic amine (TAA) removals through anaerobic/aerobic stage

Aromatic amine was measured as benzidine through the reductive cleavage of DR 28 dye. The TAA analysis results showed that, although this metabolite was considerably reduced (40-60%) at high dye concentrations under anaerobic conditions (750-4000 mg/l) it was mainly removed in the aerobic stage (See Figure 3.22). Figure 3.23 gives the aromatic amine levels and the recoveries in the whole system. TAA concentrations in UASB and CSTR reactor effluent were 238 mg benzidine/l and 47 mg benzidine/l at a DR 28 concentration of 4000 mg/l, respectively. These values corresponded to 54% and 9 % TAA recoveries in the UASB and CSTR effluents, respectively, The TAA measured in the UASB effluent were at high concentrations, such as 47 and 106 mg benzidine/l at DR 28 dye concentrations of 500 and 2000 mg/l, respectively, indicating that the aromatic amines produced were not converted further in the anaerobic stage. The TAA analysis results showed that the greater part of TAA was removed through the aerobic phase in the complete UASB/CSTR system. TAA removal efficiencies varying between 91-93% were obtained at a DR 28 concentration of 4000 mg/l in whole system. Since the dye concentrations in the textile and dye industries varied between 100 and 300 mg/l it can be assumed that the TAA removals would be 100%.

This shows that the TAA produced from the cleavage of the DR 28 dye could be ultimately mineralized. However, the TAA concentrations were at low levels when the DR 28 concentration was raised to 4000 mg/l, a small amount of amine (47.3 mg benzidine/l) was detected in the aerobic effluent, indicating the mineralization of amines. This result is similar to the findings of Rajaguru et al. (2000) and Tan et al. (2000) but contradicts the data of Donlon et al. (1997) and Razo-Flores et al. (1997) in which they showed the complete mineralization of TAA in anaerobic stage.

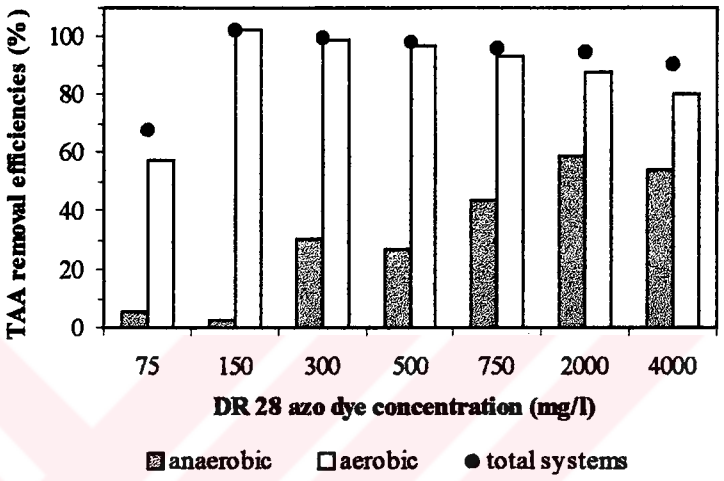


Figure 3.22. TAA removals in anaerobic, aerobic, and anaerobic/aerobic stage

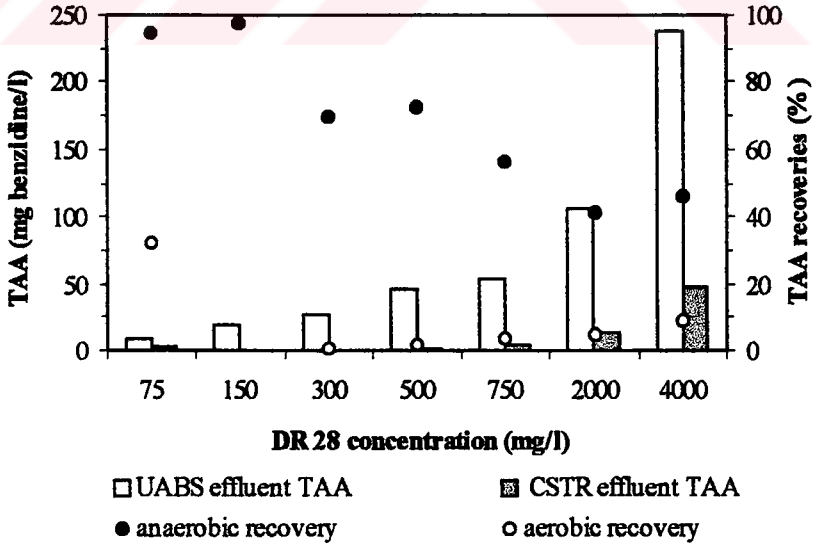


Figure 3.23. TAA values and recoveries in the system at different dye concentrations

3.2.2.6. Absorbance measurements at different wavelengths

Figure 3.24 shows the absorbance measurements at varying wavelength in the UASB/CSTR reactor system treating 4000 mg/l DR 28 dye. The UV-VIS scans showed that the maximum absorbance peak was obtained at a wavelength of 497 nm in the parent dye (influent) while the maximum wavelengths were 420 and 400 nm in anaerobic and aerobic effluents, respectively. This shows also that the parent dye converted to another form, resulting in decreases in maximum absorbance spectra indicating the decolorization of dye and mineralization of aromatic amines. Although the identification of the intermediate products were not performed, it can be concluded that the absorbency of aerobic effluents, probably contained some metabolic enzymes, products, excrete and residual mineral compounds without aromatic amines, as reported by O'Neill et al. (1999) and Knapp and Newby (1995).

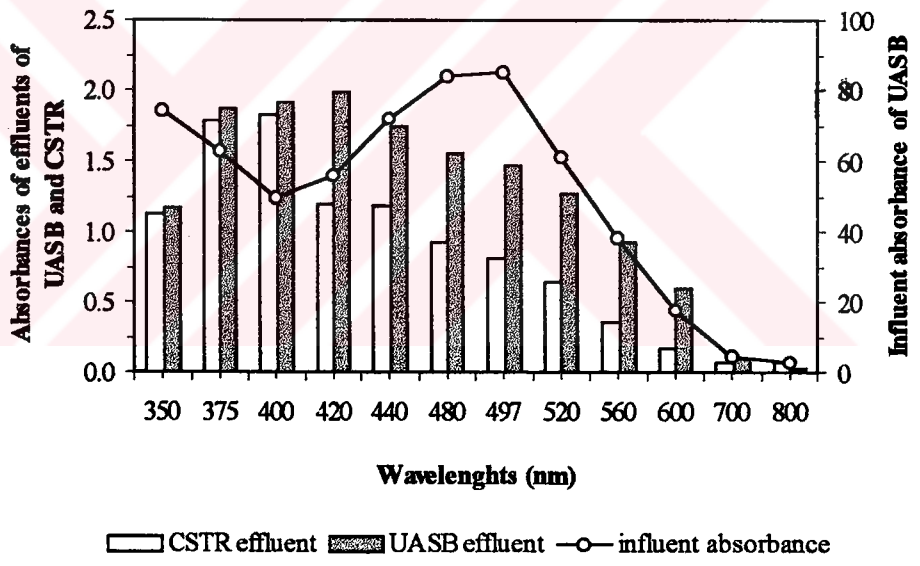


Figure 3.24. Absorbance measurements at different wavelengths

3.2.3. Effects of alkalinity and co-substrate on the performance of the UASB reactor through decolorization of DR 28 azo dye

3.2.3.1. Start-up period

After the UASB reactor was previously used for removal of synthetic wastewaters containing Reactive Black 5 and Direct Red 28 azo dyes, separately, the reactor was operated under dye-free conditions at an organic loading rate of 4.54 kg COD/m^3.day, and a HRT of 16 h in the beginning of this studies (Run 15). The volumetric organic loading rate used in this period is near the optimum organic loading rates (2-4 kg COD /m^3.day) proposed for UASB reactors treating the textile wastewater (Manu & Chaudhari, 2003). The COD removal efficiency, gas production rate, methane percentage, pH and VFA concentrations in effluent were 88%, 4280 ml/day, 80%, 7.3 and 78 mg CH_3COOH/l, respectively, indicating the steady-state conditions for effective continuous operation of UASB reactor.

3.2.3.2. Effect of glucose-COD on reactor performance

Figure 3.25 shows the effect of glucose-COD on the color and COD removal efficiencies. Color was removed very effectively (approximately 100%), at all glucose-COD concentrations varying between 100 and 500 mg/l. This shows that the electrons required for the reductive cleavage of azo dyes by anaerobic microorganisms were derived from the co-substrate glucose-COD. The electrons providing the reduced environment are sufficient for the cleavage of azo bond at COD concentrations as low as 100 mg/l and therefore for the complete decolorization. COD levels were able to perform more than 98-99% color removals in DR 28 dye through runs 16-18. In Run 19, the co-substrate was excluded in the influent in order to detect whether DR 28 azo dye could be degraded as the sole carbon and energy source by the anaerobic microorganisms in UASB reactor. As shown in Figure 3.25, almost complete (99%) decolorization was achieved even at substrate-free operation. The DR 28 azo dye breakdown to aromatic amines through the reduction and the cleavage of dye cromophore. No significant decreases in COD

removals (from 78 to 68%) were obtained as the glucose-COD concentrations decreased from 500 to 100 mg/l. The glucose-COD was decomposed into CH_4 and VFA while the DR 28 dye degraded to benzidine as aromatic amine.

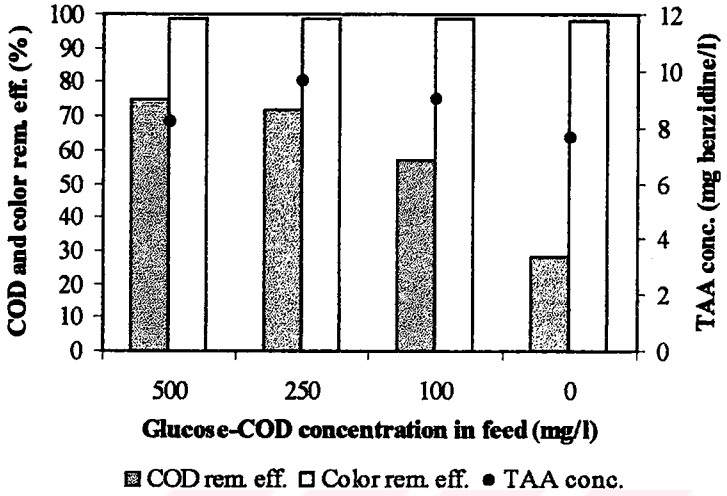


Figure 3.26. Effect of glucose-COD on color, COD removals, and TAA concentrations in UASB reactor

As shown in Figure 3.26 the methane production rates decreased from 445 to 125 ml/day while the glucose COD in the feed was reduced to 100 from 500 mg/l. The methane production was recorded as 73 ml/day at substrate-free operation. This shows that dye, the degradation products and intermediates could be used as a carbon source by methanogens as reported by Manu and Chaudhari (2002). Supporting these findings, in this study the abiotic decolorization studies with autoclaved, death granulates sludge showed that no significant color removals (E = 1-5%) were observed in co-substrate containing and co-substrate free samples. The concentrations of TAA varied between 8.2 and 9 mg benzidine/l in the effluent samples at glucose-COD concentrations of 100 and 500 mg/l, respectively, in the feed. No significant differences in TAA concentrations (7.6 mg benzidine/l) were obtained through decolorization of DR 28 dye when the UASB reactor was fed without co-substrate. The theoretical TAA concentration in the feed was calculated as 13 mg benzidine/l from the chemical reduction of 100 mg/l of DR 28 dye using

the calibration curves illustrated in Figure 2.3a. TAA and COD removal efficiencies were found as 43% and 28%, respectively, in substrate-free operation .

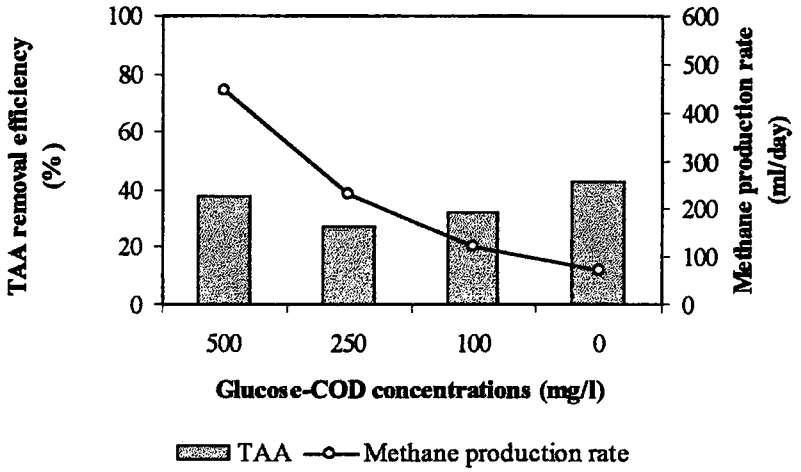


Figure 3.26. Effect of glucose-COD on the TAA removal efficiencies and methane production rates in UASB reactor

These findings in this run show that the color removal was provided by the electrons produced from the TAAs, which are degraded and used as carbon and energy source through anaerobic decolorization. These findings agree with the studies performed by Razo Flores et al. (1997). They indicated that the reducing environment was provided by the metabolism of 5-aminosalcylic acid (5-ASA) which is an aromatic amine released from the cleavage of azo dye azodisalicylate (ADS) and used as carbon and energy source in the continuous operation of UASB reactor with glucose-free wastewater. As aforementioned the aromatic amines provides electrons supporting the reduction of the dye resulting in complete decolorization even at substrate-free operation. The TAA removal efficiencies were 40 and 38% at COD concentrations 500 and 100 mg/l, respectively, indicating the partial degradation of aromatic amines under anaerobic conditions (See Figure 3.25). Supporting these findings, Kalyuzhnyi et al, (2000b) and Razo- Flores et al., (1997) also reported that the aromatic amines could further mineralized under anaerobic conditions in an UASB reactor. O'Connor and Young (1993) found that three

aromatic amines namely 2 amino phenol, 4-aminophenol and 5-aminosalisylic acid (5-ASA) can be mineralised in methanogenic consortia. Donlon et al. (1997) found that mordant orange 1 azo dye was reductively cleaved to less toxic aromatic amines (1,4–phenilenediamine and 5-aminosalicylic acid under anaerobic conditions. Contrarily, Brown and Laboureur, (1983); Haug et al. (1991) showed that aromatic amines are not degraded anaerobically. In a study performed by Bras et al., (2001) it was shown that the addition of co-substrates apparently stimulates the color removal efficiency while the color removal was explained by physical adsorption in the co-substrate-free reactor decolorizing Acid Orange 7 concentrations varying between 60 and 300 mg/l containing mixed and methanogenic biomass under batch anaerobic conditions.

The COD removal efficiencies decreased as the glucose-COD concentration was decreased. The COD removal efficiencies were found to be 75 and 40 % at glucose-COD concentrations of 500 and 100 mg/l in feed, respectively. This could be attributed to low co-substrate concentration which was a limiting factor since the methanogens have very low growth rates, and their metabolism is usually considered rate limiting in the anaerobic processes, Furthermore, the presence of dye metabolites accumulated in the UASB reactor caused lower COD depletions (Bras et al., 2001; Beydilli et al., 1998; Lun et al., 1995). In the Run 19 without co-substrate operation, the measured COD in the effluent samples resulting in low COD removal efficiencies (28%) could be mainly attributed the COD originated from the 100 mg/l of DR 28 dye together with the anaerobic extracellular and intermediate products as reported by Germirli et al. (1991).

3.2.3.3. Differences in absorbance spectra in influent and effluent samples of UASB reactor at various co-substrate levels

Figure 3.27 shows the absorbance spectra at varying wavelengths in the samples taken from the UASB reactor influent and effluent in the different runs and in the feed containing 100 mg/l of DR 28 dye with mineral medium. These spectra showed marked alterations as exemplified in the Figure. These changes could be explained

by structural modifications of the DR 28 dye molecule due to azo bond reduction under anaerobic conditions.

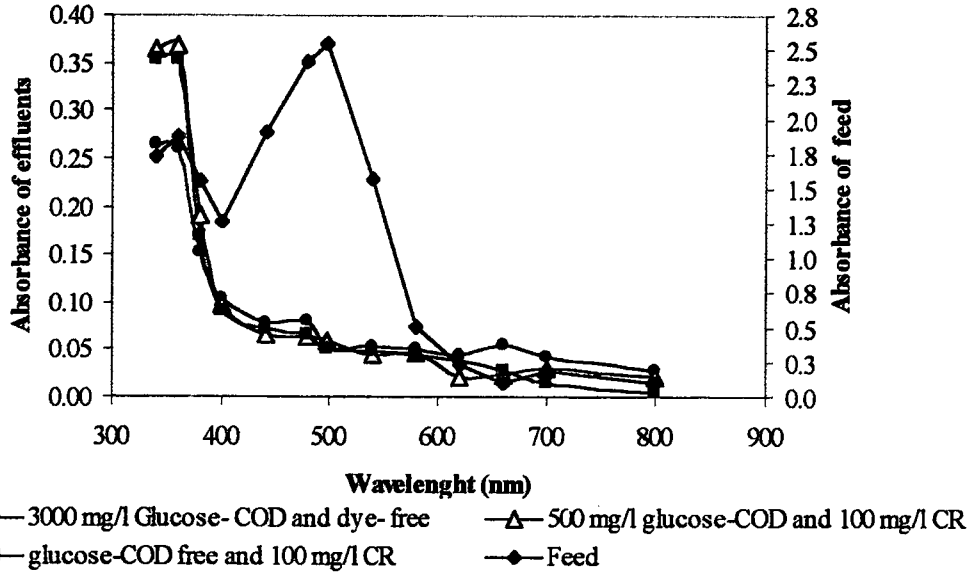


Figure 3.27. Visible absorbance spectra of DR 28 azo dye in the influent (feed) and effluent samples of UASB reactor at different co-substrate concentrations

The absorbance peak in the influent (feed) samples of the UASB reactor was obtained at a wavelength of 497 nm while the absorbency peak was at a wavelength of 340 nm in the effluents of UASB reactor through runs 16 and 19. In other words, the absorbance peak at 497 nm completely disappeared after the feed decolorized in UASB reactor. This suggests that a transformation of DR 28 dye to dye-intermediates and related organic forms had taken place under anaerobic conditions. Beside these, it is important to note that although the effluents containing dye (Run 16 and 19) and dye-free effluents (Run 15) have shown peak at the same wavelengths (340 nm). However, the absorbance levels in the effluent samples of UASB reactor were higher (0.37) compared to that dye-free samples (0.26). The absorbance level in feed samples was also recorded as 0.9. This shows that the aromatic amines produced through the cleavage of azo bond were not ultimately degraded. The partial degradation of TAAs in the anaerobic UASB reactor resulting in higher absorbencies in the effluent samples containing dye compared to dye free samples. The monitored metabolite aromatic amines, in the particular case of DR 28 dye, benzidine is expected to be obtained as intermediate products of azo bond

reduction (Rajaguru et al., 2000). Although the GS-MS spectra of breakdown products were not detected this suggest the biological mineralization of amines. Since the the aromatic amine removal efficiencies varied between 25 and 42% (See Figure 3.25) it can be conluded that most of the aromatic amines released was metabolised in UASB reactor.Lower absorbance levels in dye-free effluent samples at the same wavelengths (340 nm) indicating that structural modifications was not obtained in the feed. Razo Flores et al. (1997), Rajaguru et al. (2000), and Kalyuzhi et al. (2000a and b) showed the possibility of azo dye metabolites degradation under anaerobic conditions.

3.2.3.4. Effect of $NaHCO_3$ alkalinity on reactor performance

Traditionally, the total alkalinity in an anaerobic digester includes all the bicarbonate alkalinity and approximately 80% of the volatile fatty acids. Because only bicarbonate alkalinity is usable for neutralizing volatile acids, total alkalinity does not always represent the available buffering capacity in a digester (Anderson and Yang, 1992). A stable anaerobic treatment system requires a balance among all organisms. The maintenance of this balance is normally indicated by a low VFA concentration and a stable pH. Large molecular weight organic materials such as dyestuffs with co-substrates first have to be hydrolyzed to small molecular materials via breakdown of dye chromophore, production of aromatic amines and further was converted to volatile fatty acids, methane and degraded metabolite products. When the system is in balance, the methanogens could be inactivated by unfavorable environmental conditions, e.g., pH drop, accumulation of VFA, intermetabolites and toxicity of aromatic amines due to their toxic properties (Kuai et al., 1998)

Figure 3.28 shows that the effect of bicarbonate alkalinity on the COD, color removal efficiencies and VFA productions in the UASB system. The color was effectively removed (>99%) at all $NaHCO_3$ concentrations. No significant differences in COD removal efficiencies (E= 84-88%) were obtained while the alkalinity decreased to 550 from 3000 mg/l. However, the COD removals decreased to 68% while the alkalinity decreased to 250 mg/l in the feed since the bicarbonate

alkalinity could not buffer the pH in UASB reactor. Similarly, the VFA concentrations increased from 81 mg/l to 403 mg/l as the $NaHCO_3$ concentrations in feed decreased to 250 mg/l from 3000 mg/l. Generally the volatile fatty acid concentrations in the effluent of the UASB reactor were less than 100-300 mg/l with the exception of 250 mg/l of influent alkalinity. High levels of VFA concentration will be reflected low COD removals under high COD concentrations in effluent of UASB reactor through insufficient alkalinity in the influent (Speece, 1996).

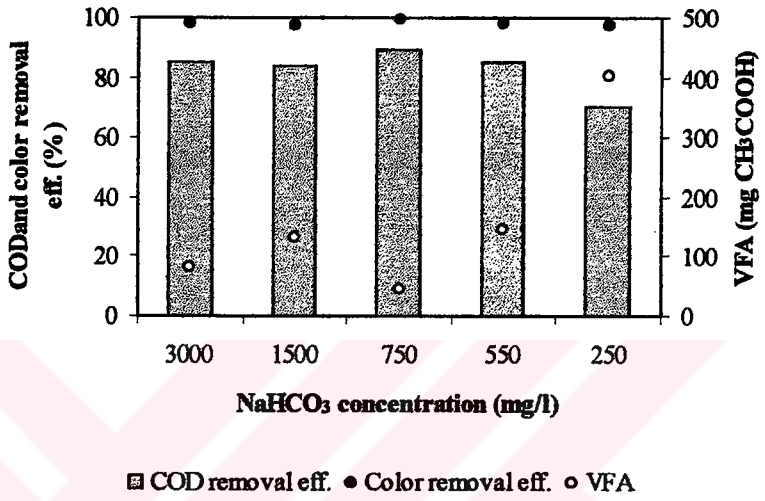


Figure 3.28. Effect of decreasing concentrations of $NaHCO_3$ alkalinity on COD,color removal efficiencies, and VFA concentrations in UASB reactor

If an UASB reactor is stable, the VFA/B.Alk ratio of reactor effluent is lower than 0.4 (Behling et al., 1997). As shown in Figure 3.29, the VFA/B.Alk. ratio were between 0.03 and to 0.28 at $NaHCO_3$ concentrations varied between 550 and 3000 mg/l in the feed. However this ratio increased to 0.68 when the $NaHCO_3$ concentrations decreased to 250 mg/l in the feed, indicating the instability of the UASB reactor. The pH ranges decreased from 7.3 to 6.2 as the bicarbonate alkalinity decreased from 3000 to 250 mg/l (See Figure 3.29). The well-being of a UASB reactor can often be judged by the effluent pH. The pH in a normally functioning reactor is close to neutral and controlled by a system that consist of bicarbonate and volatile fatty acids. The observed decreases in COD removal efficiencies (from 88 to 68%), could be due to low level of pH monitored in the UASB reactor, VFA

accumulation, resulting in inhibition in the methanogenesis due to the deactivation of sensitive methanogenic bacteria when the bicarbonate alkalinity in influent was 250 mg/l. The acidogenic phase of the overall digestion process will generate acids, but under normal circumstances this would be buffered by the alkalinity in the reactor (Behling et al., 1997). Although acidogenic phase together with methanogenic phase could be dominant, color was removed effectively in the UASB reactor. This can be explained by color removal both acidogenic and methanogenic bacteria as reported by Chinwekitvanich et al. (2000). Cruz and Buitron (2000) reported no change in color removals (80-100%) when the alkalinity decreased from 900 to 300 mg $CaCO_3$/l, in a sequencing batch anaerobic biofilter treating Disperse Blue 79 azo dye, suggesting that this parameter is not important for the elimination of color.

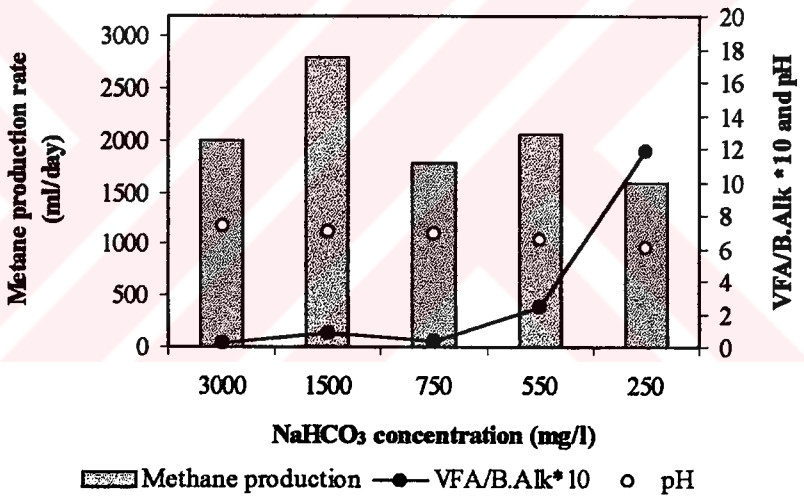


Figure 3.29. Effect of decreasing concentrations of $NaHCO_3$ alkalinity on VFA/B.Alk ratio, methane productions and pH levels

Figure 3.30 shows the TAA concentrations released through cleavage of DR 28 dye and corresponding removal efficiencies and recoveries. The TAA concentrations increased to 10.1 from 5.83 mg/l as the bicarbonate concentration in influent was increased from 250 to 3000 mg/l. The DR 28 breakdown products as benzidine were greatly removed and the corresponding aromatic amine was recovered by less than

44 %. This indicates that the aromatic amines released from azo dye cleavage were being partly metabolized.

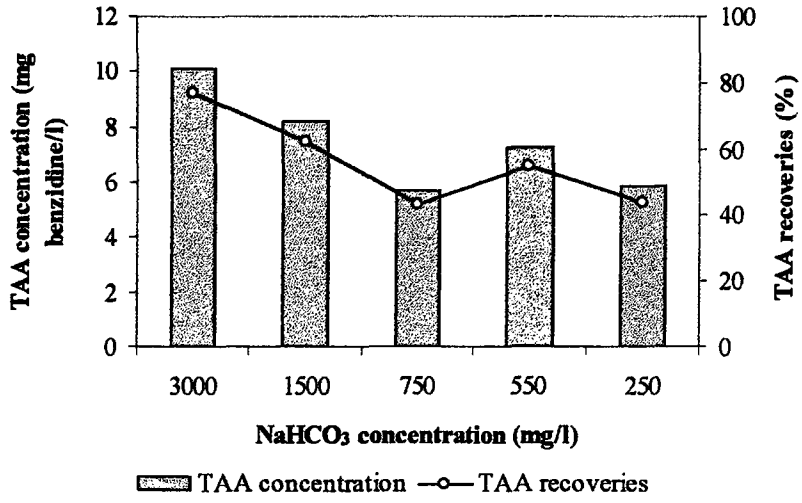


Figure 3.30. Effect of decreasing concentrations of $NaHCO_3$ alkalinity on the TAA productions and recoveries

As shown in Figure 3.29, no significant differences in methane production rates (2000-2400 mg/l) was observed as the $NaHCO_3$ concentrations decreased from 3000 to 550 mg/l in the feed. However the methane production rate was recorded as 1590 ml/l when the UASB reactor was operated with 250 mg/l of $NaHCO_3$. Since 550 mg/l of $NaHCO_3$ concentration (as 327 mg $CaCO_3$/l) provided the optimum buffering capacity to convert effectively glucose-COD to methane and VFA, it could be suggested as optimum bicarbonate alkalinity concentration in influent to maintain the pH above 6.6. This value corresponds to an Alk/COD ratio of 0.163 for optimum operation, which is lower than the ratios proposed by Gonzales et al., (1998) (0.4), Souza et al. (1992) and Moosbruger et al. (1993) (0.5), and Speece (1996), (1.2). Table 3.4 summarizes the total and bicarbonate alkalinity levels in the influent and effluent samples of UASB reactor. Increases in alkalinity concentrations were observed in the effluent of the UASB reactor compared to influent samples. For example when 1500 mg/l $NaHCO_3$ (93 mg CaCO3/l) was added to the feed the bicarbonate and the total alkalinity concentrations were 1510 and 1556 mg $CaCO_3$/l.

This shows that the alkalinity was regenerated in the upper layer (effluent) of UASB reactor. The VFA will be consumed faster than it can be produced by the methanogens and subsequently the VFA was converted to methane and the VFA alkalinity will be regenerated to bicarbonate alkalinity. Furthermore, since the NH_4-N concentration (NH_4Cl=400 mg/l) in the feed is high, the generated ammonium bicarbonate alkalinity contributes to total alkalinity in effluent samples. On the other hand, since the reserve alkalinity is the bicarbonate alkalinity maintaining the pH above 6.2-6.6 in the UASB reactor, it can be assumed that this alkalinity was provided in our system since the lower pH level was recorded as 6.3.

Table 3.4. Variations of alkalinity in influent and effluent samples of UASB reactor

$NaHCO_3$ mg/l in feed	**As mg$CaCO_3$/l equivalent**	**Eff. bicarbonate alkalinity (mg $CaCO_3$/l)**	**Eff. total alkalinity (mg $CaCO_3$/l)**
3000 dye-free	1786	1857	1870
3000	1786	2387	2406
1500	893	1510	1556
750	446	1127	1133
550	327	682	720
250	149	378	595

3.2.3.5. Differences in absorbance spectra in influent and effluent samples at various bicarbonate alkalinity levels

Figure 3.31 displays the change of UV-visible spectra of the influent (feed) and effluent samples containing different bicarbonate alkalinity levels in the UASB reactor. The feed spectrum has a peak in the visible region at 497 nm, which accounts for the color and a peak at 390nm. In the anaerobic treated samples containing different bicarbonate alkalinity levels and in the dye-free samples there is a peak in the ultraviolet region of the spectra (λ=380 nm). The absorbance peak at 497 nm completely disappeared after anaerobic decolorization. In other words when comparing the UV-VIS scans of the feed and the effluent samples it was observed that the aforementioned spectrums were quite different suggesting that DR 28 dye was degraded and exhibits maxima absorbance peak at a wavelength of 380 nm. Similarly the absorbance spectrum in the dye-free samples was obtained at a

wavelength of 380-390 nm. Although different bicarbonate alkalinity and dye containing UASB reactor effluent samples exhibit similar maxima spectra the observed absorbance levels was found to be quite different. For example the absorbance levels increased to 0.88 from 0.4 as the bicarbonate alkalinity levels increased to 1500 mg/l from 250 mg/l in the effluent of UASB reactor with the exception of 3000 mg/l of bicarbonate alkalinity containing effluents (A= 0.65). This finding demonstrated that the variation in alkalinity could change the chemical structure of the intermetabolite products releasing through cleavage of DR 28 dye and could affects the breakdown products of aromatic amines since the dominated acidogen and methanogen bacteria could varied. The modifications in the bacterial genus and species from methanogenic and acidogenic bacteria probably affect their metabolic pathway through degradation of aromatic amines and DR 28 dye resulting in different metabolic products given different absorbencies as the alkalinity increased from 250 to 3000 mg/l. Lower absorbance values at lower alkalinity level could be attributed to VFA accumulation, low methane production rates and low aromatic amine removal efficiencies.

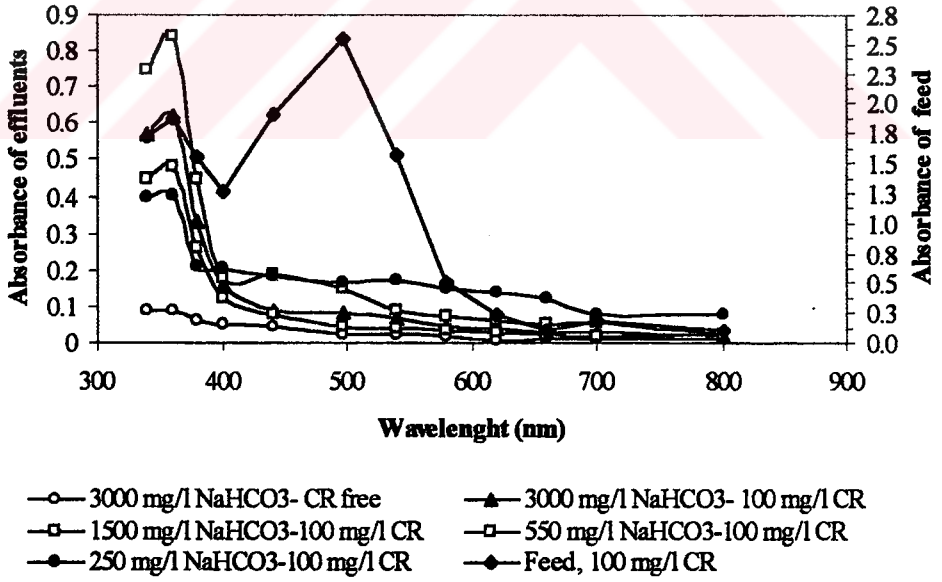


Figure 3.31. Visible absorbance spectra of DR 28 azo dye in influent and effluent samples of UASB reactor at different initial $NaHCO_3$ concentrations

3.2.4. Removal of Direct Black 38 azo dye

Since the IC_{50} value of DB 38 was found to be >3200 mg/l in anaerobic toxicity test 100 mg /l DB 38 was used at the beginning of continuous operations of the UASB reactor. In this part of the study, HRTs were approximately constant (at between 15 and 16.5 hours) and the DB 38 concentrations were raised from 100 to 3200 mg/l. Operation parameters such as organic loading rates ratios and SRTs applied in aerobic and anaerobic reactor are summarized in Table 2.4c in the section Material and Methods.

3.2.4.1. COD, color removals, volatile fatty acid accumulation (VFA), variation in alkalinity and methane gas productions in UASB reactor

Previously, the UASB reactor was operated at an organic loading rate of about 2.93 kg COD/m^3.day (HRT= 18.4 hours) for 53 days in order to evaluate the effect of alkalinity (between 250 and 3000 mg $NaHCO_3$/l) on color and COD removal in the system treating DR 28 azo dye of 100 mg/l. The COD level was reduced to 638 mg/l from initial COD concentration of 2120 mg/l indicating a COD removal efficiency of 70 %, at the end of the anaerobic operation in order to evaluate the effect of 250 mg $NaHCO_3$/l on UASB reactor performances. However, the color removal efficiency was 97% during the aforementioned period, indicating that the color removal was not affected from the low alkalinity.

The reactor system was operated during 53 days in order to asses the effect of increasing dye loadings on the performance of UASB reactor in terms of COD, color removals, VFA accumulation and methane gas productions. The COD loading was kept constant at nearly 2.91 and 5.37 kg COD/m^3.day and the DB 38 dye loadings were increased from 6 to 213 g dye/m^3.h. Figure 3.32 shows the dye loading rates, the color and the COD removal efficiencies measured in the anaerobic UASB reactor treating DB 38.

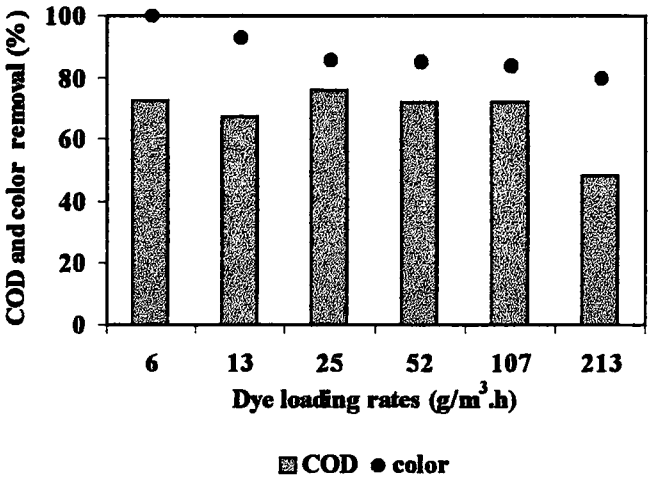


Figure 3.32. COD, and color removals in UASB reactor

At the beginning of the anaerobic continuous course (at dye loading of 6 g dye/ m^3.h), the COD level reduced to 530±120 mg/l from the initial COD concentration of 2000 mg/l, indicating a COD removal efficiency of 72.5 %. However, the color removal efficiencies were 100 % during the aforementioned period. Very high color removals could be attributed to the catabolism of glucose-COD which is ultimately responsible for the production of reduced enzyme cofactors through cleavage of azo bonds, resulting in decolorization as reported by Carliell et al. (1995). In last period, when the dye loadings were still being loaded up to 213 g dye/m^3.h, the COD removals decreased to 48 from 72 %. However, the color removal efficiencies were affected negatively from these high loadings resulting in approximately 80% color removals. The COD removal efficiencies reduced to 48 from 73% while color removal decreased to 80 from 100 % at dye loading rates varying between 6 and 213 g dye/m^3.h. The optimum dye loading rate for COD and color removal was determined to be 107 g dye/m^3.h in UASB reactor. At this dye loading rate, COD and color removals efficiencies were 72% and 84%, respectively in the UASB reactor.

As shown in Figure 3.33, the VFA, the B.Alk and the methane production rates varied as the dye loading rates was increased in the UASB reactor. 1000 mg/l of $NaHCO_3$ alkalinity was increased to 2000 mg/l after the dye loading rate was increased to 6 g dye/m^3.h in order to prevent the pH fluctuations. The bicarbonate

alkalinity measured in the UASB reactor decreased slightly from 1200 to 1100 mg/l as the dye loading increased while the VFA concentrations increased from 400 to 820 mg/l indicating the unsteady-state conditions for operation while when the dye loading rates increased. The alkalinity production at higher COD loading rates was reported to be hardly found in the acidogenesis step by the Embden-Meyerhof Patway (Fenchel & Finlay, 1995), or in the methanogenesis stage (Fenchel & Finlay, 1995, Müller& Gottschalk, 1988). The facultative anaerobes and methanogenic bacteria which can release bicarbonate during their metabolisms could be possible explanation for this happening. The VFA/B.Alk. ratios were 0.49±0.22(SD) (n=17 data) through operation, indicating stability and/or moderate unstability of UASB reactor (Behling et al., 1997). The VFA/B.Alk. ratio was 0.74 resulting in a competition between acetogens and methaogens at high dye loadings as reported by Behling et al. (1997).

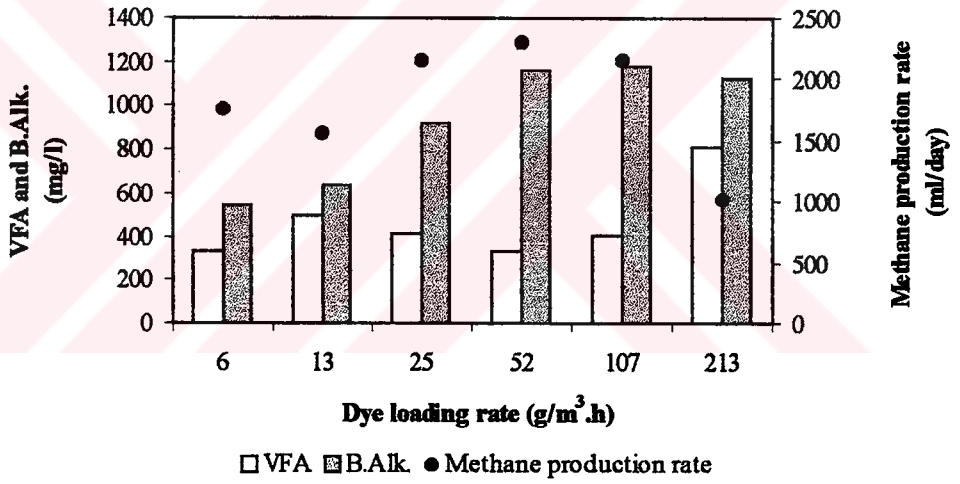


Figure 3.33. Variations in VFA, B.Alkalinity, and methane production rate through increasing in dye loadings on UASB reactor

Increases in dye loadings caused decreases in methane flowrates (See Figure 3.33). The methane flowrates measured through the 53 days of operation period at high dye loadings are significantly lower than that produced under low dye loadings since 2160 ml methane gas was produced per day at a dye loading rate of 107 g dye/m^3.h while 1020 ml/day methane gas was produced at dye loading rate of 213 g dye/m^3.h. This indicates that the DB 38 dye was not used and biodegraded.

Moreover, it has a toxic effect on methanogenesis at dye loading rates greater than 107 g dye/m^3.h.

The results of the study showed that high dye loading rates affect much more the performance of anaerobic compared to the low dye loadings. Therefore dye loading rates are more important operating parameters for inhibition of the methanogenesis and lowered the treatment efficiency of UASB reactor. Since the accumulation of VFA is higher and the COD removal is lower at the high dye loadings it is important to note that anaerobic microorganisms responsible from the methanogenesis are more sensitive than acid forming bacteria. Therefore high dye loadings rates applied to the UASB reactor caused accumulation in VFA and consumption of much more in alkalinity lowering in pH resulting in lower COD removals.

3.2.4.2. Performance of aerobic reactor

COD removal efficiencies varying between 73 and 67.7 % were observed in the aerobic reactor depending on the COD removal efficiencies and dye metabolites obtained in the effluent of the UASB reactor. This corresponded to COD loading rates varying between 0.2 and 0.77 kg COD/m^3d between days 1 and 53, respectively in the CSTR reactor. Figure 3.34 shows the COD values, removal efficiencies and the DB 38 concentrations in the feed of system throughout operation time in aerobic reactor. Since the aerobic reactor was fed with the effluent of the UASB reactor operated under constant glucose-COD loading and increasing dye loadings, the COD removals reduced to 67.7% in the CSTR reactor effluents at a DB 38 concentration of 3200 mg/l. This could be explained by the accumulation of COD, which was not removed under anaerobic conditions and probably contained high concentrations of aromatic amines or the cleavage products of azo bonds at high dye concentrations in the UASB reactor. The reason for accumulation of COD in the aerobic reactor on days between 40 and 53 is the higher dye loading rates coming from the anaerobic reactor. Probably a part of aromatic amines were not mineralized or slowly degraded to other cleavage products as mentioned by Tan et al. (2000).

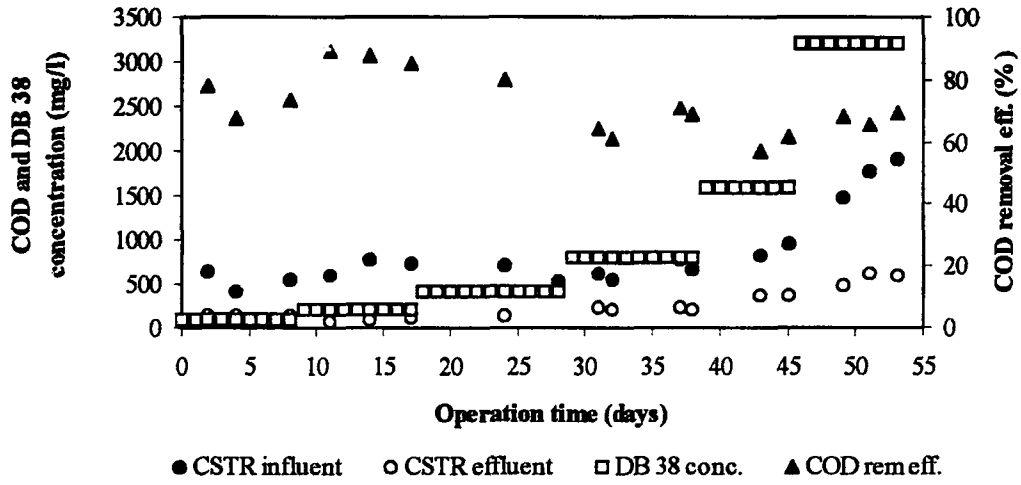


Figure 3.34. Removal of remaining COD from the anaerobic stage through aerobic period

3.2.4.3. Sequential UASB/CSTR total system performance

93% COD removals were obtained in the complete anaerobic/aerobic total system at the beginning period of the study since the anaerobic reactor was operated under low dye loading conditions. However 100% color removals were observed in the complete system. Figure 3.35 shows the removal efficiencies in terms of COD and color with increasing dye loading rates in UASB/CSTR system. The COD removal in the total system were nearly 84±2.5 % since the high COD in anaerobic reactor reduced the COD removals while 86±3 % color removals were obtained at 46.4 g m^3.h of dye loading rate . The contribution of dye concentrations to the influent COD (from 100 to 3200 mg /l) was 1184 mg /l at a dye concentration of 3200 mg/l. However the effluent COD was reduced to 555±76 in the effluent of the system (E_{COD}= 84%) when reactor was fed with a DB 38 concentration of 3200 mg /l (this is equal to 1184 mg COD/l). This shows that a significant part of glucose, and dye-COD was removed at a dye concentration of 3200 mg /l in the total anaerobic/aerobic system.

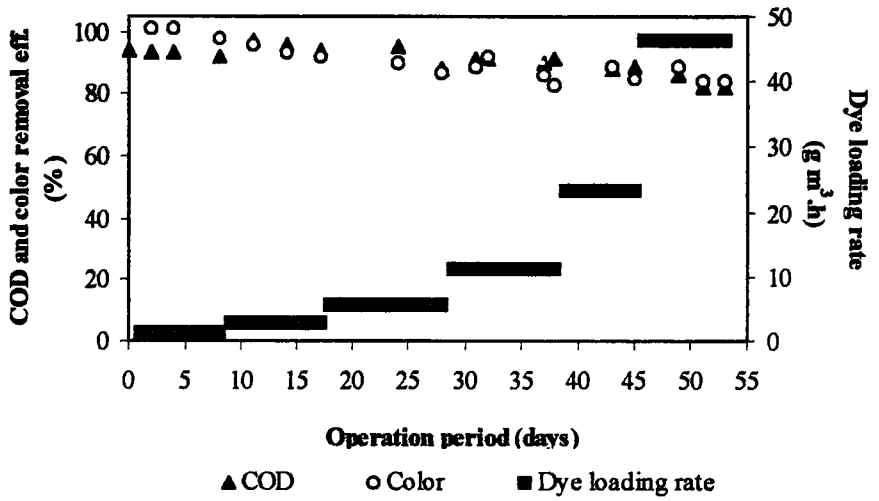


Figure 3.35. The performance of the total system in terms of COD and color removal efficiencies

3.2.4.4. Variation in total aromatic amines (TAA) in anaerobic and aerobic effluents

The DB 38 breakdown products (benzidine, aniline vs.) were recovered with a high yield of the DB 38 removed (97, 85, and 95 %) under anaerobic conditions in the UASB reactor at DB 38 concentrations of 800, 1600, and 3200 mg/l, respectively (See Figures 3.36 and 3.37). At these concentrations, TAAs was detected at high concentrations (40, 70, and 155 mg/l, respectively), indicating that further metabolism of the aromatic amines did not occur since the removal efficiencies was between 5 and 15% at high dye concentrations such as 1600 and 3200 mg/l. This indicates that the TAAs (the sum of the benzidine, aniline, 1,2,5-triaminobenzene, and 2,8,9-triamino-1-hydroxynaphthalene-3,7-disulphonic acid which are theoretical breakdown products of DB 38 when azo bonds on dye are cleaved) released from DB 38 azo dye cleavage were not metabolized under anaerobic conditions.

Aromatic amines were detected at low levels in the aerobic effluent comparing to the UASB effluents indicating the mineralization of breakdown products of azo dyes under oxygenated environments. The DB 38 degradation products (aromatic amines) were recovered with a yield of 45 and 48 % in the UASB/CSTR system treating DB 38 concentrations of 1600 and 3200 mg/l, respectively. Similarly, Rajaguru et al.

(2000) reported that the products of C.I. Acid orange 10, C.I. Napthol Blue Black, C.I. Direct Red 2, and C.I. Direct Red 28 released from the anaerobic stage were completely mineralized under aerobic conditions.

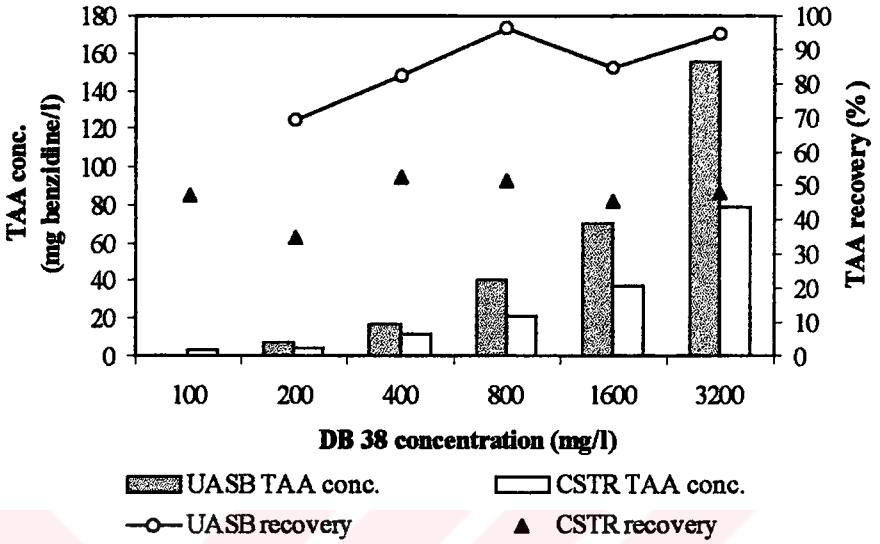


Figure 3.36. TAA values and recoveries in the system at different dye concentrations

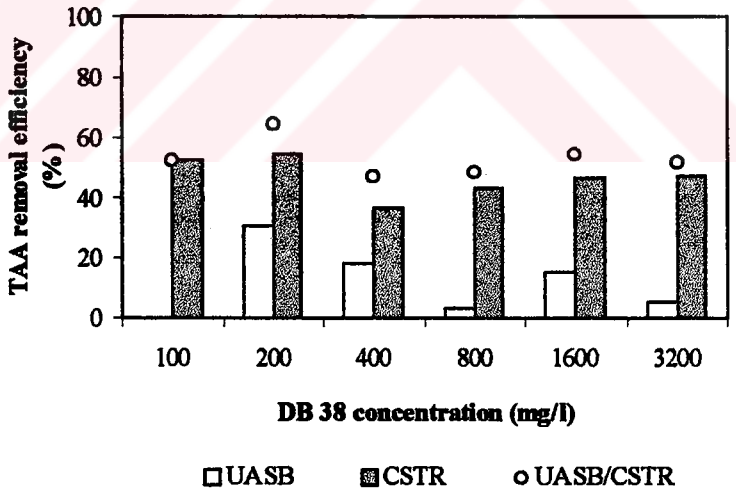


Figure 3.37. TAA removal efficiencies in UASB, CSTR and UASB/CSTR sequential system

3.2.4.5. UV-VIS scans under anaerobic and aerobic conditions

The decolorization of DB 38 dye appeared at all dye concentrations in the UASB reactor was measured with the decrease in light absorbance from the color

measurement. Figure 3.38 shows that there is only one dominant wavelength (λ_{max}) for maximum peak, hence the alteration in λ_{max} implied a relatively constant change of color in UASB/CSTR system treating 400 mg/l of DB 38 azo dye. These findings demonstrated a change in the chemical structure of DB 38 azo dye. The λ_{max} at the end of the aerobic step differed from that than the end of the anaerobic stage for DB 38 dye meaning that an alteration of dye structure took place in the aerobic process.

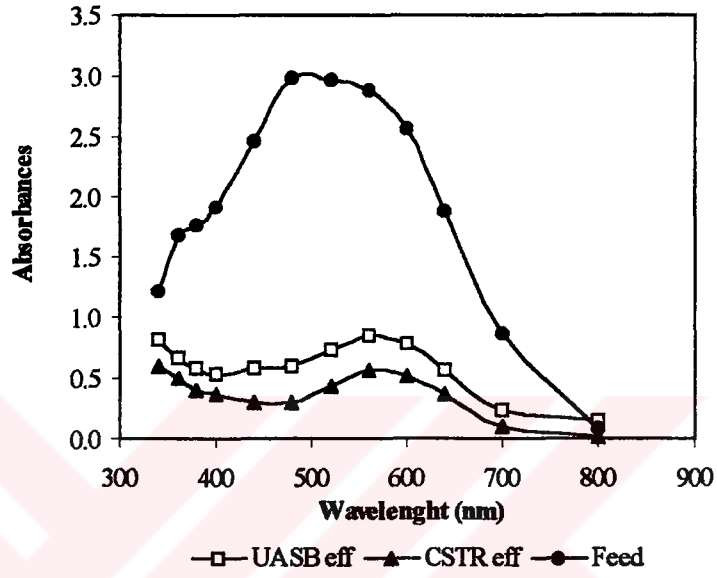


Figure 3.38. Absorbance measurements of influents and effluents at different wavelengths in UABS/CSTR system treating 400 mg/l DB 38

If dye removal is attributed to biodegradation either the major visible light absorbance peak would completely disappear or a new peak would appear. Dye absorption would result in cell materials, which are deeply colored because of adsorbed dye whereas those retaining their original colors would be accompanied by the occurrence of biodegradation (Chen et al., 1999). Abiotic batch decolorization studies showed that no significant color removals were observed under anaerobic conditions for DB 38 dye by granule organisms in UASB reactor. Figure 39 displays the change of UV-visible spectra of DB 38 dye using the supernatant fluid of the influent and effluent samples. The absorbance peak at 520 nm completely disappeared after anaerobic and aerobic process. In other words, when comparing the UV-VIS scans of dye, it was observed that the aforementioned spectrums were quite different suggesting that DB 38 dye was degraded and exhibits maximum absorbance

peak at 560 nm. These results indicate that the color removal in the UASB reactor may be largely attributed to biodegradation under anaerobic conditions. The similar spectrums in the anaerobic and aerobic effluents showed that some intermetabolite(s) products of DB 38 was not removed both in anaerobic conditions and aerobic conditions. Probably, these metabolite(s) has/have a blue color in the absorbance spectra as reported by Knapp and Newby (1995).

It is possible that the azo bonds in the DB 38 dye behaved as electron acceptors and were broken down through reduction-oxidation process, resulting in less COD removal than was evident at a higher dye concentration i.e. 84 percent. This was due to a higher non biodegradable (recalcitrant) portion of dye. The color reduction of DB 38 dye was through the alteration of the dye structure (cleavage of three azo bonds) during the anaerobic treatment. Data analysis of the visible absorption spectrum showed that anaerobic treated effluent peaks at 560 nm and the parent dye at 520 nm (See Figure 3.38). These peaks were reduced in the anaerobic stage. Data on the fate of the aromatic residues based on benzidine shows strong absorbance peaks at 560 nm. Aeration reduces these peaks as suggested by Haug et al. (1991). Their mineralization is desirable to reduce the toxicity. Aerobic organisms are exposed to a range of oxide free radicals which are neutralized by dismutase and catalyses, whereas anaerobes do not have these enzymes and it is possible these dyes induced superoxides, which could be an alternative explanation for the inhibition as reported by Shaw et al. (2002).

DB 38 was decolorized and reduced to benzidine at high efficiencies (84-95%) even at DB 38 dye loading rates as high as 107 and 213 g m^3.h in the anaerobic stage. The yield of TAA were between 40 and 60% during the whole period, indicating that products breaking down through decolorization of DB 38 were not mineralized further as shown in Figures 3.36 and 3.37. When comparing the UV-VIS scans of DB 38 dye and, aromatic amines (benzidine standards) with those of influent and effluent reactor, it was observed that the spectrum of the aerobic effluent was not exactly the same as the spectrum of benzidine alone, since the absorbance

was probably masked by the other intermetabolites spectra at 400 nm in the aerobic reactor effluent suggesting that benzidine was degraded.

Although the GS-MS spectra of breakdown products were not detected this suggest that the biological mineralization of the aromatic amines was detected at low levels in the effluent, indicating that most of the aromatic amines released were metabolized. The comparison of the UV-VIS scans of benzidine and those of the influent and effluent reactor confirmed the mineralization of a part of TAA. Furthermore, this result indicated that TAA derived from DB 38 could not be anaerobically mineralized as a sole substrate in the anaerobic reactor.

3.2.5. General discussion

The findings of the study relevant to the removal of RB 5 have shown that the sequential anaerobic/aerobic system was efficient in the degradation of diazo dye ReactiveBlack-5. In this experiment with partially granulated sludge cultures and with glucose as co-substrate, ReactiveBlack-5 dyestuff was almost decolorized under anaerobic conditions. Up to 98% of the color of the azo dyestuff was removed in the anaerobic stage. The released intermediates were mineralized in the aerobic part of the two stage system. It was observed that 85-95% of the remaining COD was removed in the aerobic stage. No color removal occurred aerobically. The COD removal efficiencies were 40-60% at organic loading rates of 4-8 kg/m^3.day while the mean methane percentage was 70% in the anaerobic UASB reactor.

The findings of this study relevant to the removal of DR 28 show that, the DR 28 dye could be completely reduced, decolorized, and the reduction products were acceptable for aerobic mineralization in the anaerobic pre-treatment. Anaerobic treatment cleaved the two azo bonds in the DR 28 dye and aerobically oxidized many amines and possibly the sulfonated products released during the breakdown of DR 28 dye. Since the limiting step of anaerobic biomineralization of any azo dye is the degradation of aromatic amines, anaerobic/aerobic systems might be effective in achieving the complete biodegradation and detoxification of azo dyes.

The findings of this study demonstrated that the DB 38 dye could be decolorized, in the anaerobic pre-treatment with glucose which acting a electron donor for the enzyme cofactors transferring electrons for azo dye reduction during the breakdown of DB 38 dye and that the reduction products were acceptable for aerobic mineralization. These findings have important implications for the environment since the amines are an important widespread contaminant of aqueous ecosystem; therefore an aerobic post-treatment step would be required for the complete mineralization of DB 38 dyes containing textile and dye industry effluents.

Several researchers have shown that the aromatic amines could be degraded under anaerobic conditions (Razo-Flores et al., 1997, Battersby & Wilson, 1989; O'Connor &Yang, 1993). Our results relevant to partial mineralization of benzidine metabolism released from the DR 28 azo dye cleavage provides electrons to support continued the reduction of DR 28 azo dye coincide with the published references given above. However these results conflict with the studies performed by Haug et al. (1991); Brown and Hamburger (1987), and Field and Stams (1995) reporting the aromatic amines could not be degraded further in anaerobic reactors.

The removal of the anaerobic effluent was demonstrated with higher aromatic amine and COD removal efficiencies through aerobic stage. Therefore coupled anaerobic-aerobic systems have proven to be successful in achieving the complete biodegradation of azo dyes. In such systems, azo dyes are reduced anaerobically, followed by subsequent aerobic degradation of the aromatic amines produced (Razo Flores et. al., 1997; Chang et al., 2001)

The findings of glucose-COD and alkalinity effects on color and COD removals showed that the decolorization of DR 28 dye in an UASB reactor did not correlate with COD concentrations, COD removals and bicarbonate alkalinity. It seems that the reducing environment prevailing in the UASB reactor by the electrons releasing from the DR 28 dye and its inter-metabolite products, mainly from the benzidine aromatic amine provides the color removal. The aromatic amines provide electrons supporting the reduction of the dye resulting in complete decolorization even at

substrate-free operation. The TAA removal efficiencies were 40 and 38% at COD concentrations 500 and 100 mg/l, respectively, indicating the partial degradation of aromatic amines under anaerobic conditions.

In an UASB system treating textile wastewaters containing azo dye alkalinity is lost through NH_4-N conversion to organic nitrogen, the buffering of H_2CO3 acidity and VFA generation by the granules. VFA build up with VFA generated, increased the acidity and reduced the alkalinity allow the partial phase separation of acidogenesis from methanogenesis in the lower active zone in UASB reactor. The NH_4-N loss and VFA cause a drop in pH levels. In the upper active zone of the granular bed the VFA converted to methane. Thus, regenerates the alkalinity and increases the pH.

Although some studies showed that the implementation of external carbon source to the anaerobic reactor improved the color removal which helps a reducing environment and possible increase in the concentration of enzyme cofactors (Carliell et al, 1995; Gingel and Walker, 1971; Banat et al., 1996; Brown and Laboureur, 1983; Chinwekitvanich et al. 2000) the remaining studies showed that the dyes can be used as sole carbon source (Chang et al., 2001; Razo-Flores et al., 1997, Kalyuzhnyi et al., 2000; Blümell et al., 1998; Coughling et al., 1997) which are in agreement with our findings in this study.

Because the textile sector in Turkey is one of the most important industrial sectors both in terms of its contribution to economy and environmental emissions, besides cost-effective anaerobic treatment process the additional external carbon sources and alkalinity requirements should be kept in mind that could not offer available option in terms of economical costs. If at least an alkalinity is required to buffer the textile industry wastewaters containing azo dyes, this could adversely affect the anaerobic treatment if the wastewater is diluted. However, this study showed that 100 mg/l of DR 28 azo dye could be used as substrate and a bicarbonate alkalinity as low as 500 mg/l is enough for simultaneous decolorization (E=100%) and COD removal (E= 56).

3.3. Treatment of Real Textile Wastewaters

3.3.1 The Treatment of Acid Dyeing Wastewater Taken from a Wool Fabric Manufacturing Plant

The treatment of the wastewater taken from a wool dyeing processing in a wool manufacturing plant located in Izmir was investigated using an anaerobic/aerobic sequential system following the treatment of azo dyes in the same system. The samples taken from the wool dyeing unit of the mill was transported to laboratory in order to feed to sequential anaerobic/aerobic reactor system. In this fabric acid dyeing had been performed with the treatment of 15 kg of dye, 15 kg of sulfuric acid, about 2 l of antifoam for prevent to foam, and 30-35 kg of salt (sodium sulphate) after 550-600 kg of the wool put into boiler in order to obtain the desired color (personal reviewing). The characterization of the real wastewater taken from the fabric is summarized in Table 3.5. As can be seen in this table, the dissolved COD, inert COD and TOC were 476, 200 and 170 mg/l, respectively, in Run 4.

Table 3.5. Characterization of acid dyeing wastewater during the operation period

Run	Run 1(1-7)	Run 2 (8-17)		Run 3 (18-33)		Run 4 (34-49)	
	addition*	Raw	addition*	raw	addition*	raw	addition*
Total COD (mg/l)		606	1593	-	-	499	
Soluble COD (mg/l)	1908	375	1414	212	1054	476	1445
inert COD (mg/l)		-		-		200	
TOC (mg/l)		88	463	-	-	170	487
Alkalinity (mg/l)	1930	380	1460	110	1350	120	1220
pH	8.67	7.26	8.43	5,86	7.68	6.41	8.33
λmax (nm)		480	480	540	520	500	500
Absorbance		1.015	0.929	0.080	1.728	1.874	1.945

* If it is necessary, glucose, alkalinity, and dyes were added to feed. The values given in second colon represent the data measured through the experiments.

The lab-scale UASB/CSTR reactor system was fed with 2000 mg/l of glucose-COD, 3000 mg/l of $NaHCO_3$ (Run 1), and suitable Vanderbilt mineral medium

during 7 days of start-up period. After that, the UASB reactor was fed with wastewater taken from the acid dyeing processing. Since acid-dyeing wastewater did not contain significant COD and color, 1000 mg/l of glucose-COD was added to the feed of UASB reactor for growth of microorganisms. Furthermore 2000 mg/l of alkalinity was added to the feed of the UASB reactor since the raw wastewater had low alkalinity as can be seen in Table 3.5. Suitable alkalinity provides stable conditions for the microorganisms through effective treatment. Table 3.6 summarize the dye used in the dyeing process of the fabric and added to the lab-scale reactor system.

Table 3.6. The dyes used through acid dyeing at period of operation

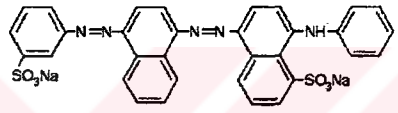
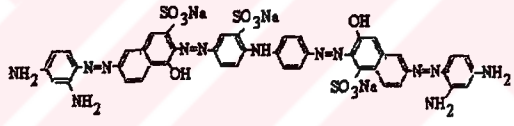
Name	Formula of dyes	Information
Acid Blue 113)C.I. 26360	 $C_{32}H_{23}N_5O_6S_2$ 682 g/mol	This dye was used in acid dyeing processing in the fabric (Run 2). This dye was added to the raw wastewater in Run 3 as 100 mg/l .
Direct Black 22 C.I. 35435	 $C_{44}H_{32}N_{13}Na_3O_{11}S_3$ 1084 g/mol	This dye was used in acid dyeing processing in the fabric (Run 2). This dye was added to raw wastewater in Run 3 as 100 mg/l.
Sarasit Blue SR	Chemical formula was not found	This dye was used in acid dyeing processing in the fabric (Run 3). The dyes mentioned above were added to wastewater since the synthetic wastewater had low absorbance.
Acid Wool Black ATT	Chemical formula was not found	This dye was used in acid dyeing processing in fabric (Run 4)
Black 10B	Chemical formula was not found	It was used in acid dyeing processing (Run 4)

Figure 3.39 illustrates the 200 mg/l inert COD measured through Run 4. It is important to note that the color of the raw wastewater could not be removed through aerobic inert COD experiments since the azo bond probable could not be cleaved during 30 days.

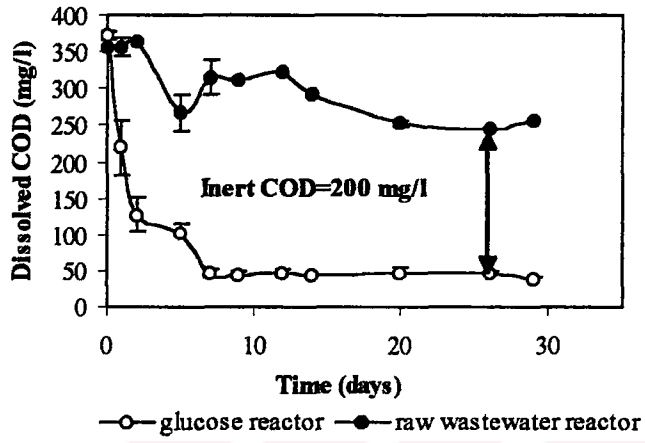


Figure 3.39. Determination of inert COD in raw wastewater through Run 4

3.3.1.1. Variations in methane production rate, VFA and B.Alk. in UASB reactor

The variations of VFA, B.Alk and methane production rate in effluent of UASB reactor are shown in Figure 3.40 through all Runs. VFA, B.Alk. values and methane production rates found to be 40 mg CH_3COOH/l, 1593 mg $CaCO_3$/l and 1437 ml CH_4/day through Run 3 in which Acid Blue 113, and Direct Black 22 of 100 mg/l added to raw wastewater. In this Run reactor system operated at favorable conditions (low VFA and high methane production rates). The ratios of VFA/B.Alk and pH values are illustrated in Figure 3.41. In Run 4, the VFA concentrations increased slightly but did not exceed the critic value for VFA/B.Alk (0.4). The VFA/B.Alk. ratios were about 0.15 in Run 4, indicating the stability of the UASB reactor (Behling et al., 1997) while the VFA/B.alk. ratio was determined to be 0.025 in Run 3. pH values obtained were suitable for optimum anaerobic treatment through all Runs (6.5-7.5)

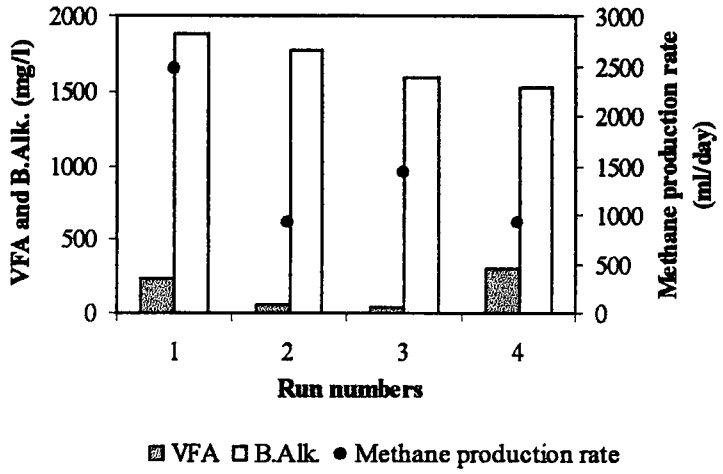


Figure 3.40. Variation in methane production rate, VFA, and B. Alkalinity concentrations in four operation runs

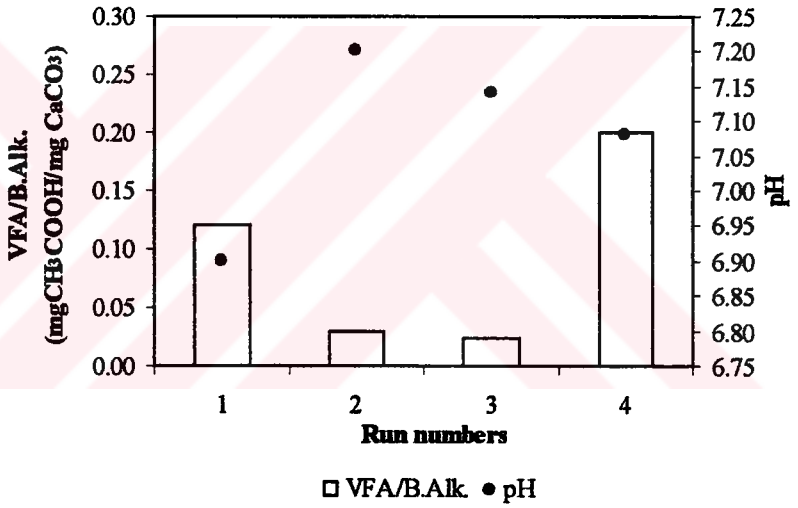


Figure 3.41. Variation of pH and VFA/B.Alk. ratios in the effluent of UASB reactor

3.3.1.2. COD removal efficiencies of UASB/CSTR reactor system

The COD removal efficiencies in total system are shown in Figure 3.42. It may be said that the COD removed perfectly in the system during the operation except Run 4. In Runs 2, 3 and 4, 1000 mg/l of the glucose-COD was added to raw wastewater

since the wastewater contained only glucose-COD of 2000 mg/l. Since the glucose is easily degradable organic matter the initial glucose-COD could be reduced to 60 mg/l at a total HRT of 2.3 days in UASB/CSTR system. At the following Runs, final COD values were higher than the COD values obtained in Run 1 since the raw wastewater might be contained considerably inert COD values. In Run 4, the effluent COD of the system was decreased to about 257±27 mg/l since the inert COD level of raw wastewater was 200 mg/l. The system could be removed all degradable COD from he total soluble COD fraction.

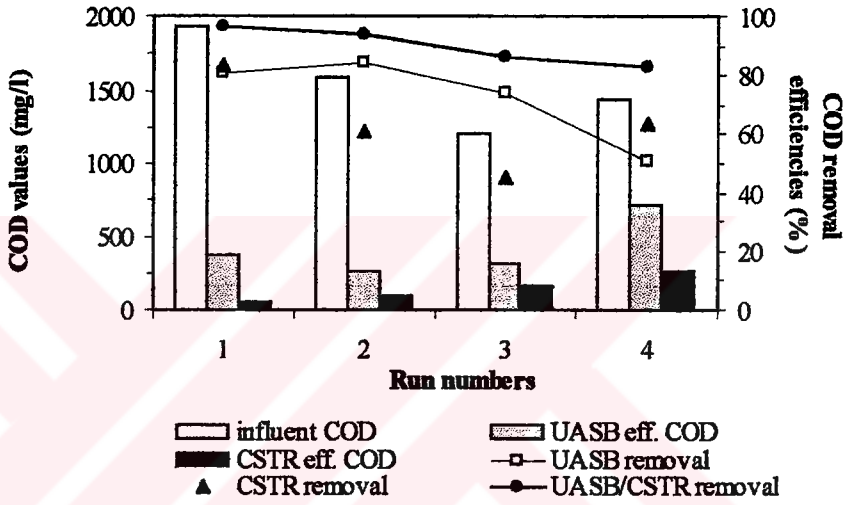


Figure 3.42. The COD removal performance of the UASB/CSTR sequential system

3.3.1.3. Performance of color removal in UASB/CSTR reactor system

The removal efficiency of color was not decreased lower than 80% in total system as shown in Figure 3.43. Color was reduced mainly in anaerobic stage. In Run 3 and 4, aerobic stage helped to the removal of color since the intermediate products released from the degradation of azo dye was ultimately decolorized. For instance, in Run 3 the color of UASB influent, and effluent and CSTR effluent were 285, 93 and 44 m^{-1} corresponding to color removals in UASB (E=83), CSTR (E=23) and UASB/CSTR (E=91) reactor system. In Run 1 the influent color originated from

Vanderbilt mineral medium except dye wastewater. However the color in anaerobic and aerobic effluents produced from the metabolic activity of the biomass.

Similar results obtained in a study performed by Kuai at al. (1998) concerning with the treatment of the textile wastewater taken from a carpet dyeing and printing factory by in a lab scale anaerobic (UASB)/aerobic SCAS (Semi-continuous activated sludge) reactor system. The average COD and color was decreased to 79-219 mg/l and 0.02-0.07 absorbance unit (at a wavelength of 500 nm) from 6000 mg/l and 0.8 absorbance unit in the aforementioned system at a total HRT of 1-2 days.

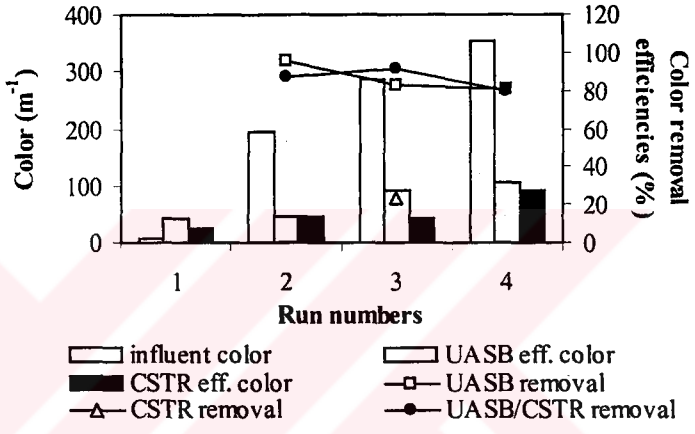


Figure 3.43. Color removal efficiencies of the system through 50 days when the reactor was fed with acid dyeing wastewater

Figure 3.44 shows the absorbance measurements at varying wavelength in UASB/CSTR system treating acid dyeing wastewater in Run 4. The influent spectrum had two peaks in the visible region at 500 nm and 620 nm. In the anaerobic treated sample spectrum the peaks occurred in the UV region at 360 nm, indicating the cleavage of the azo bond, which thus providing colorless of dye. The effluent sample of CSTR reactor exhibited maximum absorbance spectra at low wavelengths (325 nm) due to the aromatic amines produced through the dye degradation. This shows that the parent dye converted to other intermediate organic forms in UASB reactor, and then these intermediates could be degradated in CSTR reactor.

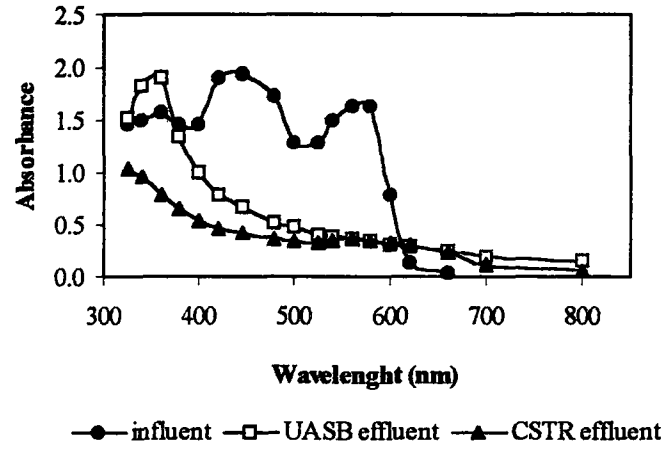


Figure 3.44. Absorbance spectra in influents and effluents of the reactor system at different wavelengths in Run 4

3.3.1.4. Variation of TAA in UASB/CSTR sequential system

Since the dye concentration of raw wastewater was not known, the TAA content of the wastewater could not been determined by the calibration lines given in Chapter 3. Therefore, The TAAs removal efficiencies in UASB and UASB/CSTR reactor system could not be calculated. However the aerobic stage had ability to decrease TAA values lower than 5 mg /l of TAA as shown in Figure 3.45. This result shows that the aerobic stage is very important for the removal of aromatic amines.

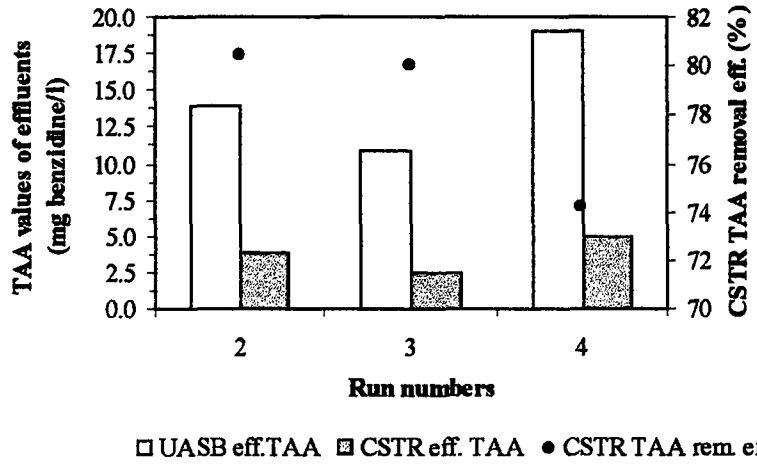


Figure 3.45. TAA removal efficiencies in CSTR reactor

3.3.2. Treatment of real textile mill wastewater

Real wastewater of a textile cotton mill located in Söke was treated using the lab scale anaerobic/aerobic reactor system after the wool dyeing wastewater was treated in the same reactor system. The pH of the wastewater coming from the all processing of the textile mill was adjusted to 7 in a equilibrium tank of the real treatment plant. The wastewater was weekly taken from the equilibrium tank, was transported to laboratory, and the reactor system was fed with this real wastewater. The treatability of wastewater with a flowrate of 2 l/day was investigated during 87 days of operation period. The UASB/CSTR reactor system was fed with the textile wastewater after characterization was performed. In the last period, glucose-COD of 1000 mg/l was added to the influent of system keeping stable anaerobic conditions in the anaerobic reactor since the raw wastewater had low COD content. The color of the wastewater coming from the cotton textile mill was found to be very low than the color of synthetic and acid dyeing wastewaters studied previously. This can be explained by the dilution of dyeing processes wastewater with wastewaters arisen from the other processes in this textile mill. Therefore, the dyes used in the previous studies were added to the influent of the system at a level of 50 and 100 mg/l. The characterization of wastewater taken from the mill was illustrated in Table 3.7. All the necessary micro and macro elements were provided from the real wastewater for microbial growth since it contains domestic wastewater. Therefore, mineral elements was not added to wastewater.

In this part, the inert COD of wastewater taken from the textile mill was determined for evaluation the treatability of this wastewater. The experiments of inert COD at different samples were shown in Figure 3.46. It was observed that the fraction of inert COD was about the half of the total COD during batch tests.

3.3.2.1. COD removal efficiencies in whole reactor system

The COD removal efficiencies of the total reactor system exhibited variations during operation period since the characteristic of the wastewater from the cotton mill was variable as shown in Table 3.7. The biodegradable part of the COD in wastewater was removed effectively in the system by taking into consideration the inert COD fraction of the influent samples (see Figure 3.46)The influent COD and effluent CODs of the whole system are illustrated in Figure 3.47 for each run.

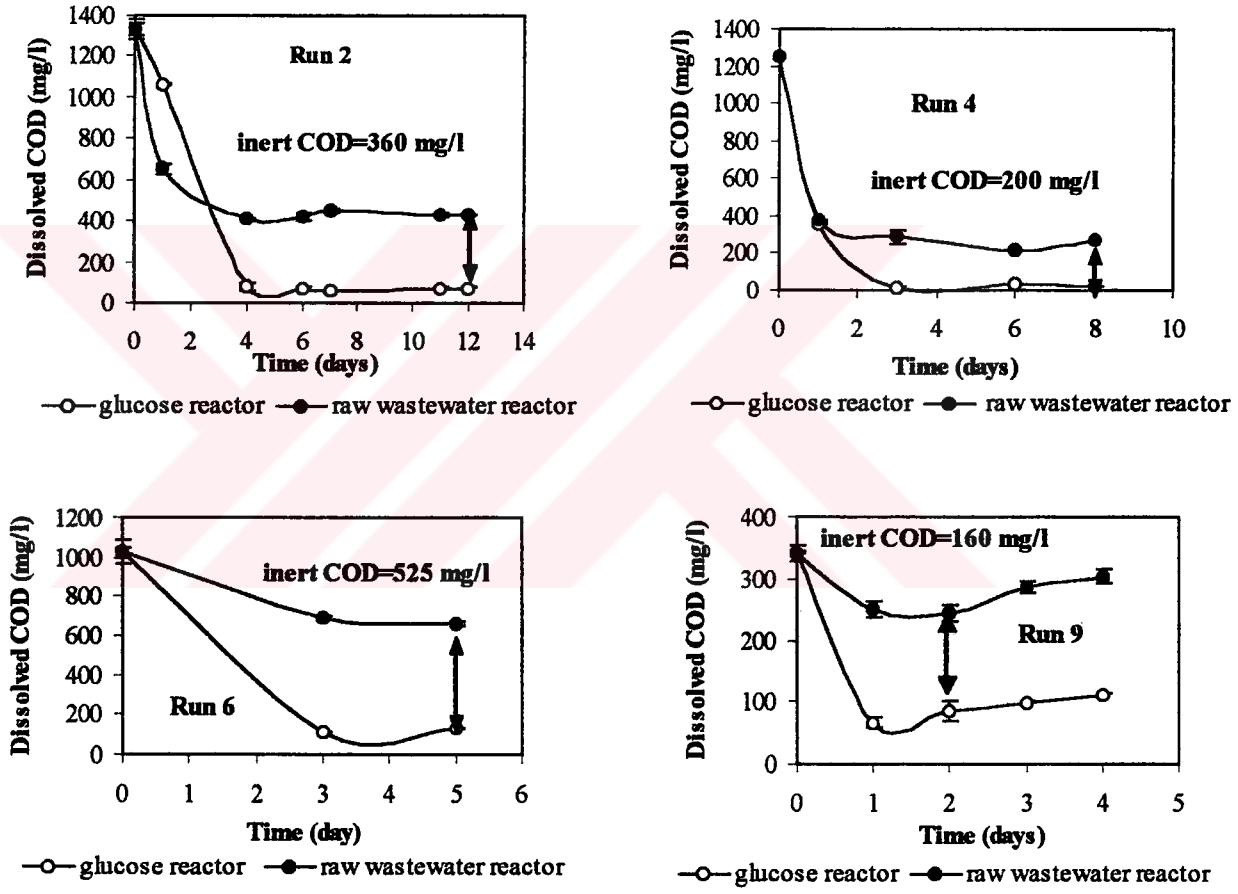


Figure 3.46. Determination of inert COD concentrations in wastewater samples of a cotton textile mill

Table 3.7. Characterization of wastewater of the cotton textile mill during operation period

Run	Run 1[1]	Run 2	Run 3	Run4	Run 5	Run 6	Run 7	Run 8		Run 9	
								Raw	Adition[2]	Raw	Addition[3]
Total COD (mg/l)	604	1708	1431	673	615	1215	1038	-	-		-
Soluble COD (mg/l)	463	1284	1112	482	493	997	952	305	518	342	1342
Inert COD (mg/l)		360		200		525				160	
TOC (mg/l)	143	388	292	147	157	289	348	76	-		283
Alkalinity (mg/l)	500	800	600	520	600	650	320	-	400	530	-
pH	7.48	7.25	7.17	7.31	9.2	7.27	7.23	6.89	7.15	7.22	7.46
Color (m^{-1})	20	52	65.5	111	24	79	37	-	935	37	758
λ_{max} (nm)	-	400	400	400	400	400	420	-	480	530	497
SS (mg/l)	131	130	167	106	107	103	-	-	-		

[1] The UASB system was fed the synthetic wastewater with glucose-COD of 2000 mg/l instead of wastewater in this run.

[2] The dyes was added to influent at a concentration of 100 mg/l for each dye (Reactive Black 5, Direct Red 28, Direct Black 38, Direct Brown 2, and Direct Yellow 12)

[3] The dyes and glucose was added to influent at a concentration of 50 mg/l for each dye (Reactive Black 5, Direct Red 28, Direct Black 38, Direct Brown 2, and Direct Yellow 12) and 1000 mg glucose-COD/l, respectively.

Textile wastes are characterized by high volume and extremely variable composition which can include non-bidegradable dyes and toxic substances. The variability arises both from the diversity of the types of industrial processes employed and the immense range of chemicals and materials involved within each category. Generally, textile wastewaters contain 1000 mg/l of COD and the BOD_5 is 300-500 mg/l corresponding BOD_5/COD ratio of 0.3-0.5 as reported by Correia et al. (1994). Germirli et al. (1991) reported that textile wastewater with a soluble COD of 1000 mg /l included a inert soluble COD of 190 mg/l indicating that COD was not degraded in textile wastewater. In the Run 2 the influent soluble COD of 1248 mg/l decreased to 598 mg/l and 226 mg/l (HRT of 30 h) in the effluents of UASB and CSTR reactor (HRT of 4.50 day), respectively. In this run, since wastewater had 360 mg/l of inert COD under aerobic condition, it is suggested that the anaerobic stage improved the biodegradability of wastewater under aerobic conditions. One of the most reasonable explanation for this improvement is that anaerobic process was apparently changed the molecular structure of substances (dyes), which readily degradable under anaerobic conditions as reported by An et al. (1996). Similar results obtained in Run 6 in which The COD of textile wastewater reduced to 376 mg/l using UASB/CSTR system with an initial COD of 1025 mg/l and inert COD of 525 mg/l at the same HRTs. This corresponded to 37, 40 and 62% removal efficiencies in UASB, CSTR and UASB/CSTR reactor, respectively.

The soluble organics in the effluent of a biological treatment process may include not only the biodegradable and non-biodegradable compounds originated from the raw wastewater, but also the compounds formed by microorganisms in the treatment system itself. Non biodegradable organics formed by the microorganisms can be classified as compounds produced as a result of substrate metabolism and microbial growth, or compounds released during the lysis and degradation of microorganisms (Germirli et al., 1990). When the system was fed only with glucose-COD (2000 mg/l) the effluent of the system included a COD of 126 mg/l in Run 1. This shows that the fraction of non biodegradable organics from substrate metabolism and microbial growth in the system were about 5% through anaerobic/aerobic incubation. Kua et al. (1996) found that normalized production of soluble microbial products ranged from 0.6 and 2.5% of the glucose fed concentration.

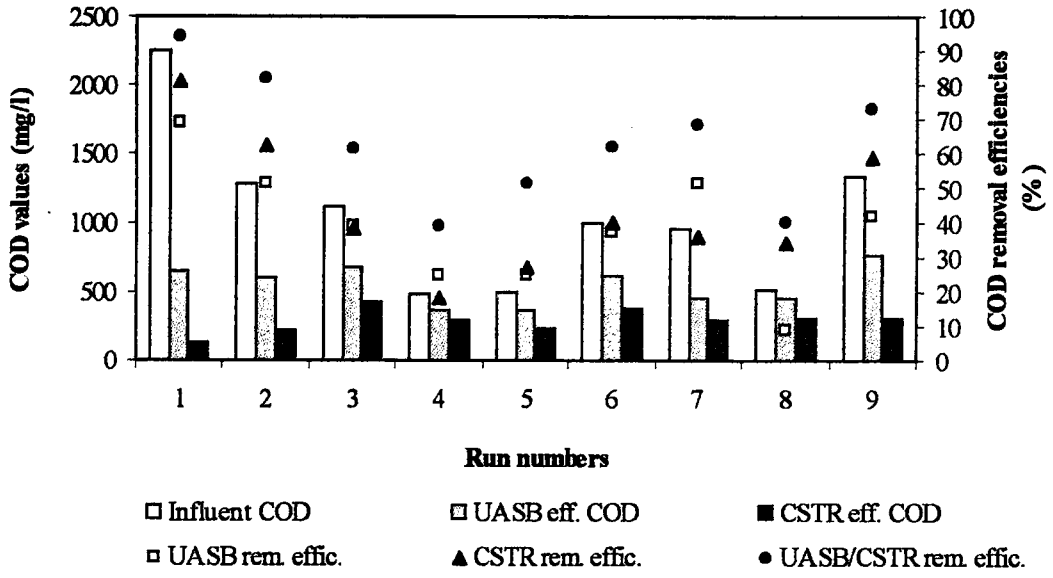


Figure 3.47. COD removal performance of the UASB/CSTR sequential reactor system

3.3.2.2. Variations in methane production rate, VFA and B.Alk. in UASB reactor

The variations of VFA, B.Alk and pH in effluent of UASB reactor are shown in Figure 3.48 through all runs. VFA concentrations in Runs 1 and 9 were slightly higher than the other runs because glucose was added to the feed in these runs. Except Runs 1 and 9, the VFA concentrations were very low in the UASB reactor effluent due to low COD concentrations in influents, and excellent reactor performance. The ratios of VFA/B.Alk and pH values are shown in Figure 3.49. VFA concentrations found to be 389, 58 and 286 mg CH_3COOH/l while the methane production rates were 938, 360 and 372 ml CH_4/day for Run 1, 6 and 9, respectively. In Run 1 and Run 9, the VFA concentrations increased slightly but did not exceed the critic value of VFA/B.Alk ratio (0.40) indicating the reactor stability. This shows

that the organic fraction of COD in wastewater was successfully converted to methane by methanogenic bacteria.

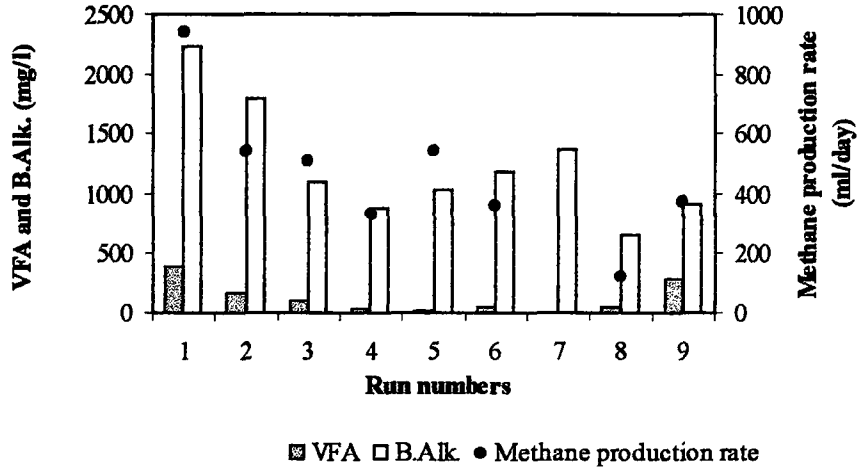


Figure 3.48. Variations in methane production rate, VFA, and B. Alkalinity concentrations at runs of operation

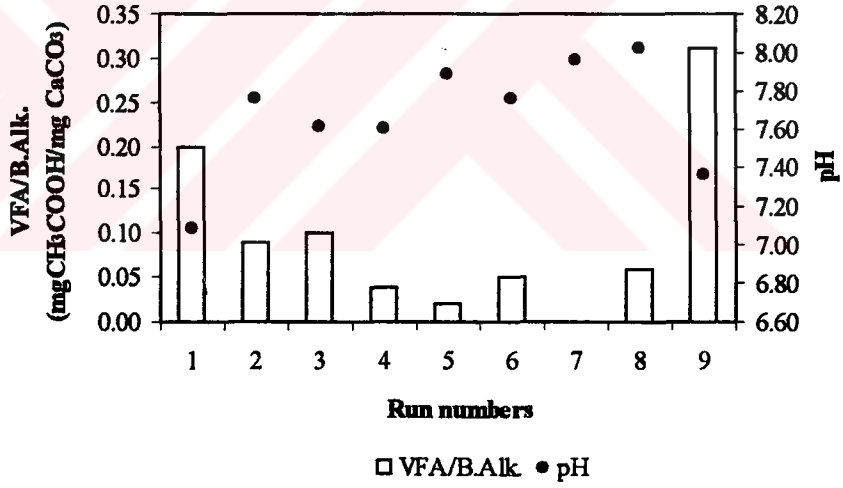


Figure 3.49. Variation of pH and VFA/B.Alk. in the effluent of UASB reactor

3.3.2.3. Performance of color removal in UASB/CSTR reactor system

The color of real textile industry wastewater was slightly purple, and exhibited red color. The performed color measurements based on the sum of absorbances at 445, 540, and 660 nm of wavelength are shown in Figure 3.50. Since the color of the

wastewater is not enough compared to the aforementioned values in previous studies, dye was added to the feed. Figure 3.50a shows the results taken from the real textile wastewater. The color of influent, UASB and CSTR effluents were found to be 111, 57.5, 32 m^{-1}, respectively, corresponded to 48, 38, 71% color removals in the UASB, CSTR and UASB/CSTR system, respectively, in Run 4. In order to investigate the effect of characterization of textile wastewater on decolorization process, the mixture of azo dyes were added to wastewater at a concentration of 500 mg/l (100 mg/l*5) and 250 mg/l (50mg/l*5) in Run 8 and 9, respectively. Figure 3.50b shows the results obtained from the study with synthetically colored wastewater. The color removal efficiencies of UASB, CSTR and UASB/CSTR reactor system were 74, 54 and 88% in Run 8 while they were 59, 5 and 61% in Run 9, respectively. The results of this study showed that although the color was mainly removed in the anaerobic stage, sometimes the aerobic stage also could reduce the color in the system. An et al. (1996) reported that COD and color were removed by more than 83% and 90% at a COD loading rate of 5.3 kg COD/m^3.day in the anaerobic stage, at a HRT of 6-10 hours for anaerobic stages and 6.5 h for aerobic stage when a wastewater from a dye manufacturing factory with color of 500 degree (dilution factor) an UASB reactor (4.5 liters) and an activated sludge tank (5 liters, adjustable),. It was reported that >99% color removal in a sequential anaerobic/aerobic biological filter system treating Basic Red at HRT of 1 day (Başıbüyük et al., 1997).

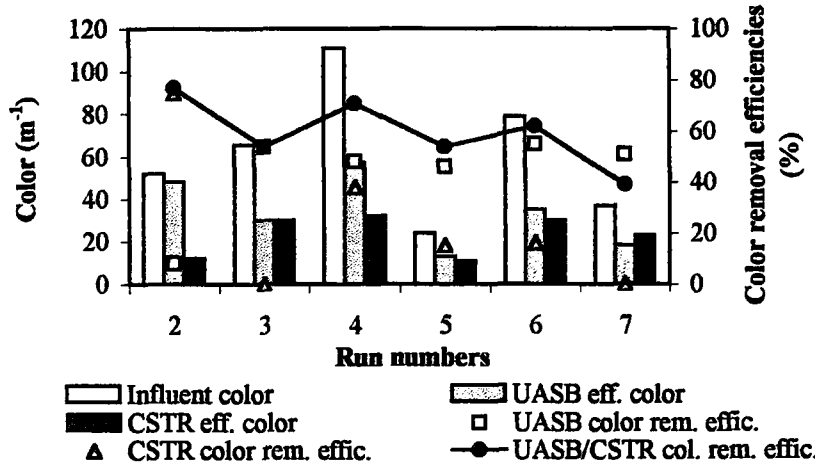


Figure 3.50a. Color removal efficiencies of the system through all runs when the reactor was fed with textile mill wastewater

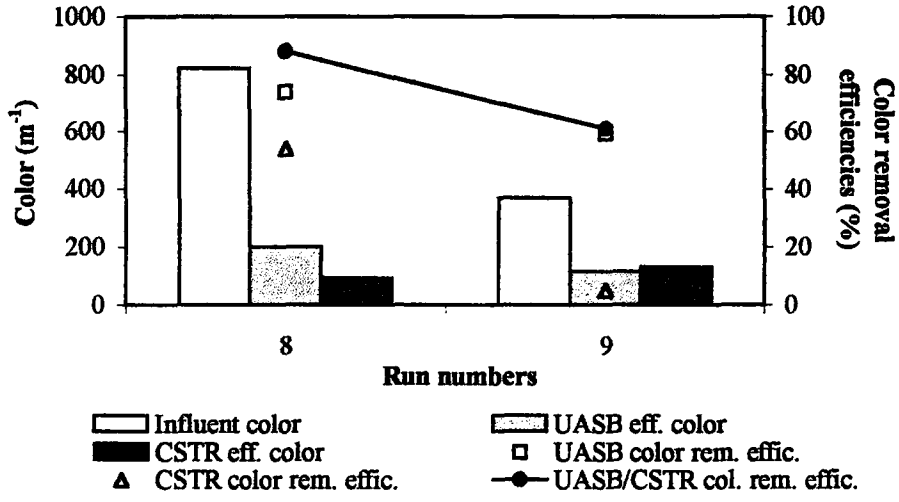


Figure 3.50b. Color removal efficiencies of the system through all runs when the reactor was fed with colored textile mill wastewater

Absorbance spectra of influent and effluents samples are shown in Figure 3.51 for UASB/CSTR system. This figure shows that the absorbance of the real wastewater exhibited low values. It is important to note that although the levels of the color was low (<100 m^{-1}), it did not changed through inert COD test under aerobic conditions. This shows that anaerobic conditions are significantly necessary for the removal of the color of dyes. Gingel and Walker (1971) proposed that reduced soluble flavins acted as electron shuttles to ferry electrons from the flavoproteins of the microbial electron transport chain to the acceptor azo compound. That is why the azo dye acted as the terminal electron acceptor in the microbial electron transport chain, being reduced and decolorized concurrently with re-oxidation of the reduced flavin nucleotides. In the presence of oxygen, the cleavage of azo bond can be inhibited since the oxygen plays a role as terminal electron acceptor.

3.3.2.4. Variation, and removal efficiencies of TAA in the reactor system

Although the dye formulas in fabric dyeing wastewater are not known, the presence of aromatic amines is doubtless in the structure of dyes. TAA measurements in the effluent samples are shown in Figure 3.52. All tests showed that the effluent of the UASB reactor had higher TAA values than the effluent of the

CSTR reactor. These results show that the aerobic stage is very important for the removal of aromatic amines.

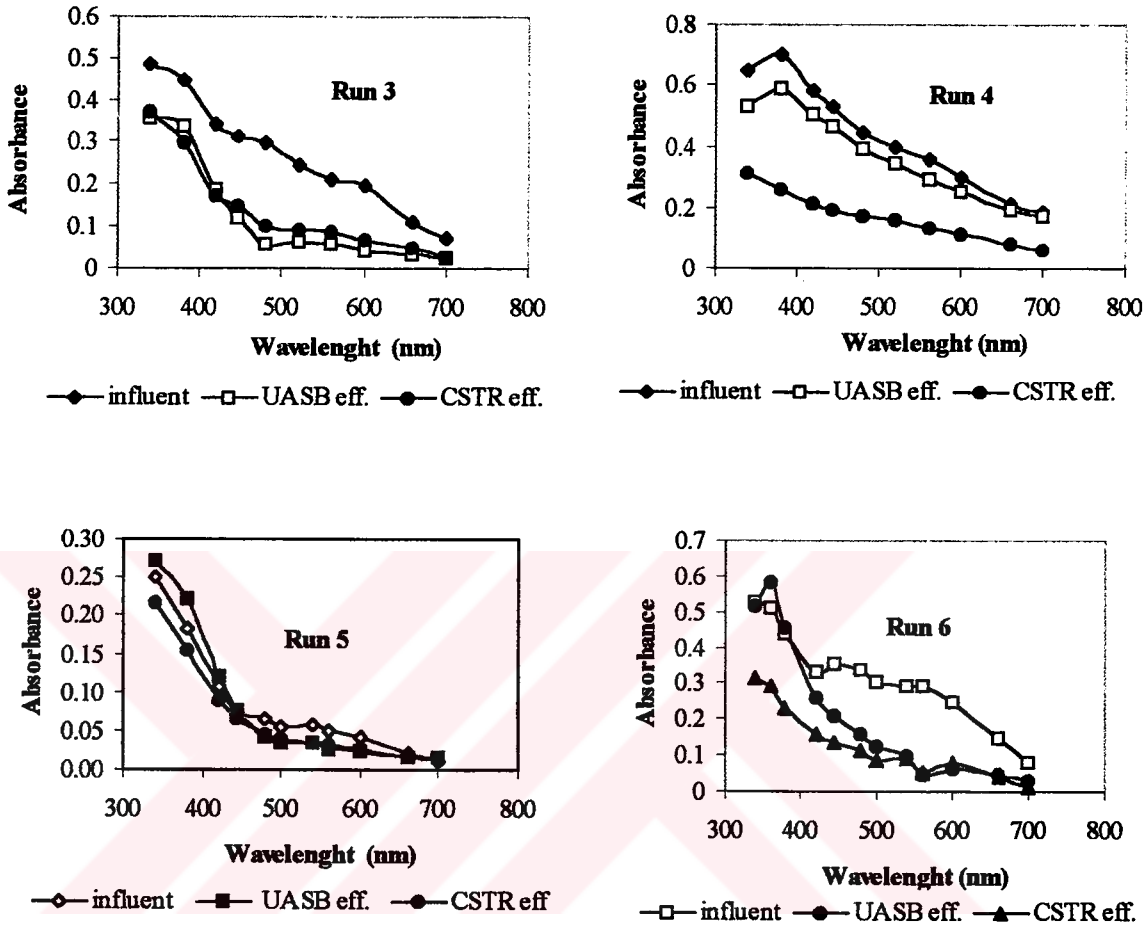


Figure 3.51. Absorbance spectra in influent and effluent of reactor system at different wavelengths

UASB effluent samples had higher TAA concentrations particularly at last two periods when synthetic dyes were added to the feed (See Figure 3.52). It was reported that the maximum dye concentration in textile industry wastewater is about 100-500 mg/l (O'Neill, 1999). The comparison of the last two periods with the other ones indicated that the real textile wastewater contains low dye concentrations.

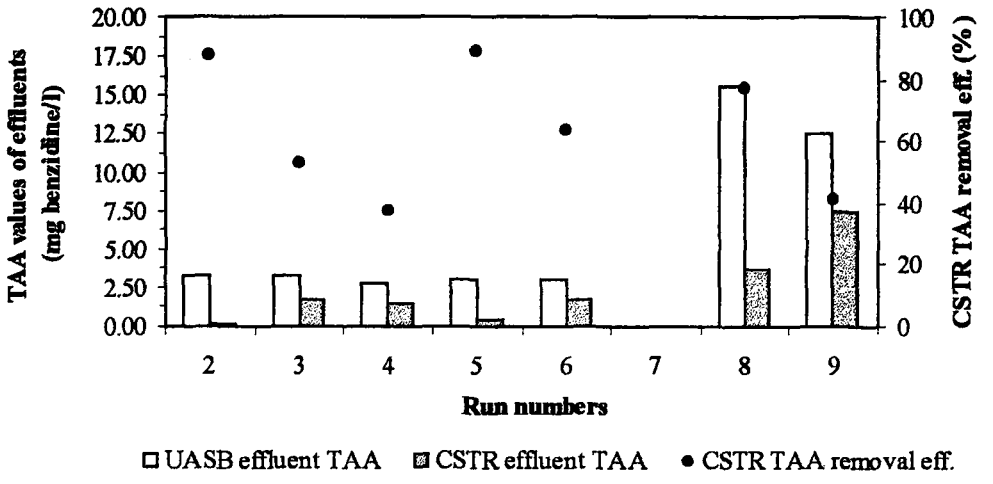


Figure 3.52. TAA removal efficiencies of CSTR reactor

3.3.3. General discussion for treatment of real wastewater

Textile processing industries produce COD at varying high quantities and composition in effluent depending on wet processes employed. Water consumption was summarized by Correia et al. (1994) and varies between 3 and 9 l/kg textile for cotton desizing and up to 334-835 l/kg textile for wool washing. Through acid dyeing for wool the major pollutant types may consist of the salts, (sodium chloride and sodium sulphate for neutralize the zeta potential of the fibre) acids, (acetic and sulphuric acid for pH control), bases (sodium hydroxide for control pH), and dyes (for dyeing fibre). Residual dyes coupled with the auxiliary chemicals are responsible from the color, dissolved solids and from the COD in dyeing wastewaters. The reported data show that the characteristics of acid dyeing wastewater depend on the type of dye, fiber and dyeing method. The characteristics of wastewater used in this study were similar to typical acid dyeing wastewaters produced from the wool dyeing. Correira et al. (1994) reported that wool acid dyeing wastewaters contained averagely a color of 3200 ADMI (American dye Manufacturers Institute unit), a TOC of 210 mg/l, a suspended solids (SS) of 8 mg/l,

and a pH of 4 through different dyeing process. In generally dyeing effluent have a high color content, high dissolved solids (DS) and moderate BOD values.

The major pollutant types identified in textile wastewater are summarized as organics (starches, enzymes, fats, greases, waxes, surfactants), color (dyes, scoured wool impurities), nutrients (ammonium salts, urea, and phosphate buffers), pH and salt effects (NaOH, mineral/organic acids, sodium chloride, silicade, sulphate, carbonate), sulphur (sulphate, sulphide and sulphiric acid), toxicants (heavy metals, reducing agents such as sulphide, oxidizing agents such as chloride, peroxide, dichromate and persulphate), and refractory organics (surfactans, dyes, resins, synthetic sizes such as polyvinyl alcohol, carboxylmethylcellulose, chlorinated organic compounds, carrier organic solvents) (Delee et al., 1998).

The partial treatment of dyes by classical aerobic biological systems (conventional and extended activated sludge) was attributed to precipitation or adsorption on the sludge. The oxygen consumption and sludge yield are generally high resulting in high operational costs (Kuai et al., 1998).

In the treatment studies with acid dyeing wastewaters, the glucose-COD (2000 mg/l in Run 1 and 1000 mg/l in other Runs) and $NaHCO_3$ (3000 mg/l in Run 1 and 2000 mg/l in other Runs), and dye types given in Table 3.6 were added to raw wastewater since raw wastewater contained low COD and/or TOC and alkalinity through experiment period in order to evaluate the feasibility of UASB/CSTR reactor system. The COD and color removal efficiencies in UASB reactor were between 51 and 84%, and 81-96% at a HRT of 15 hours while the UASB/CSTR system exhibited removal efficiencies of 83-97% and 80-91% at a HRT of 3 day, respectively. Furthermore, aromatic amines accumulated in anaerobic stage were removed effectively in the aerobic stage with TAA removal efficiencies varying between 74 and 80%. In the treatment of textile wastewater, COD removal efficiencies were found to be between 40 and 85 % at a total HRT of 5.75 days in the UASB/CSTR reactor. Color removal efficiencies were 39-81% in real and colored real textile wastewater through the operation period. TAAs were removed effectively by aerobic

CSTR reactor. The methane yield, pH and VFA concentration of UASB effluents showed that inhibition was not observed during experimental studies.

Since a number of studies about real wastewater containing dye were available in the literature removal efficiencies obtained in this study could be compared with following data:

An industrial wastewater from a food dye manufacturer fed a laboratory scale anaerobic baffled reactor at a concentration of 5% (v/v) and then increased to 10%. Anaerobic degradation of the wastewater was efficient. Methanogenic activity was high, the organic content of the influent reduced to about 70% and color reduced to almost 90% at a HRT of 20 h and an organic loading rate of 1.2 kg/m^3.day (Bell et al., 2000).

An experimental anaerobic/aerobic treatment plant was found to be successful in the treatment of a polyester and cotton mill wastewaters. The effluent was principal composed of dyes, auxiliaries, polyvinyl alcohol, detergents, acids, and alkali salts with COD levels of 600-900 mg/l. The mean efficiencies for COD and color removals were 78% and 72% respectively when the HRT and COD loading of Anaerobic Rotating Biological Contactor (RBC) and aerobic RBC were 7-8 hours, 40-50 g/m^2.d and 30-40 g/m^2.day, respectively (Zaoyan et al., 1992).The anaerobic stage consisted of a rotating fixed film reactor and loading rate varied between 1.66 and 2.71 kg COD/m^3.day. Good color removal (80%) and COD removal (92%) were reported for the treatment of a cotton processing effluent in an anaerobic/aerobic pilot plant followed by activated carbon as tertiary treatment. 55% the color removal was achieved in the anaerobic stage. The pilot plant was run for 10 months with average load of 2.13 kg COD/m^3.day and 1.71 kg COD/m^3.day for the aerobic biofilter (Yang et al., 1991).

3.4. Treatment of Simulated Textile Wastewater

In this run, simulated textile wastewater was treated using an lab-scale anaerobic/aerobic sequential reactor system. The characterization of simulated cotton textile wastewater is given in detail in section 2.3.3. (See Table 2.3). The UASB reactor was initially started to operate after one month of adaptation period of simulated wastewater since the aforementioned reactor had been previously used to treat the azo dyes and the real textile wastewater at an organic loading rate as high as 25 kg COD /m^3.day.

3.4.1. VFA accumulation, variations in B.Alk., and pH in UASB reactor

The organic loading rates were increased stepwise from 1 to 15.8 kg/m^3.day in UASB reactor through 46 days of operation period. Figure 3.53 shows the VFA, B.Alk. and pH levels during decreases in HRT from 100 to 6 hours in UASB reactor.

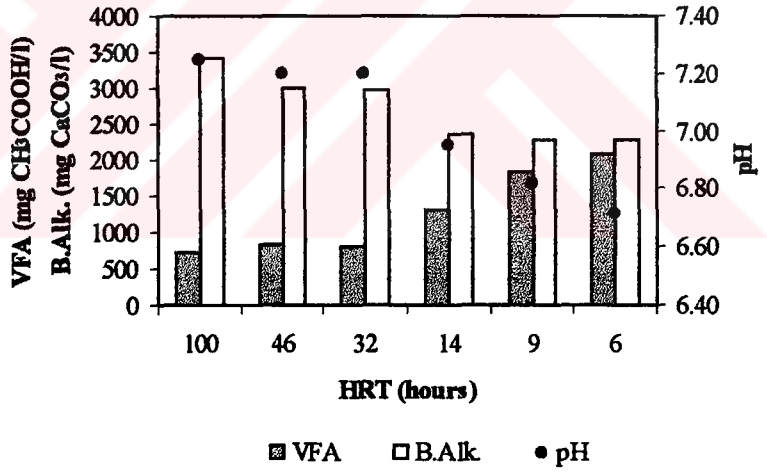


Figure 3.53. Variations in pH,VFA and B.Alkalinity through decreases in HRT

At the beginning, the VFA concentrations were higher than 50-300 mg/l as threshold level for desirable anaerobic operation as reported by Carliel et al, (1996) and Speece (1996). This might indicate that the population of methanogenic, and acetogenic bacteria present in the UASB reactor were not enough to convert the higher molecular weight acids (butyric and propionic acids) and acetic acid into

methane, respectively. Furthermore, it has also been suggested that the acetogenic and methanogenic bacteria could be inhibited by the presence of VFA (Hu et al. 2002 & Speece, 1996). The sensivity of the methanogens to pH, coupled with the fact that VFAs are intermediates in the stabilization of organic matter can result in an unstable response by anaerobic system to a decrease in pH (Grady et al., 1999). Although the pH remained in the optimal working range for anaerobic conditions (6.5-7.5) the VFA concentrations were increased from 700 to 2073 mg CH_3COOH/l through the operation. This could be explained by the hydrogen anion releasing from the volatile fatty acid and carbonic acid dissociation reacted with bicarbonate alkalinity in reactor, and therofore pH remained values between 6.5 and 7.5 (Speece, 1996).

3.4.2. Variations in total gas, methane gas productions, methane fraction and VFA/B.Alk ratios in UASB reactor

When the rate of acid formation exceeds the rate of break down to methane, a process unbalance results in which the pH and CH_4 content of biogas decreases, and the gas production falls off (Eckenfelder, 1989). This fact was observed in UASB reactor in this run as shown in Figure 3.54.

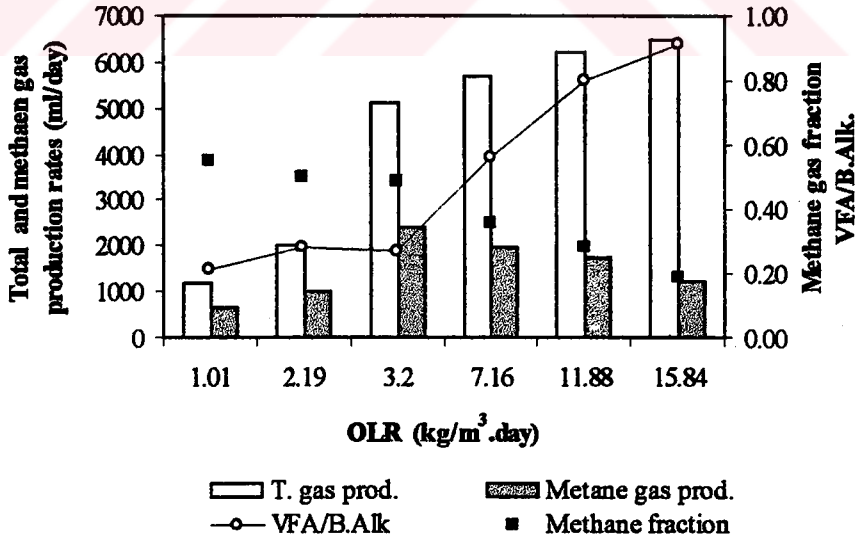


Figure 3.54. The variations of gas production rates, methane fraction, and VFA/B.Alk. ratios with increasing OLRs in the UASB reactor

Total gas production, methane gas fraction, and VFA/B.Alk. ratios were found to be 1176 mg/l, 0.55, and 0.21 at the beginning of the operation at an OLR of 1.01 kg /m^3. When the OLR increased to 15.84 kg /m^3 the aforementioned parameters were found to be 6480 mg/l, 0.188 and 0.91, respectively. The VFA/B.Alk. ratio was greater than 0.4 at an organic loading rate of 7.16 kg/m^3.day, indicating the moderate instability of the UASB reactor (Behling et al., 1997). The optimum organic loading rate for optimum pH, VFA/B.Alk ratio, and methane fraction was about 3.2 kg /m^3.day in UASB reactor. Under stable operating conditions, bicarbonate is the primary form of alkalinity in anaerobic reactor. However, under unstable operating conditions VFAs will react with bicarbonate alkalinity, both reducing its concentration and producing carbon dioxide, which increases the carbon dioxide content of the gas (Grady et al, 1999) as shown in Equation (3.1). At increasing OLRs in UASB reactor VFA occurred at high levels, B.Alk reacted with bicarbonate alkalinity and reduced to 2273 mg/l, therefore the methane content of biogas decreased to 18.8%.

$$HCO^-_3 + HAc \Leftrightarrow H_2O + CO_2 + Ac^- \qquad (3.1)$$

where; HAc represents the nonionized acetic acid and Ac^- represent the acetate ion.

Adrews & Pearson, (1965) suggested that the nonionized form of the VFAs is actually inhibitory, with concentrations on the order of 30 to 60 mg/l having an negative effect on the anaerobic microorganisms. It is appear that the inhibition caused by VFAs will be of little concern as long as the pH remains within normal range for the growth of methanogens (pH=6.8-7.4). For pH values below this range, pH impact themselves significantly was compounded by any inhibition caused nonionized VFAs (Grady et al., 1999). It is suggested that non ionized VFAs as low as 20 mg/l might be caused to slight inhibition of methanogenesis in UASB reactor at OLR of 15.84 kg/m^3.day.

3.4.3. COD and color removals in anaerobic UASB reactor

The effects of HRT and organic loading rates on the color and COD removal efficiencies are shown in Figure 3.55. At a HRT of 100 h, 80% COD and 91 % color removal efficiencies were obtained. Decreases in HRT caused decreases in COD removal while no significant variation in color removal was observed. This result can be explained by the complete cleavage of azo bond of azo dyes in very short HRTs (6 hours).

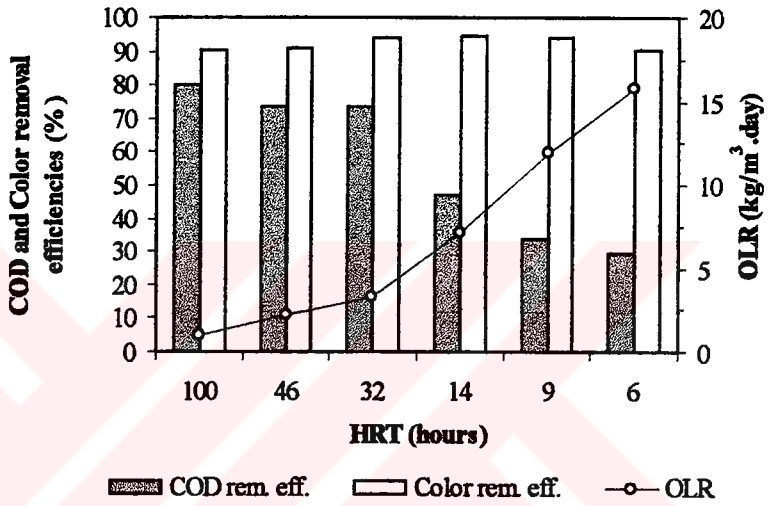


Figure 3.55. Color and COD removal efficiencies versus at varying HRTs in UASB reactor

Talarposthi et al. (2001) reported that a two stage mesophilic anaerobic upflow packed bed reactor can remove up to 90% of the color from 1000 mg/l of mixed cationic dye at a HRT of 5 day and an OLR of 1 kg/m^3.day. COD values of influent and effluent of the reactor were reported as 5000 and 4200 mg/l, respectively through this operation. Data from this anaerobic reactor indicated that the inhibition of the reactor (acidogenesis and methanogenesis) increased as the COD loadings increased, and HRT decreased.

O'Neill et al. (1999) used a simulated wastewater which contained one reactive azo dye (1.5 g/l), starch (1.9 g/l), NaCl (1.5 g/l) and acetic acid (0.53 g/l) together with nutrients and trace metals, to investigate the treatability of azo dyes in an

anaerobic/aerobic reactor system. COD was decreased from 3480 mg/l to 2252 mg/l giving a mean COD removal efficiency of 32% at a HRT of 2 day in the UASB reactor. Similarly color removal efficiencies were reported as 79%. UASB reactor used in this study had higher performances based on COD and color removal efficiencies as compared to the aforementioned UASB reactor.

3.4.4. Performance of CSTR reactor at different HRTs

It was observed that the COD removal efficiency was 88 % at a HRT of 0.96 days while the COD removal efficiency increased to 82 % for HRT of 15 days in the aerobic CSTR reactor through treatment of simulated wastewater as shown in Figure 3.56. High removal efficiency of CSTR reactor at low HRTs could be attributed to high values of COD in UASB reactor effluent which is used as the feed in the CSTR reactor. COD values of aerobic influent could be decreased to 150 and 361 mg/l at HRTs of 15 and 0.96 days, respectively. O'Neill et al., (2000) reported that effluent COD value of UASB reactor treating Procion Red H-E7B of 0.150 g/l and starch of 3.8 g/l could be reduced from 1462 mg/l to 444 mg/l giving a COD removal efficiency of 70 % in the aerobic stage at a HRT of 16 hour.

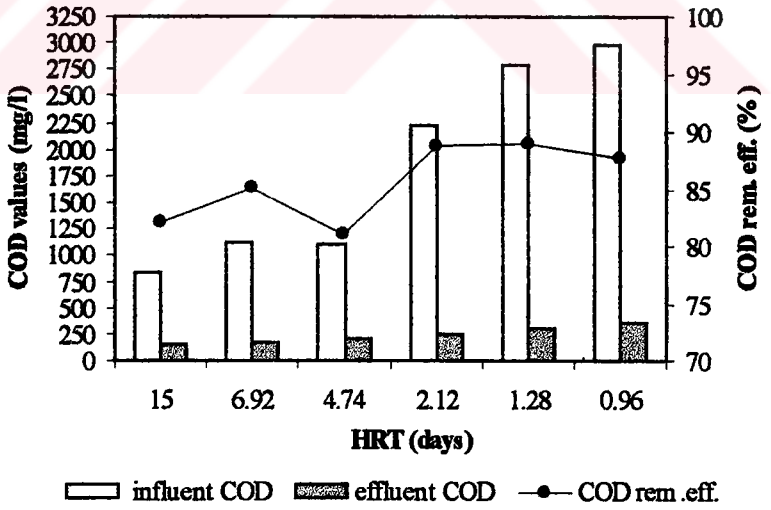


Figure 3.56. The performance of CSTR reactor at different HRTs

3.4.5. Overall performances of UASB/CSTR reactor system

Figures 3.57. shows the COD and color removal efficiencies at different HRTs in UASB/CSTR reactor system. COD and color removal efficiencies varying between 97-91 % and between 84-91 % were obtained at a total HRT of 19.17 and 1.22 days in combined anaerobic/aerobic system, respectively. Maximum color and COD removal efficiencies of 91% and 97% was achieved at a total HRT of 19.17 days in combined system treating simulated wastewater.

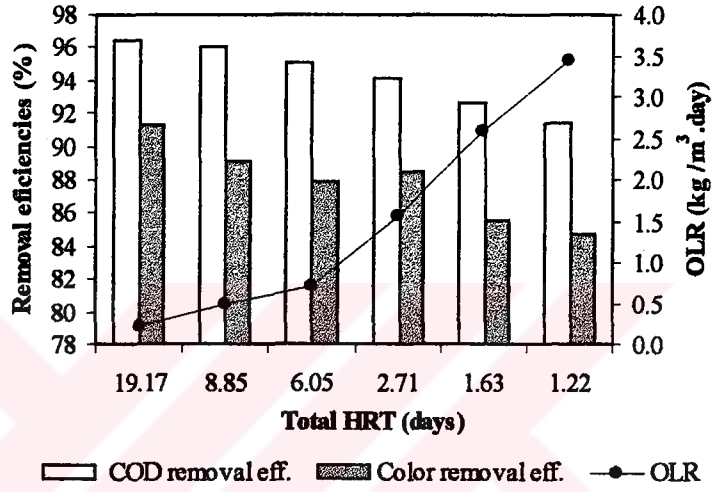


Figure 3.57. COD and color removal performances of UASB/CSTR reactor at different HRTs and OLRs

Figure 3.58 shows the aromatic amines and its removal efficiencies in system through operation at decreasing HRTs. TAA values found to be 12.7 and 2.5 mg/l in UASB and CSTR effluent, respectively at a total HRT of 8.85 days in whole system. This corresponded to % 25.6 and 85.2 of removal efficiencies, respectively, in UASB and UASB/CSTR, respectively. As the HRT was decreased, the TAA removal efficiencies decreased in the system while the removal oF TAA was not carried out in UABS reactor. For instance, at a HRT of 1.22 days, TAA removal efficiencies found to be zero and 42 % for UASB and UASB/CSTR effluents, respectively. Anaerobically, Mordant Yellow 10 was reductively cleaved and the resulting aromatic amines, 5 aminosalicylic acid (5-ASA) and sulphanilic acid (SA) were both recovered in high stoichiometric yields under anaerobic conditions as reported by

Tan et al., (2000). After anaerobic reduction of azo dyes to 5-ASA and SA could be ultimately mineralized at HRTs of 0.67 days in aerobic conditions.

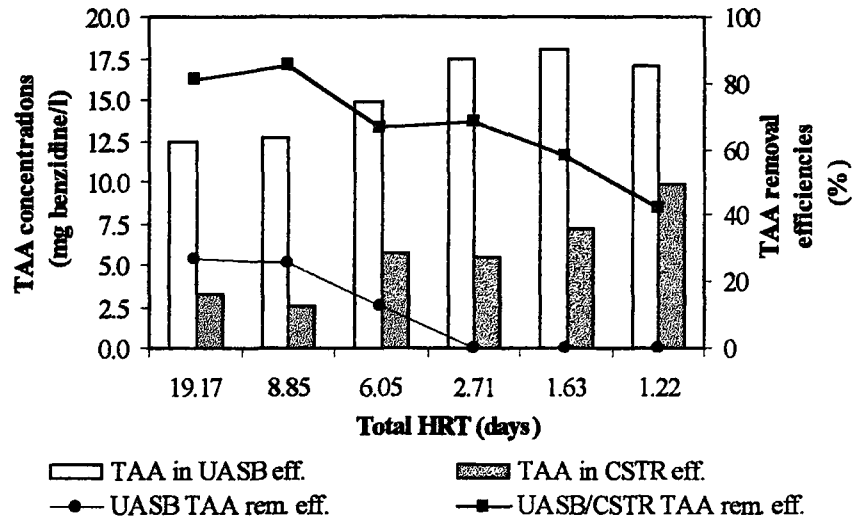


Figure 3.58. TAA values and TAA removal performance of UASB/CSTR at different HRTs

Oxidation Reduction Potential (ORP) values in UASB and CSTR reactor effluents were monitored through operation period in order to evaluate the effect of ORP on reactor performances as shown in Figure 3.59. At all HRTs, ORP values were between -380 and -420 mV which represent the anaerobic strictly reductive conditions in UABS reactor. In the aerobic reactor, ORP measurements indicates the more oxidative conditions (-90 and -100mV). The redox potential reported as below -450 to -500 mV through azo dye reduction. If nitrate is present, the redox potential is higher and no decolorization was observed until the nitrate removal is completed. The presence of sulphate as an additional electron acceptor has no apparent negative effect on azo dye reduction (Carliell et al., 1995). Beydilli et al, (1998) reported that reduction of azo dye took place when low redox potential conditions prevailed in the bioreactors since the culture activity level was decreased.

The pH within CSTR tank was between 8 and 9 (see Figure 3.59). This was due to the high pH of the simulated textile wastewater containing large quantity of $NaHCO_3$ and Na_2CO_3 used for neutralizing the pH in UASB reactor in which pH was between 6.7 and 7.30 due to produced VFA and CO_2 inside UASB reactor. However, under aerobic conditions, the VFAs produced in anaerobic stage was metabolized, and CO_2

volatilized resulting in a rise in pH level. Therefore pH control in CSTR reactor would be required to provide the conditions more suitable for the growth and settling of aerobic consortia as reported by O'Neill et al., 1999. In this study CSTR reactor were operated successfully at slightly high pH for aerobic process when the sludge volume index values, COD and TAA removal efficiencies is taken into consideration through operation. Dissolved oxygen concentrations were recorded greater than 3 mg/l in aeration tank of CSTR reactor during operation period as shown in Figure 3.59.

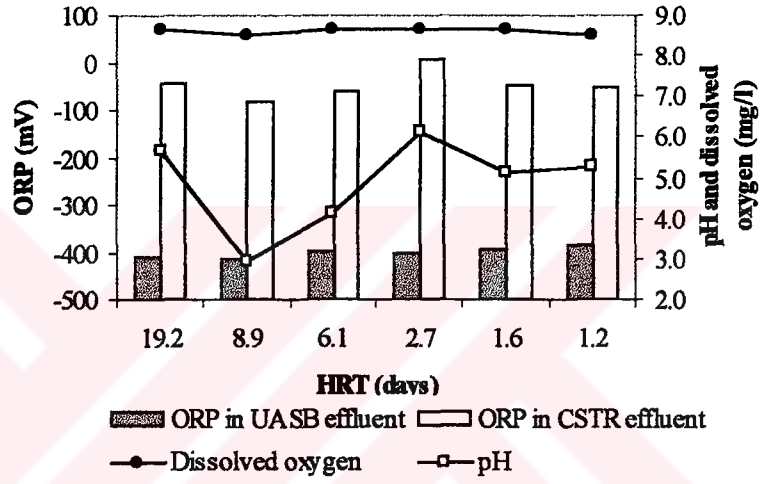


Figure 3.59. Variations of ORP, pH, dissolved oxygen concentrations

Figure 3.60 shows the absorbance measurements at varying wavelength at HRTs of 8.85 and 1.22 days in system treating simulated wastewater. The effluent samples of UASB and CSTR reactors exhibited maximum absorbance spectra at low wavelengths (UV region) due to aromatic amines produced through dye degradation Manu et al., 2002). This shows that the parent dye converted to other intermediate organic forms. Aerobic effluent at a total HRT of 1.22 days had higher absorbance than anaerobic effluent while it had lower absorbance than anaerobic effluent at a total HRT of 8.85 days. It can be suggested that the dye metabolites from cleavage of azo bond was colored or produced new colored products in aerobic tank. Some aromatic amines like phenylenediamines, aminophenols, aminonaphthol and o-

aminohydroxyl naphthalenesulfonic acid tend to autoxidize under aerobic conditions (Kudlich et al. 1997). These products could be degraded slowly in aerobic tank as could be seen in TAA measurements (see Figure 3.58). Therefore the spectra in aerobic reactor effluent was lower at higher HRTs than the spectra of aerobic reactor effluent measured at lower HRTs in the whole system treating the simulated textile wastewater.

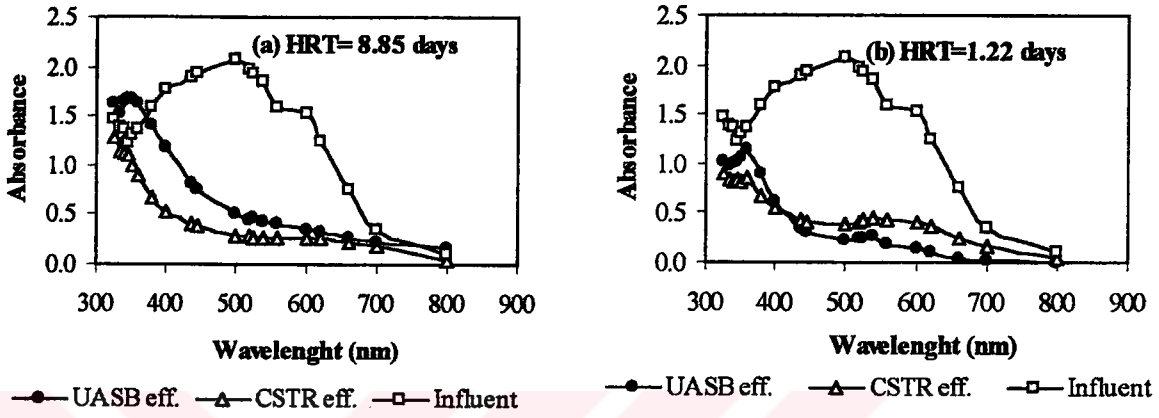


Figure 3.60. Absorbance specta in influent and effluent of whole reactor system at HRTs of 8.85 and 1.22 days, respectively.

3.4.6. Kinetics of UASB reactor

In order to obtain the kinetic coefficient for different kinetic models the UASB reactor was operated with simulated wastewater containing desizing agents, dye, salts etc. at six different HRTs through 46 days of the operation period. The results obtained for steady state conditions during the reactor operation at six different HRTs are summarized in Table 3.8.

3.4.6.1. Monod kinetic model

Six steady state sets of data were used to determine the kinetic parameters required for applying for all kinetic models. Figure 3.61 was plotted from the Eq. 2.30 (See section 2.6) for determining the values of Y and K_d for Monod and Contois model.

Table 3.8. Experimental data obtained under steady state conditions at six different HRTs used to calculate the kinetic coefficients in UASB reactor

Parameter	HRT (hours)					
	100	**46**	**32**	**14**	**9**	**6**
Operation period (days)	0-9	10-17	18-27	28-32	33-38	39-46
Sludge Retention Time (days)	736	339	232	104	63	47
pH	7.25±0.04	7.20±0.13	7.20±0.02	6.95±0.06	6.82±0.06	6.71±0.06
VFA(mg CH_3COOH/l)	709±106	840±5	797±64	1314±30	1837±190	2073±157
B.alk (mg $CaCO_3$/l)	3425±30	2993±135	2969±136	2348±4.9	2291±100	2273±90
VFA/B.Alk.	0.21±0.02	0.28±0.01	0.27±0.03	0.56±0.01	0.80±0.05	0.91±0.10
Effluent COD (mg/l)	835±93	1118±63	1108±54	2233±256	2785±230	2975±61
COD removal efficiencies (%)	79.9±2.36	73.5±1.5	73.7±1.3	47±6.1	33.9±5.4	29.4±1.5
Total gas production rate (ml/day)	1176±154	2000±211	5130±1275	5680±277	6223±1616	6480±635
Methane gas production rate (ml/day)	647±107	997±78	2390±692	1973±175	1756±475	1209±164
Methane percentage (%)	54.8±1.8	50.3±2.2	48.7±1.2	35.8±3.18	28.2±1.6	18.7±2.08
Methane yield (l methane/g COD added) at STP	0.293±0.055	0.225±0.013	0.364±0.103	0.215±0.047	0.165±0.063	0.094±0.012
Methane yield (l methane/g COD added)	0.318±0.060	0.248±0.015	0.404±0.114	0.238±0.052	0.182±0.070	0.104±0.013

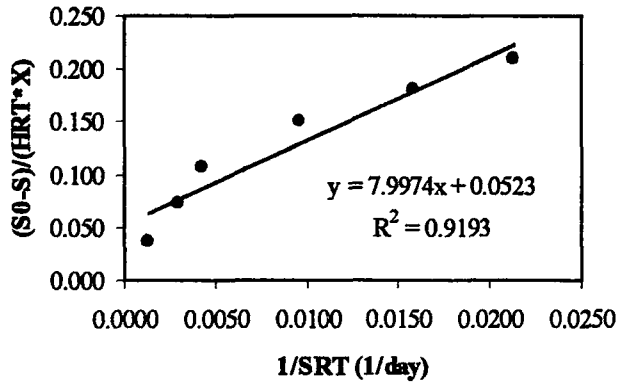


Figure 3.61. Determination of yield coefficient (Y) and death rate constant (K_d)

Y and K_d values calculated from intercept and slope of the straight line illustrated in Figure 3.61 as 0.125 g VSS /g COD and 0.0065 1/day, respectively. The value of μ_{max} and K_S were determined from Figure 3.62 using Eq. (2.31) as 0.105 1/day and 10340 mg/l, respectively.

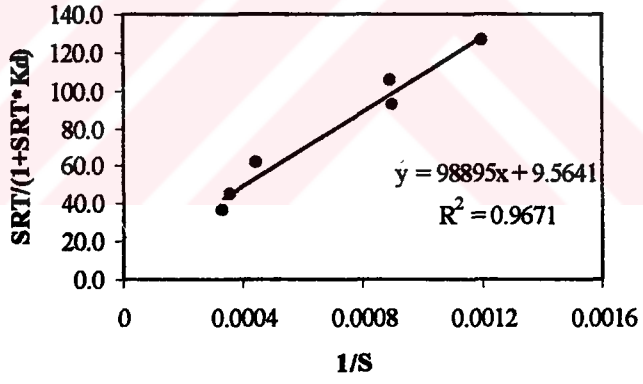


Figure 3.62. Determination of maximum specific growth rate (μ_{max}) and half saturation constant (K_S) for Monod kinetic model

3.4.6.2. Contois kinetic model

For Contois model the values of specific growth rate (μ_{max}) and β were determined from the Figure 3.63 by plotting Eq. (2.35) and the kinetic parameters,

μ_{max} and B were calculated from the intercept and the slope of the straight line as 0.1046 1/day and 0.465 mg COD/mg VSS, respectively.

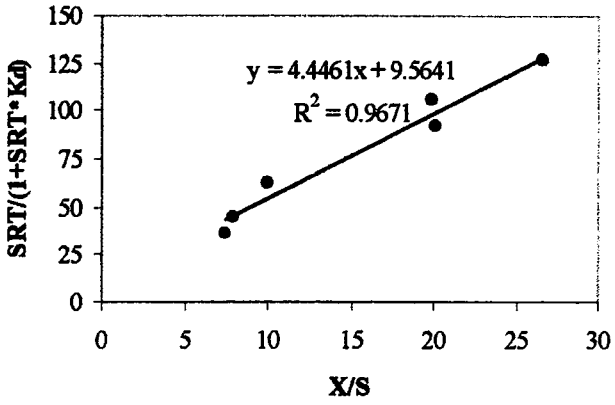


Figure 3.63. Determination of maximum specific growth rate (μ_{max}) and kinetic constant (β)

3.4.6.3. Chen-Hashimoto kinetic model

Equation 2.39 (see Section 2.6) was used to determine the volume of methane (B_0) produced under normal conditions at pressure of 1 atm and a temperature of 0°C per gram of substrate added at an infinite retention time. This equation indicates that,

if $(\mu_{max}-K_d)*\theta_C$ is higher than (1-K),

the plot of B versus 1/SRT should be a straight line since

$B \rightarrow B_0$ as $\theta_C \rightarrow \infty$ (infinite time)

Figure 3.64 shows the graph of B (l CH_4/g COD) against $1/\theta_C$ (per day). Since the plot of B versus $1/\theta_C$ was found to be linear, linear regressions (least squares method) were used to determine the intercept B_0. B_0 value was found to be 0.301 l CH_4 /g COD from the intercept of the line plotted on graph given in Figure 3.64. Figure 3.65 shows the values of θ_C plotted against the quotient $[B/(B_0-B)]$ for

determining the kinetic coefficients. The pairs of values (θ_C and B/B_0-B) fall on straight lines with the exception of one data, when using the last squares method. In such way the kinetic parameters μ_{max} and K obtained from the intercept and the slope of the adjusted lines (see Figure 3.65). According to equation 2.40 μ_{max}-K_d and K is equal to 1/intercept and slope/intercept, respectively. According to aforementioned equations and assumptions μ_{max} and K was found to be 0.0165 1/day and 0.179 (dimensionless), respectively from the intercept and the slope of the line on graph given in Figure 3.65.

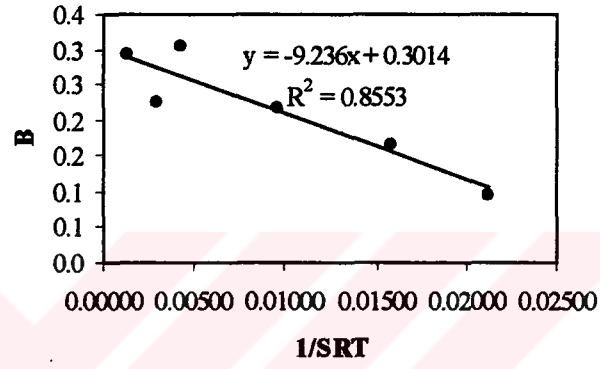


Figure 3.64. The effect of reciprocal sludge retention time on methane yields in UASB reactor

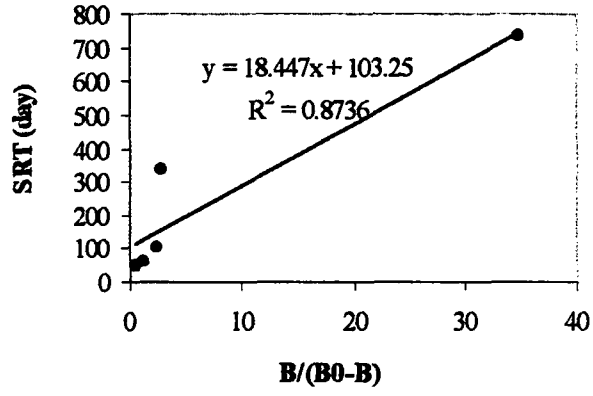


Figure 3.65. Determination of maximum specific growth (μ_{max}) rate and kinetic constant (K)

3.4.6.4. Grau first order substrate removal method

In order to determine the kinetic coefficients (p, k_f, and k_f'), Equation 2.43 was plotted in Figure 3.66. The values of p calculated from the slope of the line given on graph. The values of p, k_f, and k_f' were found to be 0.486 1/day, 2.050 g /l.day and 0.092 1/day with correlation coefficient (R^2) of 0.757.

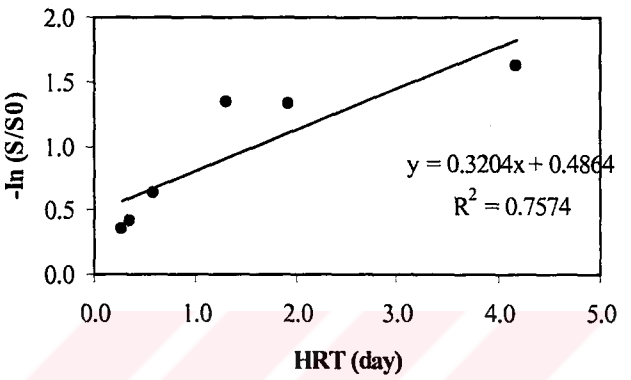


Figure 3.66. Determination of kinetic constants (p, k_f and k_f') for Grau first order substrate removal model

3.4.6.5. Grau second-order multicomponent substrate removal model

In order to determine the kinetic coefficients (a, b and k_s) Equation 2.48 was plotted in Figure 3.67. The values of a, and b calculated from the intercept and slope of the straight line on graph. The values of a, and b were found to be 0.562 and 1.095 with high correlation coefficient of (R^2) 0.995. Multicomponent substrate removal rate constant (k_s) was then calculated from the equation a= $S_o/(k_s.X)$ equation as 0.337 1/day indicating the substrate removal for each unit of microorganism depending on second order substrate removal rate constant (k_s).

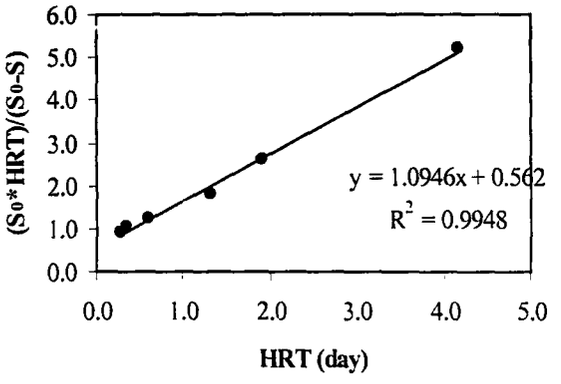


Figure 3.67. Determination of kinetic constants (a, b and k_s) for Grau second order multicomponent substrate removal model

3.4.6.6. Modified Stover-Kicannon model

Figure 3.68 shows the graph plotted between reciprocal of total organic loading removal rate, [V/(Q.(S_o-S)], against the reciprocal of total organic loading rate, V/(Q.So). Since the plot of [V/(Q.(S_o-S)] versus V/(Q.S_o) was found to be linear, linear regressions (least squares method) were used to determine the intercept and the slope. A straight line portion of intercept, $1/R_{max}$ and a slope of K_B/R_{max} results on graph. Saturation value constant (K_B) and maximum utilization rate (R_{max}) were calculated from the line plotted on graph in Figure 3.68 as 8.2 g/l.day and 7.5 g/l.day, indicating the substrate removed by microorganisms during time and the maximum substrate removed by the anaerobic organisms versus time, respectively. The data obtained in this study are quite similar with those obtained by Büyükkamacı and Filibeli (2002) and Yu et al. (1998).

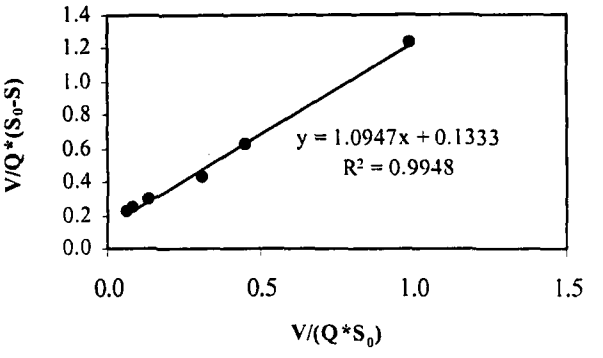


Figure 3.68. Stavor-Kincannon model plot

3.4.6.7. Zero order Substrate removal model

The value of k_0 was obtained from the slope of the line by plotting S_0-S versus HRT in Eq. (2.53). Figure 3.69 shows the plot between S_0- S and HRT. k_0 was calculated as 519 mg/l.day with correlation coefficient of 0.671.

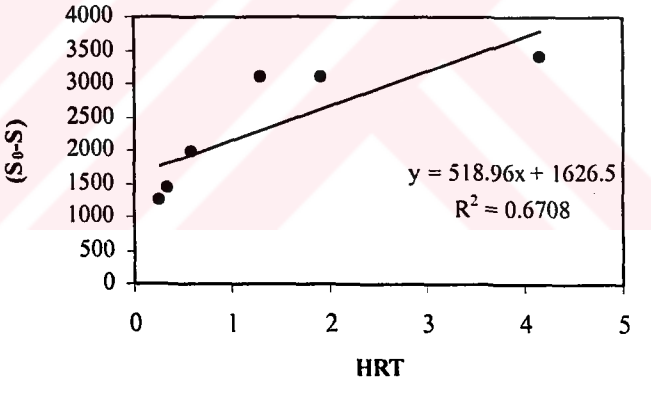


Figure 3.69. The model plot of zero order substrate removal

3.4.6.8. First order substrate removal model

The value of k_1 was obtained from the slope of the line by plotting S_0-S/HRT versus S in Eq. (2.55). Figure 3.70 shows the plot between (S_0- S)/HRT and S. k_1 was calculated as 0.615 1/day with correlation coefficient of 0.932.

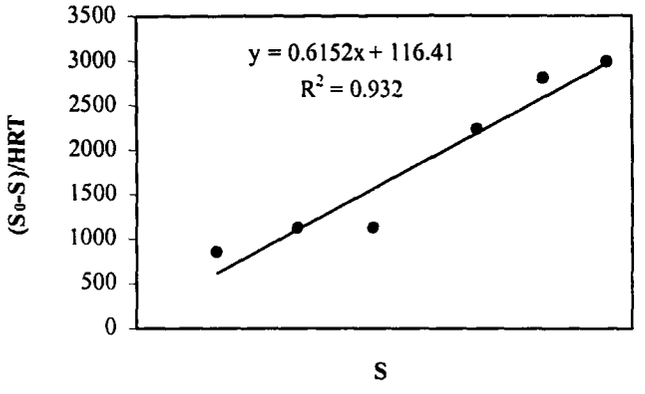


Figure 3.70. The model plot of first order substrate removal

3.4.6.9. Second order substrate removal model

The value of k_2 was obtained from the slope of the line by plotting S_0-S/HRT versus S^2 in Eq. (2.57). Figure 3.71 shows the plot. k_2 was calculated as 2327 l/mg.day with correlation coefficient of 0.908.

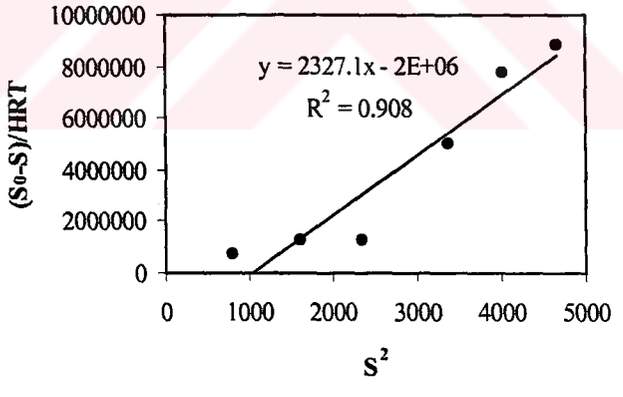


Figure 3.71. The model plot of second order substrate removal

All kinetic coefficients calculated from models are summarized in Table 3.9 with correlation coefficients. The kinetic data showed that Stover-Kincannon and second order multicompenant substrate removal kinetics were more appropriate models than

other models for predicting the performance of the lab scale UASB reactor when the regression coefficients and kinetic coefficients were compared with each other.

Table 3.9. Kinetic parameters of UASB reactor treating simulated cotton textile wastewater

Kinetic models	Kinetic parameters	Values	Regression Coefficients (R^2)
Monod	Y (mg VSS/ mg COD)	0.125	0.912
	K_d (1/day)	0.0065	0.912
	k_{max} (μ_{max}/Y) (1/day)	0.84	0.967
	μ_{max} (1/day)	0.105	0.967
	K_S (mg/l)	>4000	0.967
Contois	Y (mg VSS/ mg COD)	0.125	0.912
	K_d (1/day)	0.0065	0.912
	β (mg COD/ mg VSS)	0.465	0.967
Chen-Hashimoto	B_0 (l CH_4 /g COD)	0.301	0.855
	μ_{max} (1/day)	0.016	0.874
	K	0.179	0.874
Grau first order	P (1/day)	0.486	0.757
	k_f(mg/l.day)	2.050	0.757
	k_f'(1/day)	0.092	0.757
Grau second order	a	0.562	0.995
	b	1.095	0.995
	k_s (1/day)	0.337	0.995
Modified Stover-Kincannon	K_B (g/l.day)	8.211	0.995
	R_{max}	7.501	0.995
Zero order	k_0 (mg/l.day)	519	0.671
First order	k_1(1/day)	0.615	0.932
Second order	k_2 (l/mg.day)	2327	0.908

3.4.7. General discussion for treatment of simulated wastewater

The SS and VSS in the UASB reactor were 31.7 g/l and 22.4 gVSS/l, respectively. The maximum sludge loading rate was between 0.114 -1.718 kg COD/ kg. VSS. day. These values exhibited similar data achieved by An et al. (1996) in a UASB reactor fed with dye wastewater with of organic loading rate of 0.269 kg COD /kg VSS.day. In the studies performed by Zhu et al. (1994) and O'Neill et al. (2000a) the UASB reactors were operated at organic loading rates of 0.75 kg COD/kg VSS.day and 0.15 kg COD/kg VSS.day treating dye manufacturing and simulated textile wastewaters, respectively.

VFA levels is extremely high for a stable operation in UASB reactor indicating the accumulation of VFAs and resulting in low COD removal efficiencies, such as 60% at a HRT of 6 hours. However, the azo dyes decolorized under reductive anaerobic conditions in very short HRTs but the breakdown products such as aromatic amines were not ultimately metabolized and accumulated under anaerobic conditions. The optimum retention time and organic loading rate of UASB reactor were about 32 h and 3.2 kg COD /m^3.day, respectively, giving 94% color and 74 % COD removal efficiencies.

In the sequential aerobic stage the significant part of COD was removed successfully while the color removal slightly increased with TAA removal efficiencies of 85%. In the studied HRTs, the COD removal efficiencies in aerobic tank were not mainly affected since the readily biodegradable COD in anaerobic effluent could be removed at a HRT as low as 0.96 day. The effluent COD and TAA values were 150 mg/l and 3.26 mg benzidine/l at a HRT of 15 days while the same parameters were 360 mg/l and 9.26 mg benzidine/l at a HRT of 0.96 day, respectively. TAA values decreased in aerobic effluent at higher HRTs due to the slow degradability of azo dye metabolites. Small part of TAA were removed in UASB reactor particularly at longer HRTs such as 100, 46 and 32 hours. Increases in HRT provide enough time for partial mineralization of COD and intermetabolites in anaerobic and/or anaerobic/aerobic systems. The optimum hydraulic retention time

was found to be 6 days in anaerobic/aerobic reactor system. Moreover, COD from the soluble microbial products (SMP) through substrate metabolism and biomass decay should be considered because the effluent soluble COD might be mostly SMP (50-90%) as reported by Kuo et al., 1995. This COD is refractory and slowly degradable (Speece, 1996) and appeared in the effluents.

Color consents for sewage treatment works in the Seven Trent area at 550 nm were between 0.012-0.025 absorbance units while a typical consents from the UK Environment Agency at 550 nm is 0.055 absorbance unit (O'Neill, et al., 2000a) Further physico-chemical treatment for color removal is required in order to provide the color consent standard for these experimental conditions.

Of relatively few studies performed relevant to treatment of simulated dyeing and textile study results could be compared with our data:

The study performed by Talarposthi et al. (2001) showed that a mesophilic anaerobic upflow anaerobic packed bed reactor can be removed up to 90 and 24% of color and COD, respectively, from a mixed 1000 mg/l of cationic dye containing wastewater and whey powder as co substrate (Influent COD of 5000 mg/l) at a HRT of 5 days. Color and COD removal efficiency raised with increased hydraulic retention times from 3 days to 5 days.

Manu et al. (2002) studied the anaerobic treatment of simulated wastewater containing 1280-2080 mg/l COD (derived from 0.53 g/l acetic acid, 1.5 g/l starch and Orange II and Black 3 HN of 100 mg/l), alkalinity of 1000-1500 mg/l as $NaHCO_3$ and Na_2CO_3, and suitable trace elements at a HRT of 10 days. The studies showed that COD and color removal efficiencies were between 80-95% and 80-98 % respectively, for Black 3HN dye while COD and color removals were between 80-90% and 80-98%, respectively for Orange II.

The treatability of simulated textile wastewater was studied by Shaw et al. (2002) under anaerobic/aerobic conditions. They reported that sequencing batch reactor had

a total HRT of 2.6 days removed 66% of the applied total organic carbon and 94 % of color derived from the Reactive Black 5 of 533 mg/l. Aromatic amines from the anaerobic breakdown of RB 5 azo dye were not completely mineralized by aerobic phase.

In the study performed by O'Neill et al., (2000a) COD and color removal efficiencies were found to be 77 and 67.2% in a UASB reactor treating a simulated wastewater containing Procion Red H-E7B dye of 450 mg/l and a COD of 3137 mg/l at an organic loading of 3.1 kg COD/ m^3.day and a HRT of 1 day.

Kalyuzhi and Skylar (2000a) reported an innovative reactor for the treatment of azo dyes in anaerobic/aerobic sequential environments. Anaerobic and aerobic phases were combined into one single unit called anaerobic-aerobic hybrid reactor. The performance of this reactor was tested with a synthetic wastewater containing Sigrusgelb azo dye (0.3 g/l as COD) and ethanol (0.82 g/l as COD) at 30°C and 56% removal of azo dye-COD was achieved at volumetric loading rate of 0.3 g azo dye COD/l.day.

In this study, based on the kinetic studies, it appears that the kinetic coefficients obtained from the anaerobic treatment of simulated cotton textile wastewater, agree with the Stover-Kincannon and second order multicomponent substrate kinetic models. Although several studies relevant to anaerobic treatment of the simulated wastewater were reported in literature (O'Neill et al., 1999; O'Neill et al., 2000a; Talarposthi et al., 2001; Manu et al., 2002; Basibuyuk & Forster, 1997) none of studies were contained a study of kinetic data revelling to anaerobic reactor treating simulated cotton textile wastewater.

As seen in Table 3.9, Grau second order kinetic model gives similar results compared to Modified Stover-Kincannon substrate kinetic model. This result is not surprising because two models are very similar as mentioned:

Second order linearized equation ($\frac{S_0 * \theta_H}{S_0 - S} = \theta_H + \frac{S_0}{k_s * X}$) could be transformed to Modified Stover-Kincannon model ($\frac{V}{Q*(S_0 - S)} = \frac{K_B}{R_{max}} \frac{V}{Q*S_0} + \frac{1}{R_{max}}$) by dividing S_0 each part the equation.

$$\frac{V}{Q*(S_0 - S)} = \frac{V}{Q*S_0} + \frac{1}{k_s * X} \qquad (3.2)$$

If equation (3.2) is simulated to Modified Stover-Kincannon model, the following equation can be obtained:

$$\frac{1}{k_s * X} = \frac{1}{R_{max}} \qquad (3.3)$$

$$R_{max} = k_s * X \qquad (3.4)$$

$$\frac{K_B}{R_{max}} = 1 \qquad (3.5)$$

$$K_B = R_{max} \qquad (3.6)$$

The calculating kinetic parameters from the plots of both two model are appropriate to the values calculated from the above equations 3.4, and 3.6. As a results of this similarity, two model have the same sensitivity in order to obtain the kinetic coefficients from the plots when the regression coefficient of the graphs are compared together.

Table 3.10 summarizes the constants determined from the models in previous studies.ll

For anaerobic system a review of common substrate utilization kinetic models was recently carried out by Kuroda et al., (1993), and also by Mata-Alvarez and Cecchi (1990). Monod is the most widely used for UASB reactors and for industrial effluents (Castillo et al., 1999). On the contrary, in this study it was found that Monod model was not appropriate for interpreted the kinetic data of UASB reactor

treating simulated textile wastewater. Although K_S values estimated from the Monod model are very large, k_{max} and Y values were acceptable values. Giraldo-Gomez et al., (1991) reported that k_{max}, Y, and K_S values were 0.77-6.67 mg COD/mgVSS.d , 0.04-0.11 mg VSS/mg COD, and 105-3180 mg COD /l, respectively, for anaerobic oxidation of long-chain fatty acids.

In this study, determined β Contois kinetic coefficient (0.465 mg COD/mg VSS) is very similar to the coefficient (0.482 mg COD/mg VSS) found by Hu et. al. (2002) although reactor configurations were different in these studies. Therefore it can be assumed that Contois model could be also applicable for determining the substrate removal kinetic of UASB reactor treating textile wastewater.

Table 3.10. Comparison of kinetic constants in the Grau second order, Modified Stover Kincannon, and Contois models

Models	Substrate	Reactor type	Influent COD(mg/l)	HRT (day)	Kinetic parameters			References
					k_s	a	b	
Grau second order	Synthetic (glucose)	UASB	560-730	0.17-1.0	1.655	0	1.099	Ubay (1991)
Grau second order	Municipal W.	UASB	230-445	0.25-1.0	0.217	0.002	1.346	Ubay (1994)
Grau second order	Synhetic (glucose)	UASB	3000-10000	0.3-1.5	13.6	0.018	1.02	Ubay (1989)
Grau second order	Brewery	AFBR	200-300	0.2-1.7	2.1	0.1	1.015	Anderson et al., (1990)
Grau second order	Synhetic (glucose)	ACR	14750	4.8-24		0.073	1.073	Anderson and Donnely (1978)
Grau second order	Landfill leachate	UASB	9000-25000	1.7-2.8	38.5	0.013	1.066	Öztürk et al., (1998)
Grau second order	Molasses	AHR	2000-15000	0.5-2	10.81	0.033	1.192	Büyükkamacı and Filibeli, (2002)
Grau second order	Simulated W.	UASB	4214	0.25-4.16	0.337	0.562	1.095	This study
					R_{max}	K_B		
Modified-Stover Kincannon	Molasses	AHR	2000-15000	0.5-2	83.3	186.23		Büyükkamacı and Filibeli, (2002)
Modified-Stover Kincannon	Soybean wastewater	AF	7520-11450	1-1.45	83.3	85.5		Yu et al., (1998)
Modified-Stover Kincannon	Simulated W.	UASB	4214	0.25-4.16	7.5	8.2		This study
					β	Y	K_d	
Contois	Ice-cream W.	CSTR	5500	2.99-7.45	0.4818	0.2116	0.0131	Hu et al., (2002)
Contois	Simulated W.	UASB	4214	0.25-4.16	0.465	0.125	0.0065	This study

AF: anaerobic filter AHR: anaerobic hybrid reactor AFBR: Anaerobic fluidized-bed reactor

ACR: Anaerobic contact reactor UASB: Upflow anaerobic sludge reactor CSTR: Continuous stirred tank reactor

3.5. Monitoring of Toxicity and Intermediate Products through Decolorization of DB 38 and DR 28 Azo Dyes

In this part of the study, synthetic wastewater containing DB 38 and DR 28 azo dye of 3200 mg/l, glucose-COD of 3000 mg/l, alkalinity of 3000 mg $NaHCO_3$/l and Vanderbilt mineral medium in given composition was treated using an anaerobic/aerobic sequential reactor system. Sludge was refreshed with refilling of UASB reactor treating the wastewater of PAK MAYA yeast factory located in IZMIR. CSTR reactors used had the similar configuration and operated with MLSS of 3000 mg/l. These reactors inoculated with the activated sludge taken from the lab scale CSTR reactor treating molasses. The UASB/CSTR reactor systems were initially started to operate after 15 days of adaptation period with free-azo dyes. Then the azo dye concentrations increased stepwise to 3200 mg/l in order to minimize the adverse effect of increasing dye loadings rates. Once steady-state condition were achieved at a organic loading rates of 1.14 and 1.79 kg /m^3.day in the UASB reactors and at HRTs of 86 and 72 h, for the treatment of DB 38 and DR 28 azo dyes. HRTs of CSTR reactors were 18 days corresponded to a flowrate of 0.5 l/day for two dyes. The data collected at steady state conditions were summarized in Table 3.11, and the treatment performances based on COD, TOC, TAAs and color removals of UASB/CSTR reactor system are given in Table 3.12.

3.5.1. Removal of organics in the UASB/CSTR sequential system

Since the influent COD contained 3000 mg glucose-COD/l, the remaining COD values was originated from the azo dyes. BOD_5 concentrations were 3018, 360 and 30 mg/l in the influent and effluent of UASB reactor, and effluent of CSTR reactor for DB 38 azo dyes, BOD_5 values concentrations 1907, 80, and 160 mg/l in the influent and effluent of UASB reactor, and effluent of CSTR reactor for DR 28 azo dyes, respectively. It can be concluded that BOD_5 measurements was inhibited by the azo dyes or by the azo dye metabolites particularly in the case of influent and effluent samples of DR 28 reactor. Inert COD measurements of samples corresponded to effluent CODs of CSTR showed that the COD values are quite

close. Since CSTR reactors were operated at long HRTs (18 days), probably the degradable fraction of the UASB effluents could be degraded by the aerobic microorganisms. Inert COD measurement showed that effluent COD of CSTR reactors constituted mainly from the inert COD under aerobic condition. Effluent COD of the system probably contained SMPs and dye metabolites. Table 3.12 shows the treatment performances of the UASB/CSTR reactor system. COD removal efficiencies were found to be 77, 64 and 92% in the treatment of DB 38 while they were 76, 63, 91% in the treatment of DR 28 for UASB, CSTR and UASB/CSTR reactors, respectively. In the DB 38 treatment, COD/BOD_5 ratio increased after the anaerobic and aerobic treatment indicating that the biodegradable material was removed as reported by O'Neill et al. (1999). Overall 81% and 84% reduction in TOC were achieved as a result of anaerobic/aerobic treatment of wastewater containing DB 38 and DR 28 azo dyes, respectively.

Table 3.11. The influent and effluent parameters of anaerobic/aerobic system treating azo dye concentration of 3200 mg/l

Parameter	Direct Black 38 (DB 38)			Direct Red 28 (DR 28)		
	Feed	UASB eff.	CSTR eff.	Feed	UASB eff.	CSTR eff.
COD (mg/l)	4100	928	335	5377	1298	475
BOD_5 (mg/l)	3018	360	30	1907	80	160
COD/BOD_5	1.35	2.6	11.6	2.8	16.2	2.96
Inert COD (mg/l)	1440	381	335	769	992	455
TOC (mg/l)	1236	496	231	1807	701	283
TAAs (mg benzidine /l)	-	121.4	23.7	-	125.3	11.7
Absorbance at λmax	21.43	4.45	1.33	27.58	0.546	1.139
NH_3-N (mg/l)	1.15	1.6	2	47	55	12.2
NO_3-N (mg/l)	16	15	84	*	10	26
VFA (mg CH_3COOH/l)	-	503	-	-	586	-
T.Alk (mg $CaCO_3$/l)	2200	2400	1500	2340	2300	1850
VFA/B.Alk	-	0.23	-	-	0.29	-
pH	8.18	6.91	8.65	8.38	6.87	8.81
ORP (mV)		-339	-57		-369	-59

*color interference

Table 3.12. Treatment performances of the UASB/CSTR sequential system

Parameter	% removal efficiencies					
	DB 38			DR 28		
	UASB	CSTR	UASB/CSTR	UASB	CSTR	UASB/CSTR
COD	77	64	92	76	63	91
TOC	60	54	81	61	60	84
TAAs	26	81	86	75	91	97
Color	81	67	94	98	-	96

3.5.2. Color removal in the UASB/CSTR sequential system

Microbial decolorization of DB 38 and DR 28 azo dyes occurred anaerobically due to reduction and cleavage of the azo bonds with the color which is associated and resulting in the production of the TAAs which was biodegradable in aerobic conditions. The peaks in visible region at 520 nm and 497 nm for DB 38 and DR 28 azo dyes in the feed, respectively, were removed by anaerobic treatment, and replaced by a small peak in UV region around 345 nm for both dyes as shown in Figure 3.72. For DB 38, color removals were 81, 67 and 94% in the UASB, CSTR and UASB/CSTR reactors, respectively showing that both anaerobic and aerobic reactor could removed color. Probably, colorful intermediates occurred from the decolorization of DB 38 azo dye in the anaerobic stage, and then these intermediates degraded in the aerobic CSTR reactor. For the DR 28; 98, and 96% color removals obtained in UASB and UASB/CSTR reactor, respectively. Intermediates of DR 28 recolored due to the oxidation and polymerization of intermediates, therefore total color removal efficiency decreased from 98 to 96% in the CSTR reactor. Similar findings was reported by O'Neill et al. (1999) and Knapp and Newby, (1995). When COD, VFA, VFA/B.Alk, ORP and pH parameters were taken into consideration, inhibition was not observed in the UASB and CSTR reactors. Color and COD could be effectively removed in the UASB/CSTR system.

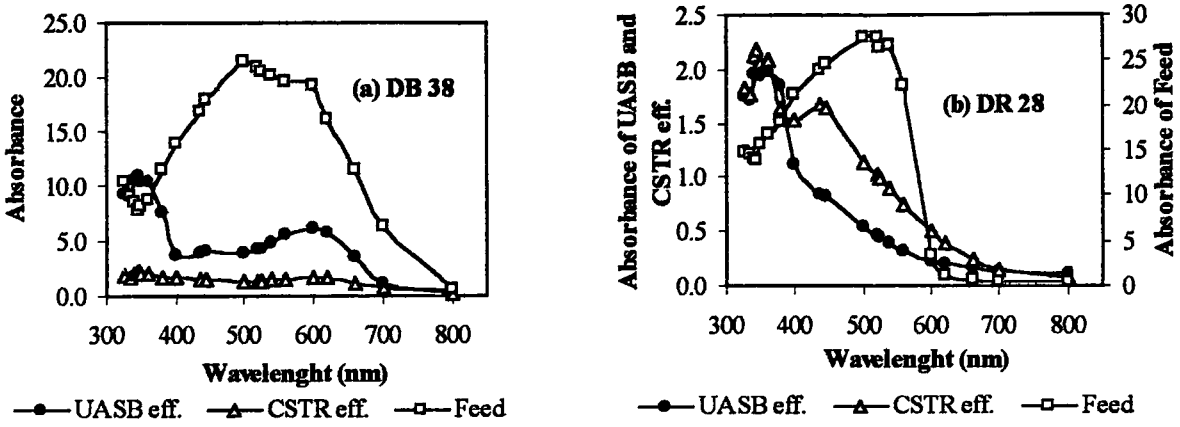


Figure 3.72. Absorbance spectra in influent and effluent samples of reactors treating (a) DB 38 and (b) DR 28 azo dyes

3.5.3. Monitoring of intermediates through decolorization of DB 38 and DR 28 azo dyes

The concentration of TAA from the degradation of DB 38 were 121 and 24 mg benzidine/l while TAA from the degradation of DR 28 were 125 and 12 mg benzidine/l in the effluent of UASB and CSTR, respectively. This corresponded to TAA removal efficiencies of 86 and 97% in UASB/CSTR reactor system for DB 38 and DR 28 azo dyes, respectively. Anaerobic breakdown products of DB 38 was recovered in high stoichiometric percentages (74%) while anaerobic breakdown products of DR 28 was recovered in low stoichiometric percentages (25%) showing that TAAs released from DR 28 could be removed in the anaerobic stage. These findings agree with studies performed by Rajaguru et al. (2000) and Razo Flores et al. (1997).

To avoid the time-consuming and analytical complex procedures necessary for the identification of each derivative, simple high performance liquid chromatography – diode array detection (HPLC-DAD) and gas chromatography- mass spectra (GC-MS) analysis were carried out to determine qualitatively whether UV-absorbing amino derivatives were formed and/or degraded during each treatment step (O'Neill.,

2000b). Similar studies were performed in this study. Figures 3.73 and 3.74 show the HPLC chromatograms of effluents of UASB and CSTR, respectively.

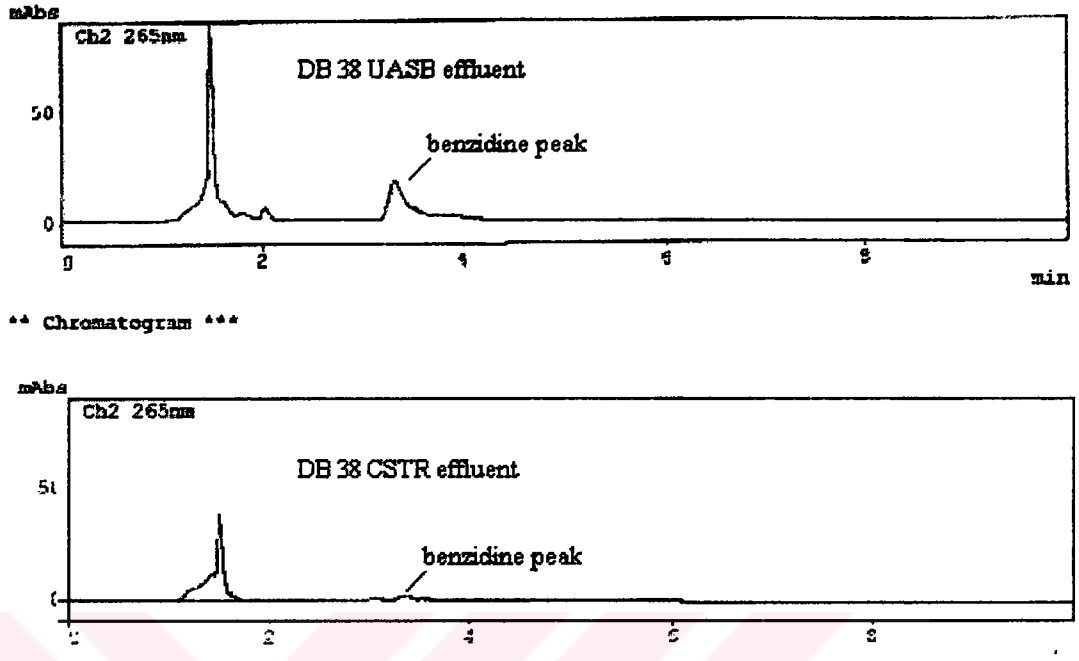


Figure 3.73. HPLC chromatograms of effluent (a) UASB and (b) CSTR for DB 38 azo dye

Figure 3.75 shows the chromatograms of benzidine standard (20 mg/l) in HPLC analysis for comparison of the samples containing dye by-products. Since DB 38 and DR 28 are benzidine based dyes it is assumed that if azo bond on these dyes is broken down, benzidine will be formed under anaerobic conditions. Figure 3.73 and 3.74 clearly demonstrate that UV absorbing by-product was degraded through aerobic treatment in the samples containing DB 38 and DR 28 by-products. The standard benzidine peaks in Figure 3.75 was obtained at retention time of a 3.5 minutes. The similar peaks are shown on the chromatograms at the same retention times of samples studied in these samples. Decreases in detectable area in the benzidine peaks during aerobic treatment indicated the degradation of benzidine.

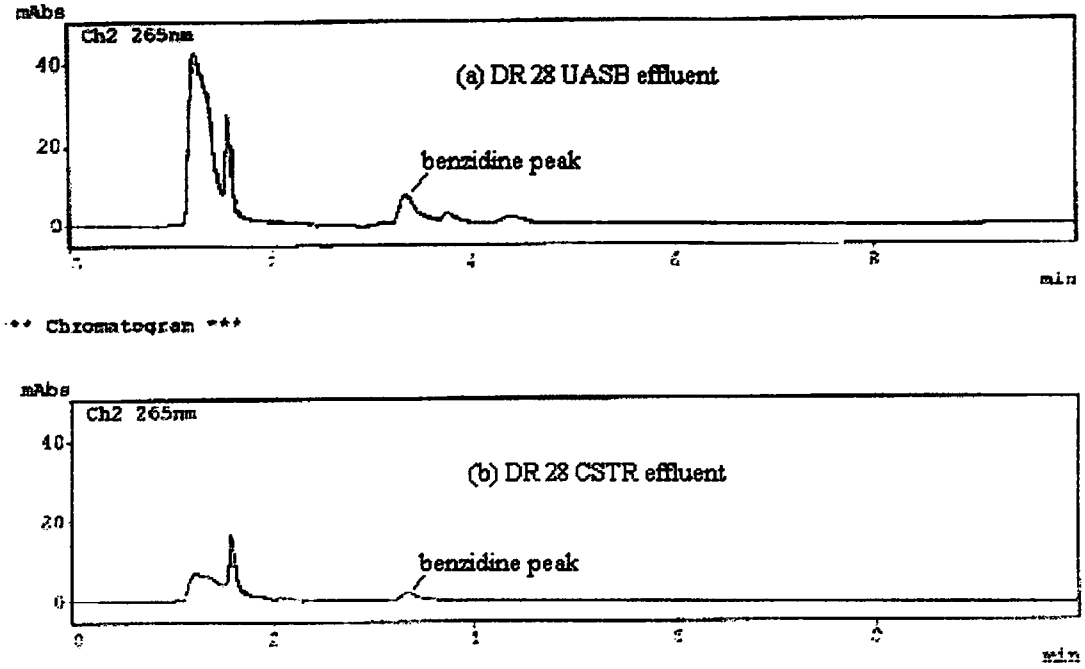


Figure 3.74. HPLC chromatograms of effluent (a) UASB and (b) CSTR for DR 28 azo dye.

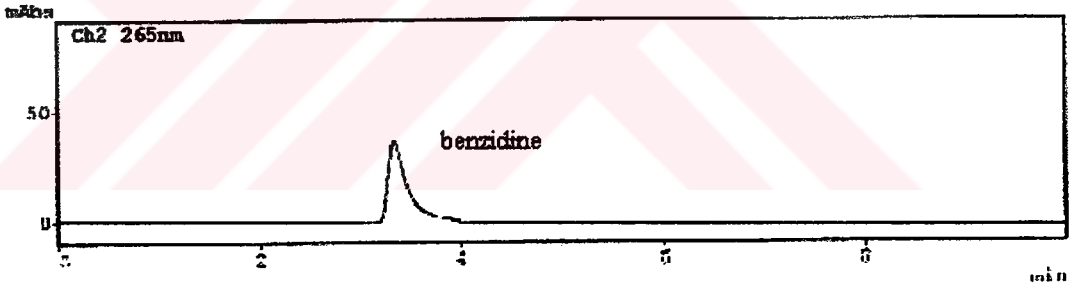


Figure 3.75. HPLC chromatogram of Benzidine (20 mg/l)

GC-MS analyses were performed in order to determine the intermediates of DB 38 and DR 28 azo dyes. In the samples only benzidine could be identified by comparison of the retention times and mass spectra of the samples and benzidine standard. Figure 3.76 and 3.77 shows the GC chromatograms of DB 38 and DR 28 effluents. The benzidine gives a peak at 19 min in all the samples. The comparison of benzidine chromatograms is depicted in Figure 3.78 in effluents of anaerobic and aerobic reactor for DR 28 showing that benzidine was removed significantly under aerobic conditions.

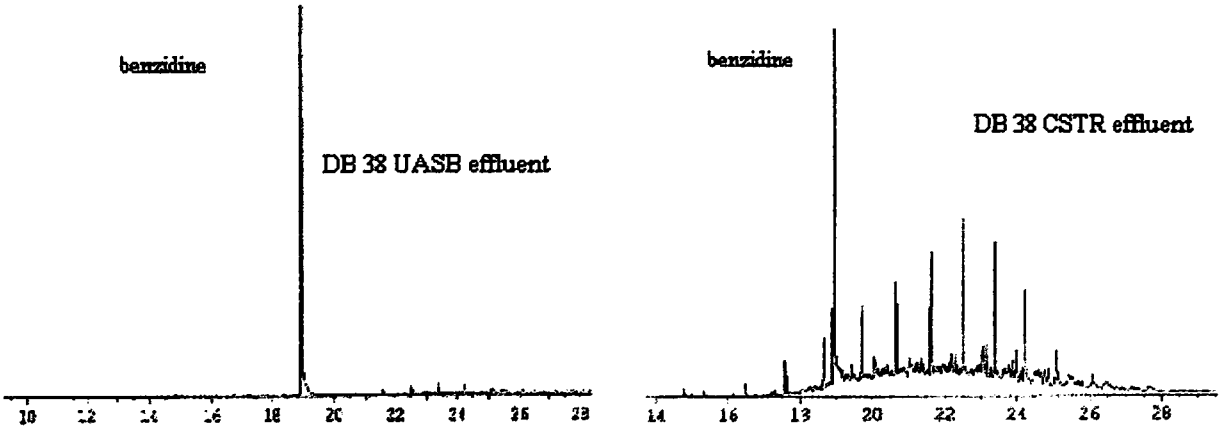


Figure 3.76. Gas chromatograms of effluent of (a) UASB and (b) CSTR for DB 38 azo dye

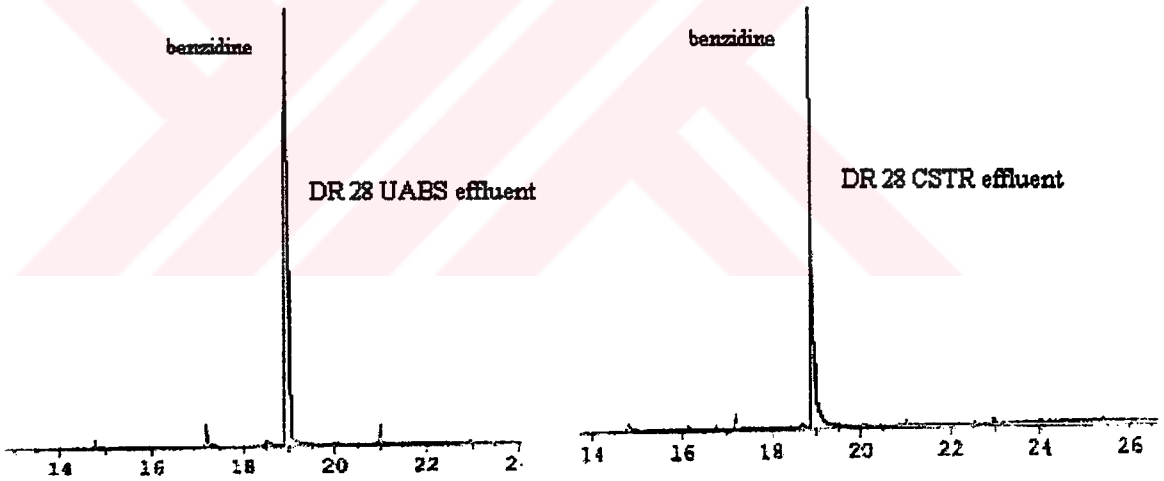


Figure 3.77. Gas chromatograms of effluent of (a) UASB and (b) CSTR for DR 28 azo dye

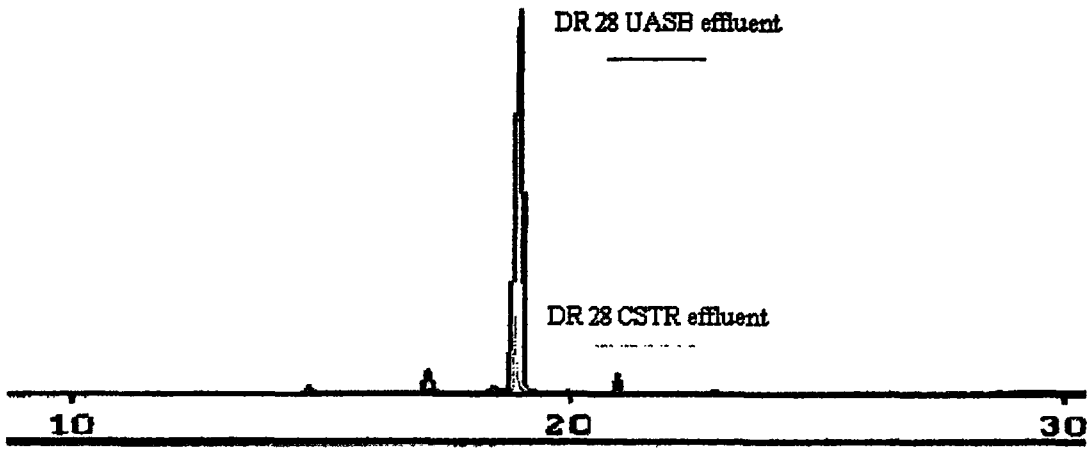


Figure 3.78. The comparison of gas chromatograms of UASB and CSTR reactor effluents for DR 28 azo dye

As shown in Table 3.11, the degradation of azo dyes and their metabolites were associated with increases NO_3-N in the aerobic effluent indicating NH_3 released from the azo dyes and their by-products mineralized to NH_3, and then NH_3 was converted to NO_3 by nitrification under the aerobic conditions. These findings show that mineralization of azo dyes by anaerobic/aerobic processes. NH_4-N values were not meaningful in samples as it might be converted to nitrate at long HRTs in aerobic tank. Ammonia is intermediate product for azo dye mineralization but nitrate is final product for the anaerobic/aerobic system treating azo dyes.

3.5.4. Monitoring of toxicity through decolorization of DB 38 and DR 28 azo dyes

ATA, Respiration/inhibition, *Daphnia magna*, and LUMIStox toxicity assays were performed in order to determine the toxicity of azo dyes through anaerobic/aerobic sequential reactor system. Toxicity tests revelling to the procedure described in Section 2.4 were carried out in the influent, and effluents of anaerobic/aerobic system. The results obtained from the toxicity tests were tabulated in Table 3.13. Toxicity test results are given as percent (%) inhibition obtained from activity of the samples compared to control containing no dyes and toxic substances.

Table 3.13. Percent Inhibition (I %) results in different toxicity tests for DB 38 and DR 28 azo dyes

Dye	Toxicity tests	Influent sample ratio (%)				Anaerobic effluent sample ratio (%)				Aerobic effluent sample ratio (%)			
	Inhibition percentage (I %)	%25	%50	%75	%100	%25	%50	%75	%100	%25	%50	%75	%100
DB 38	Respiration/inhibition	37	58	ND	75	59	65	65	53	32	NI	32	47
DR 28	Respiration/inhibition	NI	25	60	89	NI	29	ND	65	55	84	87	74
DB 38	*Daphina magna*	100	100	100	100	100	100	100	100	NI	20	20	80
DR 28	*Daphina magna*	100	100	100	100	20	100	100	100	NI	20	60	100
		%12.5	%25	%50	%100	%12.5	%25	%50	%100	%12.5	%25	%50	%100
DB 38	ATA	12	NI	37	29	NI	NI	NI	NI	NI	NI	NI	NI
DR 28	ATA	12	29	56	62	8	16	84	82	NI	NI	NI	NI
		%10		%20		%10		%20		%10		%20	
DB 38	LUMIStox	12		28		11		60		NI		NI	
DR 28	LUMIStox	80		87		83		44		13		46	

NI: no inhibition

ND: not determined

Daphnia magna inhibition showed that the aerobic effluents did not exhibit inhibition in samples containing 25% aliquot of effluents for DB 38 and DR 28 azo dyes. Samples taken from the effluent of CSTR reactor treating DR 28 azo dye was more toxic than the samples containing DB 38 azo dye metabolites. This toxicity test showed that the anaerobic degradation of azo dyes generated metabolites which were toxic to *Daphnia magna* test organisms. Toxicity was eliminated after aerobic treatment, indicating that the products causing toxicity to *Daphnia magna* partially degraded under aerobic conditions. In a study performed by Moran et al. (1997) *Daphnia magna* toxicity test results showed that aerobic treatment of textile wastewater did not remove the COD, and the non degradable portion of the dye, which is responsible from the toxicity.

ATA and SMA tests were also used to analyze the toxicity of the metabolic products of azo dyes. The values of percent inhibitions (I %) showed differences in toxicity before and after the azo dye has been metabolized. Analysis revealed significant differences between the results obtained by the influent and those obtained by the anaerobic and aerobic effluents indicating that the toxicity of the metabolic products were increased in the anaerobic stage and decreased after aerobic stage for DR 28 azo dye. Similarly the specific methanogenic activity (SMA) in dye containing mineral medium, anaerobic and aerobic effluent were 632, 442 and 1770 mg CH_4-COD/ g SS.day, respectively, indicating the anaerobic effluent has a lower methanogenic activity compared to dye and aerobic effluents.

Table 3.13 gives the toxicity analyses of degradation products by ATA tests. This observation suggests that the toxicity of the parental compound increased as the degradation proceeded. The products were more toxic than the parental compounds. Both the parental dye compounds and the metabolic products of dyes were reported to affect the aquatic microbial population (Chung and Stevens, 1993; O'Neill et al., 2000b). As seen in Table 3.13, the ATA tests results of parent dye, anaerobic and aerobic intermediates showed that reduction of toxicity could be possible through treatment of DR 28 and DB 38 azo dyes. Since the aromatic amines were not degraded under anaerobic conditions these intermediates had higher toxic effect than

the DR 28 azo dye (See Table 3.12). Since the aromatic amines were ultimately mineralized under aerobic conditions the aerobic effluents was found to be not toxic for DB 38 in aerobic effluent.

LUMIStox test and respiration/inhibition test showed similar findings indicating the effluents of UASB/CSTR reactor system had low toxicity on test microorganisms. Gottlieb et al, (2003) reported that LUMIStox toxicity was not detectable when decolorized Reactive Black 5 was metabolized under aerobic conditions. However, Decolorization of RB 5 under anaerobic conditions resulted in an increased toxicity. Differences in toxicity results may arise from the different responses of test microorganisms to toxicity in the samples.

3.5.5. General discussion for mineralization and toxicity reduction of azo dyes through anaerobic/aerobic operation

The reduction of parent azo dye compounds to the high toxic daughter aromatic amines ensured that an increasing toxicity in the anaerobic effluent. The detoxification of the anaerobic effluent was demonstrated by higher aromatic amines and COD removals through aerobic stage. Therefore coupled anaerobic-aerobic systems have proven to be successful in achieving the complete biodegradation of azo dyes. In such systems, azo dyes are reduced anaerobically, followed by subsequent aerobic degradation of the aromatic amines produced. HPLC-DAD and GC-MS analysis showed that dyes could be mineralized by a UASB/CSTR sequential reactor system.

The findings obtained in this study demonstrated that the DR 28 and DB 38 dyes could be completely reduced, decolourised, in the anaerobic pre-treatment with glucose which is acting as electron donor of reducing equivalents to enzyme cofactors transferring electrons for azo dye reduction by specific enzymes during the breakdown of DR 28 and DB 38 azo dye and that the reduction products were acceptable for aerobic mineralization. Since the main limiting operation parameter in anaerobic biomineralization is the hydraulic retention time compared to high dye

loadings, aerobic stage provide the degradation of aromatic amines in achieving a complete biodegradation and detoxification of azo dyes. Toxicity values of aerobic effluents were significantly lower compared to anaerobic effluents exhibiting low toxicity resulting in complete mineralization of carcinogenic amines. This finding has important implications for the environment since the benzidine is an important widespread contaminant of aqueous ecosystem, therefore an aerobic post-treatment step would be required for the complete mineralization of azo dyes containing textile and dye industry effluents.

CHAPTER FOUR
CONCLUSIONS AND RECOMMENDATIONS

4.1. Conclusions

In batch studies, the experimental data showed that the glucose-COD transformed to VFA resulting in methane production while the azo bonds of dyes cleaved and transformed to aromatic amines. At higher azo dye concentrations, the accumulation both in VFA and TAA resulting with low methane productions corresponding to lower rate of azo dye co-metabolism. COD was removed according to the first order reaction kinetic. Increases in dye concentrations from 0 to 3200 mg/l reduced the k_1 values from 0.0141 to 0.0019 1/h in batch studies performed with RB 5 azo dye. Similarly, the k_1 values decreased from 0.0096 to 0.0008 1/h as the dye concentrations increased from 0 to 3200 mg /l in batch reactors containing DB 2 azo dye. Increases in dye concentrations from 0 to 3200 mg/l reduced the k_1 values from 0.0187, 0.0062, and 0.012 to 0.0035, 0.0017, 0.0015 1/h in batch studies for DR 28, DB 38 and DY 12 azo dyes, respectively.

The decolorization rate constants of RB 5 dye are significantly higher than the rate constants of other dyes when first order kinetic constants considered for all dyes. RB 5 azo dye decolorized much faster than the other dyes. This difference could be attributed to the groups in the composition of other azo dyes, and intermetabolites produced could not be completely degraded and exhibited inhibition. The decolorization rates decreased slightly with the increases in dye concentrations for RB 5. For instance, the decolorization constants were found to be 0.098 1/h - 0.076 1/h at RB 5 azo dye concentrations of 200 mg /l and 3200 mg /l, respectively, while the same rate constants were 0.050-0.041, 0.0532-0.069, and 0.044-0.036 1/h at the same DR 28, DB 38 and DB 2 azo dye concentrations, respectively.

The cumulative methane gas production decreased as the azo dyes concentrations increased in batch study. This could have occurred during the solubilization of azo dye degradation products or intermetabolites, and, these products could inhibit the activity of anaerobic bacteria. 3000 mg /l of glucose–COD was completely converted to methane gas in dye-free batch reactors during 300 hours. The cumulative gas production measured at azo dye concentrations of 3200 mg /l are about 49, 70, 50, 30 and 45% of the dye-free and 200 mg /l of dye containing reactors for RB 5, DR 28, DB 38, DB 2 and DY 12 azo dyes, respectively.

At higher azo dye concentrations, with the exception of 3200 mg/l of DB 2 azo dye, while the pH and the B.Alk. levels decreased, while VFA concentrations increased. This could be due to the inhibition of methanogenesis and accumulation of inter-metabolites through simultaneous degradation and decolorization of azo dyes. Inhibition in both acetogenesis and methanogenesis was considered at the samples containing 3200 mg/l of DB 2 azo dye.

Relative methanogenic activities from the screening tests for the five dye chemicals were monitored and the order of toxicity on methanogenic activity increased as follows; DB 2>DB 38>RB 5> DY 12>DR 28.

In treatment of RB 5 azo dye by continuous fed UASB/CSTR reactor system, low COD removal efficiencies were obtained at high organic loadings since shock organic loading rates were applied before the UASB reactor reached steady state conditions. 41%-100% and 26%-91% of COD and color removal efficiencies were observed at loading rates varying between 2.5 COD/m^3.day and 25 kg COD/m^3.day, respectively in the UASB reactor. 76, 66, and 20% methane percentages were observed at loading rates of 2.5, 9.3, and 25 kg COD/m^3.day. A decrease in methane gas production was accompanied by increases in VFA/B.Alk. ratio. COD removal efficiencies of 28%, 42% and 90% were obtained at STRs of 1.7, 5.7 and 11 days in the aerobic reactor. The optimum SRT value for COD was determined to be 11 days in aerobic reactor. Although increases in SRT values significantly affect the COD

removal efficiencies in aerobic reactors only slight decreases were obtained in color removal.

88-75% color removal efficiencies were obtained for Reactive Black 5 at loading rates varying between 0.73 $g/m^3.h$ and 7.24 $g/m^3.h$ in the anaerobic/aerobic sequential reactor system. Increases in dye loading rates, did not significantly affect the color removal efficiencies. The color removal efficiencies were recorded between 75% and 88%. The total COD and color removal were 94.1 % and 80% in sequential UASB/CSTR reactor systems respectively at a HRT of 5.74 days. The influent COD of 3255 mg/l reduced to 190 mg/l by anaerobic/aerobic system at a HRT of 5.74 day. It is important to note that this COD value is lower than 250 mg/l which is the receiving water effluent discharge limit to surface waters for cotton textile industry in Turkey. Above 80% COD removals were determined for HRT higher than 2 days. The optimum HRT was detected as 2 days for the total sequential reactor system.

The treatment of DR 28 azo dye at high concentrations showed that the methane production rates increased with increases in dye-loading rates, indicating that a significant part of the DR 28 dye was converted to methane and used as a carbon and energy source by methanogens. It also was found that the VFA concentrations were lower than the studies performed with low DR 28 concentrations. These results showed that the VFA accumulation was not observed at higher dye concentrations and dye loading rates, such as 4000 mg/l of DR 28 concentration and a dye loading rate of 213 g DR 28 dye/$m^3.h$, respectively. Since the accumulation of VFA was higher and COD removal efficiencies were lower in very short HRTs (2.55 hours) compared to the condition in which high dye loading rates applied (HRT=18-22 hours), it can be assumed that short HRT values significantly affect the methanogenesis. Therefore, HRT is a much more important parameter than the high dye loadings in the inhibition of methane bacteria, although dyes are known to cause toxicity and decrease the activity of the methanogenesis.

97% COD and 100% color; 88% COD and 100% color removal efficiencies were obtained at DR 28 dye concentrations of 500 and 4000 mg/l at about HRTs of 3.7

days. TAA concentrations in UASB and CSTR reactor effluent were 238 mg benzidine/l and 47 mg benzidine/l at a DR 28 concentration of 4000 mg/l, respectively. The TAA analysis results showed that the greater part of TAA was removed through the aerobic phase in the complete UASB/CSTR system. TAA removal efficiencies varying between 91-93% were obtained at a DR 28 concentration of 4000 mg/l in whole system.

The substrate and alkalinity concentrations did not significantly affect the decolorization of DR 28 azo dye (100 mg/l). The decolorization of DR 28 dye in an UASB reactor did not correlate with COD concentrations, COD removals and bicarbonate alkalinity. It seems that the reducing environment prevailing in the UASB reactor by the electrons releasing from the DR 28 dye and its inter-metabolite products, mainly from the benzidine aromatic amine providing the color removal. The aromatic amines provides electrons supporting the reduction of the dye resulting in complete decolorization even at substrate-free operation.

In the treatment of DB 38 azo dye by continuous anaerobic/aerobic system, the COD removal efficiencies reduced to 48% from 73% while color removal decreased to 80% from 100 % at dye loading rates varying between 6 and 213 g dye/m^3.h. The optimum dye loading rate for COD and color was determined to be 107 g dye/m^3.h in UASB reactor. At this dye loading rate, COD and color removal efficiencies were 72% and 84%, respectively in the UASB reactor. High dye loadings rates (213 g dye/m^3.h) applied to the UASB reactor caused accumulation in VFA and consumption of much more alkalinity lowering in pH resulting in lower COD removals. The contribution of DB 38 concentrations to the influent COD (from 100 to 3200 mg /l) was 1184 mg /l at a dye concentration of 3200 mg/l. However the effluent COD was reduced to 555±76 in the effluent of the system (E_{COD}= 84%) when the reactor was fed with a DB 38 concentration of 3200 mg /l at HRT of 2.9 days. This shows that a significant part of glucose, and dye-COD was removed at a dye concentration of 3200 mg /l in the total anaerobic/aerobic system.

In the treatment of acid dyeing wastewater, the COD and color removal efficiencies in UASB reactor were between 51 and 84%, and 81-96% at a HRT of 15 hours while the UASB/CSTR system exhibited removal efficiencies of 83-97% and 80-91% a HRT of 3 days, respectively. Furthermore, aromatic amines (TAA) accumulated in anaerobic stage, and were removed effectively in the aerobic stage at removal efficiencies between 74% and 80%. In the treatment of textile wastewater, COD removal efficiencies were found to be varying between 40 and 85 % at total HRT of 5.75 in the UASB/CSTR reactor. Color removal efficiencies were obtained up to 39-81% in real textile wastewater through anaerobic operation period. TAA were removed effectively by aerobic CSTR reactor. The methane yield, pH and VFA concentration of UASB effluents showed that inhibition of real wastewater was not observed during anaerobic operation.

In the treatment of simulated wastewater, the optimum retention time and organic loading rate of UASB reactor were about 32 h and 3.2 kg COD /m^3.day, respectively, giving 94% color and 74 % COD removal efficiencies. COD and color removal efficiencies varying between 97-91 % and between 84-91 % were obtained at a total HRT of 19.17 and 1.22 days in combined anaerobic/aerobic system, respectively. The maximum color and COD removal efficiencies of 91% and 97% was achieved at a total HRT of 19.17 days in combined system treating simulated wastewater.

In this study based on the kinetic studies on UASB reactor treating simulated wastewater, it appears that the kinetic coefficients obtained from the anaerobic treatment of simulated cotton textile wastewater agree with the Stover-Kincannon and Grau second order multicomponent substrate kinetic models. The values of a, b and k_s were found to be 0.562, 1.095 and 0.337 1/day with high correlation coefficient (R^2) of 0.995 for Grau second order kinetic while saturation value constant (K_B) and maximum utilization rate (R_{max}) were calculated as 8.2 g/l.day and 7.5 g/l.day, respectively for Modified Stover-Kicannon model.

The results with intermediates and toxicity tests through mineralization of DB 38 and DR 28 azo dyes of 3200 mg/l in the anaerobic/aerobic system showed that the

daughter aromatic amines released from reduction of azo bonds is more toxic than parent azo dye indicating the toxicity in the anaerobic effluent. The detoxification of the anaerobic effluent was demonstrated by higher aromatic amines and COD removals through aerobic stage. Therefore coupled anaerobic-aerobic systems have proven to be successful in achieving the complete biodegradation of azo dyes. In such systems, azo dyes are reduced anaerobically, followed by subsequent aerobic degradation of the aromatic amines produced. HPLC-DAD and GC-MS, NO_3-N, UV-VS scans and TAA analysis showed that dyes could be mineralized by a UASB/CSTR sequential system.

In this study with granulated sludge cultures and with glucose as co-substrate, azo dyes were decolorized under anaerobic conditions. Up to 95-100% of the color of the azo dyestuff was removed in the anaerobic stage for all studied dyes. The released intermediates were mineralized in the aerobic part of the two stage system. Organic carbon in the dye mineralized to CO_2 and CH_4 organic nitrogen on azo bond in dye could be converted to NO^-_3-N in the anaerobic/aerobic conditions. It was observed that 85-95% of the remaining COD was removed in the aerobic stage. No color removal occurred aerobically. Since the limiting step of anaerobic biomineralization of any azo dye is the degradation of aromatic amines, anaerobic/aerobic systems might be effective in achieving the complete biodegradation and detoxification of azo dyes. High COD levels can be efficiently and cheaply removed in anaerobic stage, and can be reduced to discharge consents limits by aerobic stage. At low dye concentration (100 mg/l) discharge limits provided by anaerobic/aerobic system treating textile wastewater.

Results showed that anaerobic stages requires aerobic post treatment in order to complete the mineralization of dyes and residual COD from anaerobic stage. Anaerobic reactor/aerobic reactor system can have fallowing advantages for textile wastewater containing azo dyes;

Azo dyes reductively decolorized in anaerobic stage and mineralized in aerobic stage, methane can be obtained from anaerobic conversion of substrate in textile

wastewaters, and can be used for energy requirements in the operation of the anaerobic/aerobic system and for the other utilities. High temperatures and pH including effluents can be favourable, produced from the using alkali processing of reactive dyeing can be neutralized in the anaerobic stage. Two stage UASB/CSTR system eliminates the disadvantages of each stage. For instance, high organic loads in textile wastewater may cause bulking sludge and to expensive aeration in the aeration tank. Anaerobic pre treatment step could be a cheap alternative compared to aerobic systems. Moreover, anaerobic treatment produced a much smaller volume of sludge when compared to those obtained in aerobic treatment. The anaerobic treatment is favorable in anaerobic/aerobic stage taking consideration into the cost of the sludge treatment.

4.2. Recommendations

Currently, the treatment of textile wastewater has been carried out using aerobic biological processes, physico/chemical processes, and their modifications. The most commonly employed biological processes are conventional and extended activated sludge system. Nevertheless, these processes can not be degraded the dyes in textile industry wastewater. In the anaerobic stage, azo dyes, 60-70% of dyes in use textile dyeing processing, are easily converted to aromatic amines. Since 100-500 mg/l of dye concentration is higher than the concentrations in real textile wastewater, azo dyes can not inhibit the anaerobic treatment in the practice. However these aromatic amines exhibits resistance and/or degraded slowly under anaerobic conditions. These aromatic amines are readily degradable under aerobic conditions. The addition of an anaerobic stage in front of the aerobic stage will be favorable to treat textile wastewaters since anaerobic stage can degrade the azo dyes resulting in decolorization of textile wastewater.

As a result of this Ph. D. dissertation, sequential anaerobic/aerobic processes are recommended for the treatment of textile wastewater containing azo dyes. The anaerobic reactor can be placed in front of conventional aerobic reactors which have been still used.

CHAPTER FIVE
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CHAPTER SIX
APPENDICES

6.1. Nomenclature

A	measured absorbance value from spectrophotometer
a	$So/(k_s.X)$, (day)
A_0	the light absorbance for λ_{max} at the beginning of incubation.
A_t	light absorbance of dyes at λ_{max} for a selected time (t) of batch test;
b	constant (dimensionless)
B	volume of methane produced under normal conditions of pressure and temperature per gram of substrate added (l CH_4 / g COD added)
B_0	volume of methane produced under normal conditions of pressure and temperature per gram of substrate added at the infinitive retention time (l CH_4 / g COD added).
C_0	dye concentration at the beginning of the incubation through decolorization (mg/l)
C_t	residual dye concentration at selected time (t) of batch test through decolorization (mg dye /l)
d	sample length (cell width, 10mm)
f	factor (1000)
K	kinetic parameter equal to $Y.\beta$ (dimensionless)
k_0	zero order rate constant through co-substrate removal (mg/l.h)
K_0	zero order rate constant through decolorization (mg/l.h)
k_1	first order rate constant through co-substrate removal (1/h)
K_1	first order rate constant through decolorization (1/h)

k_2	second order rate constant through co-substrate removal (l /mg .h)
K_2	second order rate constant through decolorization (l /mg.h)
K_B	saturation value constant (g/l.day)
K_d	death rate constant (1/day)
k_f	k_f' *X (mg/l.day)
k_f'	first order multicompenant substrate removal kinetic constant (1/day)
K_M	half saturation concentration for Michaelis-Menten kinetic (mg/l)
k_{max}	maximum specific substrate utilization rate (1/h)
k_s	Grau second-order substrate removal rate constant (1/day)
K_S	half saturation concentration (mg/l)
p	k_f/S_0 (1/day)
Q	inflow rate (l/day)
Q_{UE}, Q_{CE}	effluent flow rate in UASB and CSTR reactors (l/day)
Q_{UW}, Q_{CW}	waste flow rate in UASB and CSTR reactors (l/day)
R	substrate utilization rate (mg/l.h)
R^2	regression analysis coefficient.
R_{max}	(k_{max} .X) maximum substrate removal rate (mg/l.h)
S	co-substrate concentration (mg/l)
S_0	glucose-COD concentration at the beginning of the batch incubation through co-substrate removal (mg/l)
S_I	initially inert COD (mg/l)
S_P	COD of inert soluble microbial products (mg/l)
S_R	final residual COD (mg/l)
S_S	readily biodegradable influent COD (mg/l)
S_T	effluent soluble COD (mg/l)
S_t	residual glucose-COD concentration at selected time (t) through co-substrate removal (mg/l)
t	time (h)
V	volume of the UASB reactor
V	volume of the UASB reactor
V_U, V_C	reactor volume of UASB and CSTR reactors (l)
X	biomass concentration (mg/l)

X_0, X, X_E	concentrations of biomass in the feed, reactor and effluent, respectively (mg/l)
X_U, X_C	concentration of microorganisms in UASB and CSTR reactors (mg/l)
X_{UE}, X_{CE}	effluent microbial concentration in UASB and CSTR reactors (mg/l)
X_{UW}, X_{CW}	waste microbial concentration in UASB and CSTR reactors (mg/l)
Y	the yield coefficient (g VSS / g COD)
α	color in m^{-1} unit
β	the kinetic parameter for Contois equation (g COD/g biomass)
θ_{CC}	sludge retention time in CSTR reactors (day)
θ_{CU}	sludge retention time in UASB (day)
μ, μ_{max}	specific growth rate and maximum specific growth rate (1/day)

6.2. Abbreviations

The following abbreviations are used in this study:

Ac^-	Acetate ion
ATA	anaerobic toxicity assay
B.Alk.	bicarbonate alkalinity, (mg $CaCO_3$/l)
BOD	biochemical oxygen demand (mg O_2/l)
C.I.	Color index
CSTR	continuous stirred tank reactor
F/M ratio	food to microorganism ratio
HAc	Nonionize acetic acid
HRT(θ_H)	hydraulic retention time (day or hour)
IC_{50}, IC_{25}	inhibition concentration and/or dilution ratio causing decreasing activity of 50%, and 25% compared to control, respectively.
OLR	organic loading rate (kg/m^3.day)
SMA	specific methanogenic activity (mg CH_4-COD/g.day)
SRT(θ_C)	sludge retention time (day)
SS	suspended solids (mg SS/l)
TAA	total aromatic amines (mg benzidine/l)

TOC	total organic carbon (mg C/l)
UASB	upflow anaerobic sludge blanket
VFA	volatile fatty acid (mg CH_3COOH/l)
VSS	volatile suspended solids (mgVSS/l)