



# DECOLORIZATION BEHAVIOR OF AZO DYE WITH VARIOUS CO-SUBSTRATE DOSAGES UNDER GRANULAR ACTIVATED CARBON-BIOFILM CONFIGURED PACKED COLUMN OPERATION

Soon-An Ong<sup>1,2</sup>, Eiichi Toorisaka<sup>1</sup>, Makoto Hirata<sup>1</sup> and Tadashi Hano<sup>1</sup>

<sup>1</sup>Department of Applied Chemistry, Oita University, Oita, Japan

<sup>2</sup>School of Environmental Engineering, Northern Malaysia University College of Engineering, Arau, Perlis, Malaysia

E-mail: [ongsoonan@yahoo.com](mailto:ongsoonan@yahoo.com)

## ABSTRACT

The objective of this study was to investigate the decolorization behavior of Acid Orange 7 (AO7) with different dosages of co-substrates under granular activated carbon-biofilm configured packed column (BGAC-packed column) operation. The spent granular activated carbon (GAC) was immobilized with azo dye-degrading microbes through attachment and then the GAC-biofilm was packed into a column. The system was fed with 3 l of AO7-containing wastewater for operation time of 24 h/batch. With initial 500mg/l of AO7 concentration, it was observed that complete decolorization was achieved in all runs although the co-substrates added into the BGAC-packed column system reduced until to zero. The kinetic data obtained from the removals of AO7 and COD could be explained by the autocatalytic kinetic model and Monod kinetic model, respectively.

**Keywords:** decolorization, dye, acid, orange 7, carbon, autocatalysis, biofilm, model.

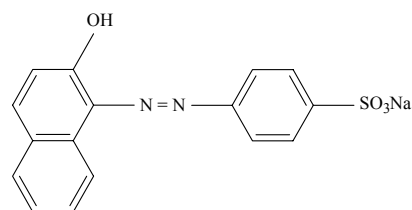
## INTRODUCTION

Removing of dyes is a major concern when treating textile-processing wastewater. The vast majority (60-70 %) of the dyes applied in textile-processing industries are azo compounds, characterized by azo (N=N) bridges linking substituted aromatic structures (Carliell *et al.*, 1995). The electron withdrawing character of the azo bond makes this class of compounds problematic for oxidative strategies of microbial degradation. Some azo dyes and their biotransformation products are toxic and carcinogenic (Brown and DeVito, 1993). Cytoplasmic azo reductases play an important role in the anaerobic biodegradation of azo dyes to produce colorless aromatic amines although complete mineralization is difficult. Once the xenobiotic azo component of the dye molecule has been removed, the resultant amino compounds are good substrates for aerobic biodegradation suggesting a choice of a sequential anaerobic-aerobic system for wastewater treatment (Banat *et al.*, 1996; Robinson *et al.*, 2001; Razo-Flores *et al.*, 1997; Manu and Chaudhari, 2001; Ong *et al.*, 2005a and 2005b). The addition of electron donors such as glucose or acetate ion apparently stimulated the reduction cleavage of azo bonds because the oxidation of these compounds produced electrons used for the formation of reduced cofactors (FAD, FMN and NADH) (Carliell *et al.*, 1995; Bras *et al.*, 2001). It is known that the co-substrate is an alternate growth substrate which when supplied to a bioreactor can enhance the degradation of some wastes or pollutants that cannot alone support the microbial growth (Atlas, 1993). Some researchers had observed that the decolorization of azo dyes follows the first-order kinetic model (Carliell *et al.*, 1995; Van der Zee *et al.*, 2001; Mechsner and Wuhmann, 1982; Willetts and Ashbolt, 2000) whereas other researchers found zero-order kinetics (Brown *et al.*, 1981; Dubin and Wright, 1975; Harmer and Bishop, 1992). Besides, the autocatalytic behavior was also

reported by some researchers in the anaerobic biological degradation and chemical reduction of AO7. The autocatalytic nature is related to the generation of 1-amino-2-naphthol, an intermediate produced after anaerobic breakdown of dye, which acts as a redox mediator favoring the reduction of the dye (Mendez-Paz *et al.*, 2003; Van der Zee *et al.*, 2000). The aim of this study was to investigate the decolorization behavior of AO7 with different dosages of co-substrates under granular activated carbon-biofilm configured packed column (BGAC-packed column) operation. The kinetic models involved in the color and organic matter removals are analyzed with autocatalytic kinetic model and Monod kinetic model, respectively.

## MATERIALS AND METHODS

The Acid Orange 7 (AO7) used as a model for evaluation in this study was obtained from Chroma Ltd. and the molecular structure is shown in Figure-1. The granular activated carbon (GAC) was supplied by Wako Pure Chemical Industries Ltd. All other chemicals were analytical grade.



**Figure-1.** Molecular structure of AO7.

The GAC from previous study in decolorization of azo dye using a sequential anaerobic-aerobic sequencing batch reactor (Ong *et al.*, 2005c) was used in this study. The spent GAC was immersed in an anaerobic sequencing batch reactor (ASBR) and a biofilm was developed after it



immersed in the bioreactor for more than a month. The ASBR has been used to treat the azo dye-containing wastewater for more than 200 days. Thus, the microbes in the ASBR were acclimatized to the azo dye and it was immobilized on the spent GAC by attachment technique. Then, the GAC-biofilm was packed into a column with 4cm Dia. X 105 cm H and the experimental set up for the BGAC-packed column is shown in Figure-2. A redox meter and a temperature controller were installed in the system to

measure the ORP values and control the temperature of synthetic wastewater, respectively. The synthetic wastewater (base mixed solution) consisted of bacto-peptone (188), sucrose (563),  $\text{NH}_4\text{Cl}$  (344),  $\text{MgSO}_4$  (49),  $\text{FeCl}_3$  (11.3) and  $\text{KH}_2\text{PO}_4$  (318) giving a COD of 800-850mg/l. The system was fed with 3 l AO7-containing wastewater daily and the operation condition is shown in Table-1.

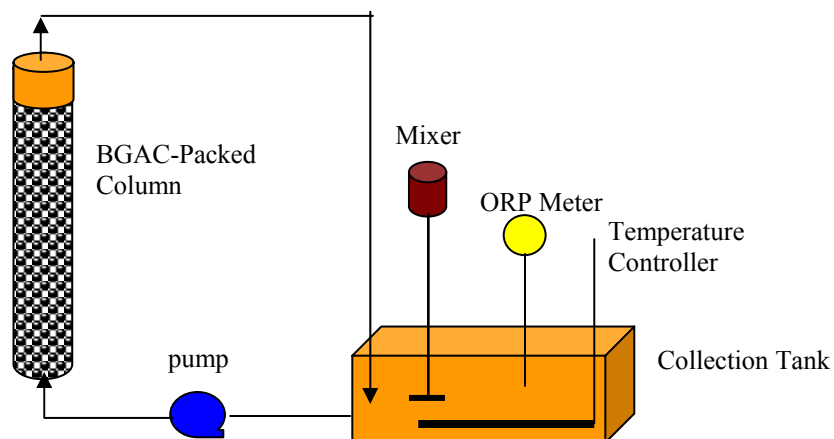


Figure-2. Schematic diagram of BGAC-packed column system.

Table-1. Operating conditions.

Term	Base mix solution (ml) (from stock solution)	Total COD con. (mg/l)	Orange II con. (mg/l)
I	100	2470	500
II	50	1700	500
III	25	1050	500
IV	10	816	500
V	5	710	500
VI	2	645	500
VII	0	620	500

\* 100ml synthetic wastewater contributes about 1700mg/l COD.

The effluent of the column was collected daily and analyzed for COD using spectrophotometry method (Hach). In the dissolved COD and color measurements, samples were prepared by filtering through a membrane filter 0.45 $\mu\text{m}$ . The AO7 concentrations were measured with the UV-Vis spectrophotometer (UV-1200, Shimadzu) at  $\lambda_{\text{max}}$  480.

## RESULTS AND DISCUSSION

### COD and color removal efficiencies with various dosages of co-substrates

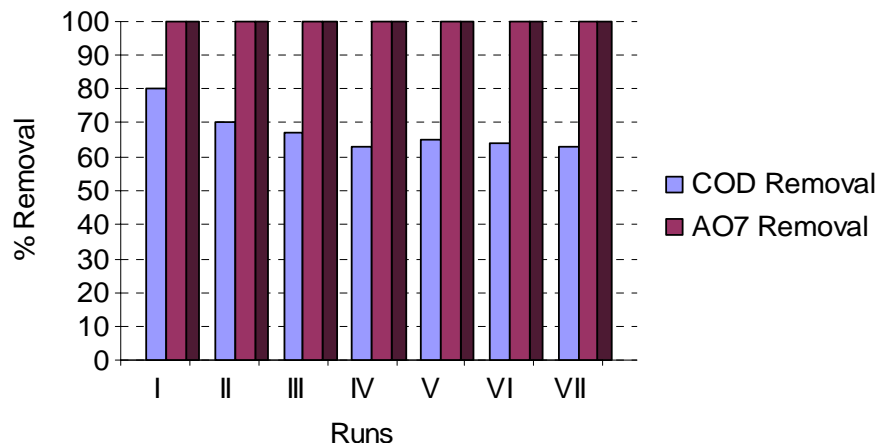
In this study, the co-metabolism of azo dye is defined as the removal of non-growth substrate (AO7) by growing microorganisms in the presence of growth co-substrate (35 – 1700mg/l of COD as sucrose equivalent). A co-substrate is defined as the carbon and energy source for microbial growth and maintenance and the release of the

electrons for cleavage of azo bond under reducing environments (Sponza and Isik, 2004). As shown in Figure-3, complete decolorization was observed for all runs under BGAC-packed column operation with initial 500mg/l of AO7 concentration. From Term I to VI, the concentration of sucrose in the synthetic wastewater was varied from 1.1 to 0.02 g/l.d. The result indicated that the decrease of co-substrate did not cause significantly dropped in the color removal efficiency by the BGAC-packed column. Moreover, it was found that complete decolorization was achieved although without the presence of co-substrate. Mendez-Paz *et al.* (2005) had reported that the low glucose concentration in the influent seems to be responsible for the lower efficiency of AO7. However, theoretically a loading rate of 0.20 g glucose  $\text{l}^{-1} \text{d}^{-1}$  is necessary to obtain the reducing equivalents needed to achieve the complete reduction of AO7 (considering the maximum AO7 loading rate). Many of the past studies show the importance of co-



substrates as electron donor in decolorization process and generally the addition of co-substrates resulted in much higher dye decolorization rates. However, the result from this study showed the ability of BGAC-packed column in the degradation of AO7 without the presence of co-substrate. In the absence of co-substrates, the endogenous lysis of biomass would supply the reducing equivalents needed for the reduction of the dye as indicated by low ORP values in the range of -300 to -400mV. Besides, the

decolorization could also due to the metabolism of aromatic amines by microbes, which yielded electrons used for reduction of azo bond. Işık and Sponza, (2004) had reported that the complete decolorization of Congo Red under co-substrate free operation could be attributed to Total Aromatic Amines (TAA) metabolism which may provide the electrons required for the cleavage of azo bond in Congo Red exist in the UASB reactor.



**Figure-3.** COD and AO7 removal efficiencies with various dosages of base mixed solution (co-substrates).

As shown in Figure-3, the COD removal by BGAC-packed column was in the range of 65 – 80 %. The percentage of COD contributed by AO7 increased as the base mixed solution added into influent reduced. However, it was observed that the COD removal efficiency able to maintain at about 65 % although the base mixed solution used reduced to zero. This indicates that the microbes immobilized on BGAC-packed column able to reduce azo bond of AO7 and subsequently degraded the intermediate products, aromatic amines, generated in the reactor. Generally, the intermediate products (carcinogenic aromatic amines) generated from dye reduction in anaerobic conditions have to be degraded by an aerobic process. However, the results from this study showed the ability of BGAC-packed column in simultaneous decolorization and biodegradation of azo dye in synthetic wastewater.

### Kinetics of Orange II biodegradation

The past research shows that the azo reduction under anaerobic conditions follows a first-order kinetic model (Carliell *et al.*, 1995; Van der Zee *et al.*, 2001; Mechsner and Wuhrmann, 1982; Willetts and Ashbolt, 2000). However, it was found that 1-amino-2-naphthol (1A2N) generated in the anaerobic degradation of AO7 could act as a redox mediator and thus giving the whole process an autocatalytic nature (Van der Zee *et al.*, 2000; Mendez-Paz *et al.*, 2003). The autocatalytic model proposed by Mandez-Paz *et al.* (2003) was used to analyze the data obtained from the degradation of AO7 in various

dosages of co-substrates under BGAC-packed column operation.

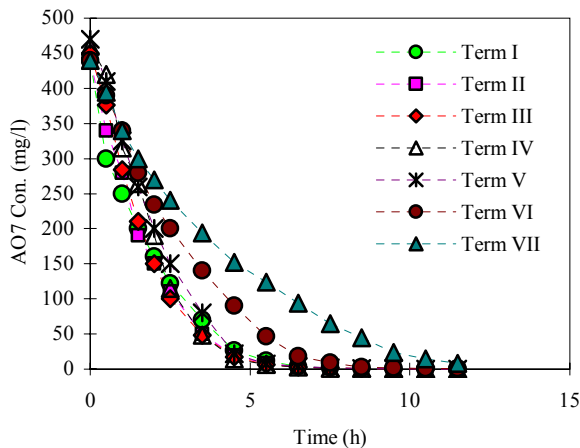
$$-dC/dt = k_1C + k_2C(C_0 - C)^a \quad (1)$$

Where  $C_t$  is concentration (mg/l) at time  $t$  (h),  $C_0$  is initial concentration (mg/l),  $k_1$  is the first-order kinetic constant ( $h^{-1}$ ) and  $k_2$  the autocatalytic constant ( $l.mmol^{-1}.h^{-1}$ ). With a equal to 1, after integral Eq. 1,

$$C_t = C_0(k_1 + k_2C_0)e^{-(k_1 + k_2C_0)t} / (k_1 + k_2C_0e^{-(k_1 + k_2C_0)t}) \quad (2)$$

The maximum removal rate ( $r_{max}$ ) ( $mmol.l^{-1}.h^{-1}$ ) can be calculated by derivation of Eq. 1 equal to 0.

$$r_{max} = k_1(k_1 + k_2C_0) / 2k_2 + (1/2)(k_1 + k_2C_0)[C_0 - (k_1 + k_2C_0) / 2k_2] \quad (3)$$

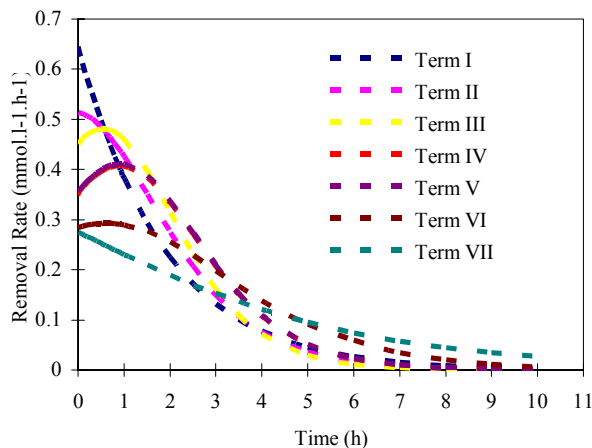


**Figure-4.** AO7 decay curves in BGAC-packed column with various base mixed solutions (co-substrate) concentrations.

Figure-4 shows the AO7 decolorization curves with various base mixed solution concentrations. Almost complete (~ 99 %) decolorization was achieved within 13h in all cases of treatment conditions. The data obtained was analyzed with autocatalytic kinetic models (Van der Zee *et al.*, 2000; Mendez-Paz *et al.*, 2003). The  $k_1$ ,  $k_2$ ,  $r_{max}$  and  $Tr_{max}$  (first-order, autocatalytic constants, maximal removal rate and time to achieve maximal removal rate) were evaluated from the autocatalytic kinetic model and the result are shown in Table-2. Based on the correlation coefficients, it was found that the data obtained well fitted with autocatalytic kinetic model. The maximal removal rates were decreased along with the decreasing in the amount of co-substrates added in the treatment system (Figure-5 and Table-2). Besides, the time to achieve maximal removal rate in the system also varied with the amount of base mixed solution used in the system (Figure-5).

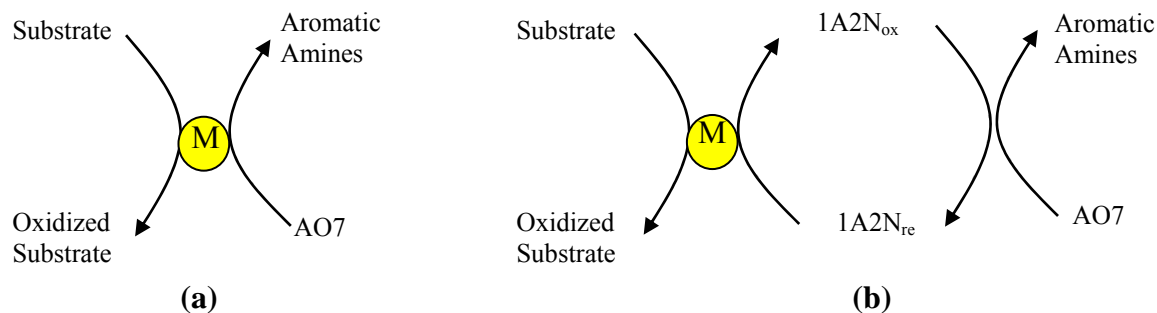
**Table-2.** Kinetic constants, correlation factor and maximum removal rates evaluated from first-order and autocatalytic models.

Term	$k_1$ ( $h^{-1}$ )	$k_2$ ( $l.mmol^{-1}.h^{-1}$ )	$R_{max}$ ( $mmol.l^{-1}.h^{-1}$ )	$Tr_{max}$ (h)	$R^2$
I	0.5291	0.0079	0.6450	0.0	0.9998
II	0.4122	0.2963	0.5141	0.0	0.9993
III	0.3495	0.4478	0.4804	0.5	0.9999
IV	0.2653	0.4263	0.4074	0.9	0.9988
V	0.2649	0.4248	0.4105	0.9	0.9995
VI	0.2268	0.2559	0.2928	0.8	0.9992
VII	0.2209	0.0449	0.2745	0.0	0.9987



**Figure-5.** Removal rates of AO7 calculated from autocatalytic kinetic model in BGAC-packed column with various base mixed solutions (co-substrate) concentrations.

As suggested by Mendez-Paz *et al.* (2003), the anaerobic biodegradation of AO7 generates 1A2N, which reacts as redox mediator in the transport of electrons to the dye, thus giving to the whole process as autocatalytic nature. As shown in Figure-6, the decolorization in anaerobic environments is a combined process of biotic and abiotic reactions. Azo dyes can be reduced in a direct chemical reaction with bulk biogenic reducing agents, but they can also be reduced by biological reactions, either directly as an enzymatically catalyzed reaction or indirectly via reduced enzyme cofactors (Van der Zee *et al.*, 2003). The indirectly biological reduction (redox mediator catalyzed) of the AO7 with 1A2N, which acts as a redox mediator, may cause the enhancement of the biodegradation rate. The sucrose used as electron donor generates reducing equivalents which needed for the reduction of azo bond. However, in the absence of co-substrate, the lysis of cells would generate enough reducing equivalents for the reduction of azo dyes.



**Figure-6.** Schematic proposed for (a) direct and (b) indirect biological dye reduction.

### Simultaneous biodegradation of co- substrate AO7

The most common rate expressions used to describe biodegradation reactions include Monod equation and its first- or zero-order approximation (Schreiber and Bahr, 2002). The substrate removal rate in a batch reactor can be expressed by Monod equation as below:

$$-dS/dt = (R_{\max} S)/(K_s + S) \quad (4)$$

By integral Eq. 4,

$$K_s \ln(S/S_0) + S - S_0 = -R_{\max} t \quad (5)$$

The Eq. 5 can be rearranged in the form of  $y = mx + c$  as below:

$$(\ln(S/S_0))(1/t) = -(R_{\max}/K_s) + (1/K_s)((S_0 - S)/t) \quad (6)$$

Where  $S$  is the substrate concentration expressed by COD (mg/l),  $R_{\max}$  the maximum substrate removal rate (mg/l.h) and  $K_s$  the half-velocity coefficient for the substrate (mg/l). At low substrate concentration ( $S \ll K_s$ ), the equation will reduced to first-order reaction.

$$-dS/dt = R_{\max} S/K_s \quad (7)$$

$$-dS/dt = R_{\max} \quad (8)$$

When  $S \gg K_s$ , the equation can be expressed by a zero-order reaction.

The zero- and first-order kinetic models are commonly applied than the full Monod kinetic model due to the simplicity of using linear expression (Salanitro, 1993). However, use of these (zero- and first-order) kinetic models may lead to inaccurate model predictions if the relative magnitudes of the substrate concentration and half-saturation constant are not within appropriate ranges (Schreiber and Bahr, 2002). By plotting  $(\ln(S/S_0))(1/t)$  vs  $(S_0 - S)/t$ , a relatively good fit ( $R^2 > 0.9$ ) for all cases was obtained indicating that the Monod kinetic model provides a reasonable correlation of the results. The parameters evaluated from the plot are summarized in Table-3. It was observed that the decrease of base mixed solution (sucrose) added into the system caused the  $K_s$  and  $R_{\max}$  values significantly reduced from 2500 to 625mg/l and 184 to 20mg/l.h, respectively. The ratio of COD contributed by AO7 would increase along with the decrease of base mixed solution (sucrose) added in the BGAC-packed column system. As a result, the COD removal rate would decrease because the biodegradability of AO7 by microbes is harder than sucrose. Besides, the huge amount of AO7 or aromatic amines generated from the reduction of AO7 might cause some extent of inhibitory effects on the microbes in BGAC-packed column.

**Table-3.** Monod kinetic constants obtained for COD removal in the treatment of various concentrations of co-substrate used under GAC-packed column operation.

Constants	Co-substrate (sucrose) (mg/l)						
	1.1	0.55	0.27	0.11	0.06	0.02	0
$R_{\max}$ (mg/l.h)	184	56	47	30	20	21	22
$K_s$ (mg/l)	2500	1428	1111	769	667	625	625
$R^2$	0.9570	0.9022	0.9848	0.9444	0.9682	0.9745	0.9856

### CONCLUSION

With initial 500 mg/l of AO7 concentration, it was observed that complete decolorization was achieved in all runs although the co-substrates added into the BGAC-packed column system reduced until to zero. This indicated that the decreased of co-substrate did not caused

significantly dropped in decolorization of AO7. In the kinetic study, it was observed that the COD and AO7 removals were well expressed by the Monod kinetic model and autocatalytic kinetic model, respectively. The indirectly biological reduction of the AO7 with 1A2N, which acts as a redox mediator, may cause the enhancement of the



biodegradation rate. It was observed that the reduced in co-substrate concentrations caused the  $K_s$  and  $R_{max}$  values decreased significantly.

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