

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Biodegrdation Kinectics of Azo Dye Mixture: Substrate Inhibition Modeling.

Jagannathan Krishnan, Anthony Arvind Kishore, Athreya Suresh, Akshay Krishna Murali and Jaikumar Vasudevan.

Department of Chemical Engineering, Sri Sivasubramaniya Nadar College of Engineering, Kalavakkam, 603110, Tamilnadu, India.

ABSTRACT

This study was focused on the substrate inhibition modeling for the biodegradation of mixture of three azo dyes using mixed microbial consortia obtained from a tannery effluent treatment plant. 15 different biokinetic models were used for the batch biodegradation experimental data. These were Haldane, Modified Haldane, Yano & Koga, Edwards, Tseng & Wayman, Luong, Han-Levenspeil, Andrews, Webb, Aiba, Modified Aiba, Michaelis-Menten, Teisser, Teisser Type and Moser models. The good fit of the degradation kinetic data of each of the three dyes with the Andrews, Aiba, Michaelis-Menten, Monod and Tessier models indicated that these models were perfect to represent the biodegradation of these azo dye mixture with mixed microbial culture used under the experimental conditions of initial concentration (20 -100 mg l⁻¹), pH (5-7) and inoculum dosage (5-10 % v).

Keywords: Biodegrdation, Azo dye mixture, Kinetic modelling, Substrate inhibition.

*Corresponding author Email id: jagannathank@ssn.edu.in



1. INTRODUCTION

Water contamination is a perpetually developing worry in our current world situation, given the sum and sorts of waste that are dumped into lakes and water bodies without proper pre-treatment, owing to their high level of biological and chemical oxygen demand, suspended solids, toxic compounds and aesthetic issues by their colour and odour. Wastewater containing dyes is a standout amongst the most effluents to treat. Huge assortment of dyes with polycyclic gatherings is utilized by the textile, leather and printing enterprises. Being xenobiotic mixes, they are hard to biodegrade. They are poisonous to sea-going species, people and creatures also. The most ordinarily utilized dyes are generally steady, hard to debase, safe to assault by microbial, physical and chemical techniques and in this way, hard to dispose of totally. Direct dyes have become a trend since the ban of azo dyes in 1997 due to their carcinogenic properties. Direct dyes offer different focal points over responsive dyes, the most essential of them all being the cost. A real weakness of direct dyes is its high solvency in water bringing about contamination in local water bodies when discharged without prior treatment. Dyes, being toxic, represent an ecological issue at the point when incorporated into waste (Kant, 2012). Attributable to their confused structures, wastewater containing dyes is for the most part treated utilizing more than one process, for example, physical and concoction medications (Türgay et al., 2011), adsorption (Yagub et al., 2014; Mahmoodi et al., 2011), or strategies like electrochemical treatment and flocculation forms (Mahmoodi et al., 2011). Be that as it may, these systems are not financially feasible and pose operational troubles. A portion of the different medicines effectively used to degrade direct dyes incorporate photocatalysis, oxidation, and so forth which are again vitality concentrated forms. By and large, treatment of such effluents should be possible utilizing natural or physical/compound treatment forms (Oller et al., 2011).

Usage of mixed microbial culture puts forth incredible favorable circumstances over the utilization of single strain societies for dye degradation. Singular strains could assault the dye particle at different introductions or could use the waste items delivered by an alternate strain to encourage assist decay (Oller et al., 2011; Solis et al., 2012). Mixed microbial culture was utilized because of their capacity to degrade the dye with finish mineralization when contrasted with pure algal or single strain microbial societies. Additionally, utilization of mixed microbial culture dodges the separation of a specific strain of microscopic organisms and gives high degradation of fragrant amines (Moosvi et al., 2007; Barragan et al., 2007). Mixed microbial societies were utilized to degrade different ecological poisons, for example, household wastes, pharmaceuticals, petroleum oil slime and so on. The advantages of utilizing mixed microbial culture over pure strain is the synergistic affiliations between the microorganisms (Kurade et al., 2017). Azo dyes are perplexing toxins; it is about difficult to absolutely debase the contaminant by a pure strain. The microbial proteins required to degrade the mixed dye arrangement may not be created by an isolated strain, a mixed microbial culture could create various proteins which can successfully degrade the azo dye mixture. Among the different treatment techniques utilized, natural treatment was picked, as it is eco-accommodating, non-harmful and very cheap (Pandey et al., 2007; Kalyani et al., 2016). Biodegradation of single dye utilizing microorganisms was accounted for widely in the writing, however thinks about on mixture of azo dyes is extremely limited. Besides, the material and dyeing enterprises discharge wastewater containing a mixture of azo dyes into nature (Saratale et al., 2011).

This research work is aims to simultaneously degrade three dyes present as a mixture. Normally single dye or two fold mixture of azo dyes are accounted for in writing. Though in this review, an endeavor was made to biodegrade a mixture of three azo dyes. Ordinarily utilized material and leather dyes to be specific RBRX-3B, DB-6 also, DB-19 were utilized as model pollutants and tried for dye evacuation in a batch shake flask by utilizing mixed microbial culture disengaged from the slop secured from a local waste water treatment effluent plant. Despite the fact that a mixture of azo dyes was utilized, the expulsion of individual dye from the mixture was measured for each dye and potrayed in this work. The impact pH, inoculum dose and introductory dye focus on the biodegradation of azo dye mixture were likewise considered efficiently.

2. MATERIALS AND METHODS

2.1. Dye sample preparation

All three azo dyes in particular, RBRX-3B, DB-6 and DB-19 utilized as a part of this review were got from a local tannery and utilized without advance filtration. Required amount of particular dye was dissolved

RJPBCS



in millipore water independently to first arrange a stock of 1000 mgl⁻¹. Successful dilutions were done in order to get dye arrangements of required fixations. Dye mixture of known concentration was prepared by mixing each dye solution in the required volumetric ratio. The UV scanning of the mixture of dyes yielded greatest absorbance peaks at three wavelengths. The 320 nm peak compared to RBRX-3B dye, the 456 nm peak compared to DB-6 and the 604 nm top spoke to DB-19. Obscure grouping of dye specimens were measured from the standard alignment plot arranged.

2.2. Inoculum and acclimatization

The mixed microbial culture was cultivated from the biological sludge got from a centralized wastewater treatment plant that treats the wastewater from more than 200 adjacent businesses. The culture was developed in a medium containing 10 g1⁻¹ glucose, 0.34 gl⁻¹ yeast, 0.84 gl⁻¹ NH₄Cl, 0.134 gl⁻¹ KH₂PO₄, 0.32 g l⁻¹ K₂HPO₄ and 0.084 g1⁻¹ MgCl₂.6H₂O (Kumar et al., 2009). Acclimatization of mixed culture was done at room temperature by continuously expanding the dye fixation with occasional perception of the decolourisation of dye. Following two weeks of acclimatization, microorganisms with high degradation capacity having a most extreme working point of 100 mg 1⁻¹ of initial concentration of the mixture of dyes at room temperature was obtained.

2.3. Degradation kinetics

To complete a kinetic study, a 20 mg l^{-1} of dye solution was included in a 250 ml Erlenmeyer flask containing the 10% acclimatized inoculum. An aggregate working volume of 200 ml was kept up by mixing 170 ml of media, 20 ml of dye arrangement and 10 ml of acclimatized inoculum. In the wake of changing the pH to 7 by utilizing hydrochloric corrosive and sodium hydroxide, the flask was covered with cotton and kept in the incubator shaker set at 180 rpm and 30°C. For each 4 h, aliquots of around 2ml specimens were withdrawn and centrifuged. The supernatant was taken out to quantify the absorbance and the residue was dried for a few hours to get its dry weight to study the development of the microscopic organisms and its enzymatic responses towards the entire procedure (Jagannathan and Siti, 2013).

2.4 Modeling the kinetics of degradation

The Monod model is one of the earliest models on microbial kinetics which relates the degradation rate of microorganism to the substrate concentration μ = f(S) via two parameters (Saravanan et al.2011): maximum specific growth rate (μ m) and half saturation constant (K_s).It is represented by the following equation:

$$\mu = \frac{\mu_m S}{K_S + S}$$

The Monod growth model can be implemented to quantitatively describe the degradation of substrate or growth associated product formation. (Kovariet al.1998). However, this model fails in explaining substrate inhibition on either growth of microorganisms or substrate degradation. The novel model that incorporated both the substrate affinity constant and the substrate-inhibition constant was proposed by Haldane (Haldane 1965). This model is applicable for representing the degradation kinetics under inhibitory substrates.

When the inhibition constant is infinitely large, the model reduces to a simple Monod's kinetics. The modified Haldane model involves the total inhibition concentration. Yano and Koga (Yano et al.1969) proposed a model based on a theoretical study on the dynamic behavior of single-vessel continuous fermentation subject to growth inhibition at high concentrations of rate-limiting substrates, e.g., the gluconic acid fermentation from glucose.

A modiefied Haldane model was proposed by Edward (Yano et al.1969). It proposes the protective diffusional-limitation of high and inhibitory substrate concentrations.

Tseng- Wayman (Tseng et al.1975) proposed a model which accounted for the fact that there would be no growth inhibition below a threshold substrate concentration, S*. However, microbial growth would



decrease linearly with respect to the concentration (S - S^*) at concentrations above S^* (Mulchandaniet al.1989).

To account for the discontinuous nature of the model, Luong (Luong 1987) proposed the application of substrate inhibition to microorganism growth. Luong model, was applicable for representing the kinetics of the inhibition of substrate, but also accounted for substrate stimulation at its low and high concentrations. Mulchandani & Luong stressed on the importance of critical and maximum substrate concentrations. They argued that the Haldane model did not prove that the microbial growth operates in a similar mechanism.

To cover the kinetics at inhibitory levels of substrate and product, Han and Levenspiel (Han et al.1998) proposed a non-linear model to express the growth. The Andrew equation is based upon the specific growth rate and it is commonly used owing to its mathematical simplicity. It is widely accepted for describing the growth inhibition kinetics of microorganisms.

The substrate inhibition effects at high concentrations are explained by the Andrews model (Andrews et al.1968), although the equation reduces to Monod's at very high inhibition constant values. The Webb model equation (Edwards 1970) is a modified form of the Andrews model. The equation is derived from enzyme kinetics, and allosteric effect ' β ' is incorporated. Teisser proposed a model that took only the growth kinetics into consideration.

Model	Equation	Reference
Haldane model	$\mu = \frac{\mu_m S}{\kappa_s + S + \frac{S^2}{\kappa_1}}$	16
Modified Haldane model	$\mu = \frac{\mu^*}{1 + \frac{K_I}{S} + K_5 \frac{S}{S^* - S}}$	17
Yano and Koga	$\mu = \frac{\mu_m S}{K_s + S + S^2 \left(1 + \frac{S}{K}\right)}$	18
Edward model	$\mu = \mu_m S \left[\exp\left(\frac{-s}{\kappa_l}\right) - \exp\left(\frac{-s}{\kappa_s}\right) \right]$	18
Tseng and Wayman model	$\mu = \mu_m \left(\frac{S}{S+K_S}\right)$	19
Luong model	$\mu = \frac{\mu_m S}{K_s + S} \left[\left(1 - \frac{S}{S^*} \right)^n \right]$	21
Han Levenspiel model	$r = k \left[\left(1 - \frac{S}{S^*} \right) \right] \frac{S * C}{\gamma_s + K_m \left[\left(1 - \frac{S}{S^*} \right)^m \right]}$	22
Andrews equation	$\mu = \frac{\mu_m S}{(K_s + S)(1 + \frac{S}{k_I})}$	23
Webb	$\mu = \frac{\mu_m S (1 + \frac{S}{K})}{(S + K_s + \frac{S^2}{k_I})}$	24
Aiba model	$\mu = \frac{\mu_m S}{K_s + S} e^{-\frac{S}{K_l}}$	25
Modified Aiba model		17

Table 1. List of kinetic models used in this study.



	$\mu = \frac{\mu^*}{1 + \frac{K_I}{S}} e^{-K^* \frac{S}{S^* - S}}$	
Michaelis - Menten condition	$V = \frac{V_m S}{K_m + S}$	26
Teisser	$\mu = \mu_m \left(1 - e^{\frac{-5}{K_s}} \right)$	27
Teisser type model	$\mu = \mu_m \left[\left(e^{-\frac{S}{K_f}} - e^{\frac{S}{K_s}} \right] \right]$	27
Moser model	$\mu = \frac{\mu m S^n}{K_s + S^n}$	28

The model developed by Aiba (Aiba et al 1968), is an empirical correlation. However albeit a simulated data with substrate inhibition, empirical data from laboratory experiments corroborates its validity. The modified Aiba model takes into consideration the total inhibition concentration. The Moser model (Moser et al.1985) includes a power function of substrate concentration. It is considered as a modified Monod equation. The degree of inhibition is determined by the power value. However, it does not indicate critical substrate concentration or inhibition constant. The list of all the 15 kinetic models used in this study are shown in Table 1.

2.5 Effect of process parameters

250 ml Erlenmeyer flasks were used to carry out this experiment in a batch mode. The acclimatized bacterial culture was used as inoculum and the initial dye concentration was varied along with the pH of the mixture. The various parameters studied were pH (5-9), inoculum concentration (5-15%) and initial dye concentration (20-100 mg l^{-1}). Samples were withdrawn from the flasks periodically and centrifuged. Supernatant thus obtained was analysed by UV-vis spectrophotometer to examine the residual concentration of the three dyes through absorbance measurements.

3. RESULTS AND DISCUSSIONS

3.1 Modeling the biodegradation kinetics of RBRX-3B

The degradation kinetic data obtained for RBRX-3B was fit into 15 models. Among the models which are generally used to represent the bio-degradation kinetics, even at substrate inhibitory levels, Andrew's, Aiba, Michaelis – Menten, Modified Aiba, Monod, Moser, Tessier, Webb and Yano & Koga models fit the data very well with R^2 values greater than 95%. However, Edwards model did not fit the data at all. The R^2 values were used as statistical measures of the goodness of fit of the data with each model for each dye. The R^2 values are measures of an excellent fit of the data with the model and hence these criteria were chosen to identify the best kinetic model(s).

It was also noted that the bio-kinetic parameters, namely μ_s , K_1 and K_s vary substantially from one model to another. This difference could be attributed to the fact that these models difference in their origin of development and were designed to test the fit of bio-kinetic data for a particular microorganism under particular experimental conditions only. (1) K_s values indicate the affinity of the microorganisms towards the substrate and hence the models with high K_s values (refer table) such as Edwards, Haldane, Modified Haldane and Moser were chosen as the ones validating this fact. On the other hand, the K_1 value is indicative of the substrate's toxicity towards the microorganisms; it is a measure of the inhibitory effect. Also, it can be described as the substrate concentration at which the bacterial growth is reduced to 50% of its maximum specific growth rate. Low K_1 values indicate a high sensitivity of the microorganisms towards inhibition. This was shown in the case of the Modified Aiba, Tseng and Wayman, Webb and Yano and Koga models compared to other models that had exceptionally high values of K_1 . The summary of kinetic models fitted for the biodegradation of RBRX-3B is given in Table 2.

May – June

2017(Suppl.)

RJPBCS

8(3S) Page



Table 2. Summary of kinetic models fitted for the biodegradation of RBRX-3B.

Models	Parameters														R ² value	
models																
	μ _m (hr⁻¹)	Kı	K _s (mg l ⁻¹)	X ₁	Км	К	Y ₁	а	b	с	d	n	X _M	α	β	(%)
Haldane	2086889.01	14172706.55	21563437135	995768.99	-	-	-	-	-	-	-	-	-	-	-	28.8
Modified Haldane	-	216593748.6	1690017498	3207.4	-	-	-	-	-	-	-	-	-	-	-	88.1
Yano and Koga	0.00692	4.682	400.47	-	-	0.00655	-	-	-	-	-	-	-	-	-	98.1
Edwards	2.529	4668344.77	-3368341.31	-	-	-	-	-	-	-	-	-	-	-	-	1.9
Tseng and Wayman	0.4085	0.5428	3.041	-	-	-	-	-	_	_	-	-	0.003 84	_	-	88.5
Luong	0.00669	-	27.569	-	-	-	-	-	_	_	-	-	-	-	-	99.9
Han levenspiel	0.00669	-	27.569	_	-	-	-	-	_	-	-	-	-	-	-	99.9
Andrew's	0.00669	9901949780	27.569	-	-	_	-	-	-	-	-	-	-	-	-	99.9
Webb	0.006401	619.481	26.341	-	-	-	-	-	-	-	-	-	-	-	1.046	99.9
Aiba	0.00671	42666.246	27.674	-	-	-	-	-	-	-	-	-	-	-	-	99.9
Modified Aiba	-	27.571	-	0.000152	-	3.7029	0.271 7	-	-	-	-	-	-	-	-	99.9
Michalis – Menten	0.00699	-	-	_	27.5 69	-	-	-	_	-	-	-	-	-	-	99.9
Tessier	0.00507	-	25.066	-	-	-	-	-	-	-	-	-	-`	-	-	98.2
Tessier type	0.005074	3521606562	25.066	-	-	-	-	-	-	-	-	-	-	-	-	98.2
Monod	0.006699	_	27.569	-	-	-		-	_	-	-	-	-	_	-	99.9
Moser	90882.57	-	114641794	-	-	-	-	-	-	-	-	0.42 77	-	-	-	96.1



Table 3. Summary of kinetic models fitted for the biodegradation of DB-6.

																R ² value
Models		1	Γ	1		Para	meters	r —				r	1	1	1	<u> </u>
	μ _m (hr⁻¹)	Kı	K _s (mg l ⁻¹⁾	X 1	Км	к	Y ₁	а	b	с	d	N	Хм	α	β	(%)
		14209056		229124												90.5
Haldane	379644.57	.13	9708748554	03.7	-	-	-	-	-	-	-	-	-	-	-	
Modified																99.9
Haldane	-	440.64	3.432	0.0218	-	-	-	-	-	-	-	-	-	-	-	
Yano and Koga	1.269	0.328	27291.99	_	-	0.0006 14	-	-	-	-	-	-	_	-	-	99.8
Edwards	0.8276	36.302	26.296	_	_	-	-	-	-	_	_	_	-	_	_	87.4
Tseng and													0.0007			97.9
Wayman	0.2473	0.303	4.44	-	-	-	-	-	-	-	-	-	22	-	-	
																99.9
Luong	0.00899	-	181.11	-	-	-	-	-	-	-	-	-	-	-	-	
Han Levenspiel	0.00899	-	181.11	-	-	-	-	-	-	-	-	-	-	-	-	99.9
· · ·		79054118														99.9
Andrew's	0.00899	6.6	181.11	-	-	-	-	-	-	-	-	-	-	-	-	
															0.85	99.9
Webb	0.00983	1749.97	198.059	-	-	-	-	-	-	-	-	-	-	-	9	
Aiba	0.009257	9716.7	186.61	-	-	-	-	-	-	-	-	-	-	-	-	99.9
						0.0031	0.0090									99.9
Modified Aiba	-	181.8	-	10.91	-	07	4	-	-	-	-	-	-	-	-	
Michalis –					181.											99.9
Menten	0.008993	-	-	-	112	-	-	-	-	-	-	-	-	-	-	
																99.9
lessier	0.00520	-	106.189	-	-	-	-	-	-	-	-	-	-	-	-	
Teesien Tune	F2 44F	25/85/.1	210200 4													90.5
Tessier Type	52.415	1	319200.4	-	-	-	-	-	-	-	-	-	-	-	-	00.0
Monod	0.008993	-	181.127	-	-	-	-	-	-	-	-	-	-	-	-	33.3
												0.82				99.2
Moser	172960.77	-	2283405495	-	-	-	-	-	-	-	-	21	-	-	-	



Table 4. Summary of kinetic models fitted for the biodegradation of DB-19.

Models							Parameters									R ² value
	µs(hr⁻¹)	Kı	K _s (mg l ⁻¹⁾	X1	Км	к	Y ₁	а	b	с	d	n	Хм	α	β	(%)
Haldane	597.194	410.063	3065565.13	7767.15	-	-	-	-	-	-	-	-	-	-	-	31.5
Modified Haldane	_	97 722	6 4002	0.0648	_	_	_	_	_	_	_	_	_	_	_	99.9
Vano and Koga	0.012	344056	18.02		_	0.00783	_	_	_	_	_	_	_	_	_	99.9
Edwards	4 009	7889.03	7920 609		_	-	_		_		_	_			_	18.1
Tseng and Wayman	0.08736	-	1.9641	_	-	-	-	-	-	-	-	_	-	-	-	76.8
Luong	0.0119	-	18.019	-	-	-	-	-	-	-	-	-	-	-	-	99.9
Han Levenspiel	0.0119	-	18.019	-	-	-	-	-	-	-	-	-	-	-	-	99.9
Andrews	0.0119	266958 5250	18.019	-	-	-	-	-	-	-	-	-	-	-	-	99.9
Webb	0.06905	0.45144	389.37	-	-	-	-	-	-	-	-	-	-	-	0.118	87.8
Aiba	0.062	30.172	117.94	-	-	-	-	-	-	-	-	-	-	-	-	96.8
Modified Aiba		5.873	-	3.382	-	3.35	0.2689	-	-	-	-	_	-	-	-	72.2
Michalis – Menten	0.01199	-	-	-	18.0 199	-	-	-	-	-	-	-	-	-	-	99.9
Tessier	0.00944	-	17.172	-	_	-	-	-	-	-	-	_	-	-	-	97.5
Tessier Type	0.00944	931148 1580	17.172	-	-	-	-	-	-	-	-	-	-	-	-	97.5
Monod	0.01199	-	18.019	-	-	-	-	-	-	-	-	-	-	-	-	99.9
Moser	0.001306	-	1.496	-	-	-	-	-	-	-	-	0.065 6	-	-	-	84.1



3.2 Modeling the biodegradation kinetics of Direct Blue - 6:

Among the different models tested for their goodness of fit with the bio-kinetic data of Direct Blue – 6, the Andrews, Aiba, Michaelis – Menten, Modified Aiba, Monod, Moser, Tessier, Webb and Yano and Koga fit the data excellently, validated by their very high R^2 values. On the other hand, as in the case of RBRX-3B, the Edwards model did not fit the data well. The R^2 values were also used as another statistical measure of the goodness of it and the values was found to be greater than 95% in the cases of Haldane, Yano &Koga, Edwards, Tseng & Wayman, Aiba, Tessier, Tessier type and Moser model equation. Thus these models fit the data very well.

Also, as in the case of RBRX-3B, no particular trend in the bio-kinetic parameter values was observed; they were very different from each other. A high affinity of microorganisms towards Direct Blue – 16 was shown by the high K_s values of the Haldane, Moser, Tessier and Yano and Koga models. With comparatively low K_l values, a high sensitivity of microbes towards inhibition was shown in the Edwards, Modified Aiba, Tseng and Wayman and Yano and Koga models. Kinetic models fitted for the biodegradation of DB-6 is summarized in Table 3.

3.3 Modeling of the biodegradation kinetics of Direct Black – 19:

In the case of Direct Black – 19, the bio-kinetic data was fit excellently in the following models: Andrews, Aiba, Michaelis – Menten, Monod, Modified Haldane, Piecewise Linear, Tessier and Yano and Koga, with R^2 values greater than 95%. However, the Edwards and Haldane models did not fit the data well. This concludes that the Edwards models did not satisfactorily fit the bio-kinetic data of any of the three dyes used for this study. Thus, the goodness of fit of the data was described using the R^2 values from which we can arrive that the models fit the data really well.

As in the case of the other two dyes, the bio-kinetic parameters varied hugely from each other. High K_S values obtained from the Edwards and Haldane models showed a high affinity of microbes towards the dye, whereas a comparatively low K_1 value obtained from the Aiba, Modified Aiba, Haldane, Modified Haldane and Webb models indicated the high sensitivity of the microbes towards inhibition when in contact with the substrate. Summary of kinetic models fitted for the biodegradation of DB-19 is shown in Table 4.

4. VALIDATION OF MODELING RESULTS

In the present study, the bio-kinetic data across the range of substrate concentration $-20 \text{ mgl}^{-1} - 100 \text{ mgl}^{-1}$ were fitted into all of the 15 models using Curve Expert Professional software. It needs to be mentioned that no similar literature could be found focusing particularly on the biodegradation of RBRX-3B, Direct Blue-6 and Direct Black-19. (No similar data for K_s, K_i and μ_s) However, a review of literature comparing similar kinetic models to describe the effect of substrate inhibition is given below:

The Aiba equation was found to be the ideal equation to illustrate ammonium inhibition of the nitration process in a system containing suspended/immobilized biomass, as shown by (Carrera et al. 2004). However, the Haldane equation which is comparable to Andrews was found to best describe nitration inhibition by nitrite in both kind of systems. (Agarry et al. 2008) proved that the Yano and Koga model was best suited to fit the data from phenol degradation kinetics studies carried out by a binary culture of Pseudomonas aeruginosa and Pseudomonas fluorescence. To compete with this, (Saravanan et al. 2008) proved that the substrate inhibition kinetics of phenol degradation using mixed microbial consortia was best fit by the Haldane and Han-Levenspiel models; Han-Levenspiel, however, was the better fit between the two. More recently it was proved that the Haldane model was best suited to describe phenol degradation by mixed microbial culture by (Dey et al. 2010) as well as for the aerobic bioremediation of hydroquinone and catechol by an activated sludge acclimatized to consume p-nitrophenol. (Pramparo et al., 2012)

The Yano and Koga model was found to describe the biodegradation of Malathion using indigenous activated sludge. (Tazdait et al. 2014). Kinetics of phenol biodegradation was modeled and was fit bet using the Haldane model when the experiment was carried out in a draw-fill suspended and attached growth reactors by olive pulp bacteria. (Tziotzios et al., 2007). The Haldane kinetic model accurately predicted the



kinetics of self-inhibitory growth (phenol) and non-growth (4-chlorophenol) substrates using Acinetobactor isolate. (Hao et al., 2002). (Han et al 1988) predicted that an extended form of the Monod model can be used to fit data belonging to all kinds of product, cell and substrate inhibition experiments very well.

5. CONCLUSION

In this study, the capability of the acclimatized mixed microbial culture to simultaneously degrade RBRX-3B, Direct Blue-6 and Direct Black-19 present as a mixture. The biokinetic data obtained from the degradation studies were modeled to fit various substrate inhibition models out of which, the Andrews, Aiba, Michaelis-Menten, Monod and Tessier were found to be very successful in expressing the inhibition kinetics. Based on this study, acclimatized mixed microbial culture could be used as a potential and economical culture to remediate azo-dye mixtures.

6. ACKNOWLEDGEMENT

Authors are very thankful to the SSN Trust for the financial support and Department of Chemical Engineering, SSN college of Engineering for providing the facilities to carry out this research work.

7. REFERENCES

- [1] Kant, Rita (2012). Textile dyeing industry an environmental hazard, *Natural science*, 4(1): 22.
- [2] Türgay, Orçun, (2011). The treatment of azo dyes found in textile industry wastewater by anaerobic biological method and chemical oxidation, *Separation and Purification Technology*, 79(1):26-33.
- [3] Yagub, Mustafa T. (2014) Dye and its removal from aqueous solution by adsorption: a review, *Advances in colloid and interface science*, 209: 172-184.
- [4] Mahmoodi, Niyaz Mohammad (2011). Adsorption of textile dyes on pine cone from colored wastewater: kinetic, equilibrium and thermodynamic studies, *Desalination*, 268(1): 117-125.
- [5] Oller, I., S.Malato, and Jab Sánchez-Pérez (2011). Combination of advanced oxidation processes and biological treatments for wastewater decontamination—a review, *Science of the total environment*, 409(20): 4141-4166.
- [6] Solís, Myrna (2012). Microbial decolouration of azo dyes: a review, *Process Biochemistry*, 47(12): 1723-1748.
- [7] Moosvi, S., Kher, X., Madamwar, D (2007). Isolation, characterization and decolourisation of textile dyes by a mixed bacterial consortium JW-2, *Dyes and Pigments*, 74: 723-739.
- [8] Blanca, E. B. Costa C, Marquez, M. C. (2007). Biodegradation of azo dyes by bacteria inoculated on solid media, *Dyes and Pigments*, 75:73-81.
- [9] Anjali Pandey, Poonam Singh, Leela Iyengar (2007). Bacterial Decolorization and Degradation of Azo Dyes, *International Biodeterioration and Biodegradation*, 59(2):73-84.
- [10] Saratale, R.G., Chang, J.S. Govindwar S.P. (2011). Bacterial de-colorization and degradation of azo dyes. A review, *Journal of the Taiwan Institute of Chemical Engineers*, 42:138-157.
- [11] Kapil Kumar, M.G. Dastidar, T.R. Sreekrishnan (2009). Effect of process parameters on aerobic decolourization of Reactive Azo dye using mixed culture, *World Academy of Science*, Engineering and Technology, 58.
- [12] Jagannathan Krishnan, Siti Rabiatul, Adawiyah Ibrahim (2013). Batch Kinetics and Effects of Process Parameters for Biodegradation of Reactive Black 5 in an Aerobic Mixed Microbial Culture, *Scientific Research Journal*, 10(2): 1-14.
- [13] Kumar, K., Dastida M. G, Sreeniketan, T. R. (2009). Effect of Process Parameters on Aerobic Decolourization of Reactive Azo Dye using Mixed Culture, *World Academy of Science*, Engineering and Technology, 28:962-965.
- [14] P. Saravanan, K .Pakshirajan, P. Saha (2011). Kinetics of phenol degradation and growth of predominant Pseudomonas species in a simple batch stirred tank reactor, *Bulgarian Chemical Communications*, 43 (4): 502-509.
- [15] K.K. Kovari, T. Elgi (1998), *Microbiology & Molecular Biology Reviews*, 62: 646.
- [16] Haldane, J. B. S. (1965), Enzymes, *MIT Press*, Cambridge, 84.
- [17] Computer applications in biotechnology, *Google books*, 256.
- [18] T. Yano, S. Koga (1969), *Biotechnology & Bioengineering*, 11: 139.
- [19] M.M. Tseng, M. Wayman (1975), *Canadian Journal of Microbiology*, 21: 994.
- [20] A. Mulchandani, J.H.T. Luong (1989), *Enzyme Microbial Technology*, 11: 66.

RJPBCS



- [21] J.H.T. Luong (1987), Biotechnology & Bioengineering 29:242.
- [22] K. Han, O. Levenspiel (1998), *Biotechnology & Bioengineering*, 32: 430.
- [23] Andrews, J. F. (1968), A mathematical model for the continuous culture of microorganisms utilizing inhibitory substrates, *Biotechnology & Bioengineering*, 10:707.
- [24] Edwards, V.H. (1970), Thelnuence of High Substrate Concentrations on Microbial Kinetics, *Biotechnology & Bioengineering*, 12: 679-712.
- [25] Aiba, S., Shoda, M., Nagalani, M. (1968), Kinetics of product inhibition in alcohol fermentation, *Biotechnology & Bioeng*ineering, 10: 845.
- [26] Jagannathan Krishnan, Anthony Arvind Kishore, Athreya Suresh, B. Madhumeetha, D. GnanaPrakash (2017), Effect of pH, inoculum dose and initial dye concentration on the removal of azo dye mixture under aerobic conditions, *International Biodeterioration and Biodegradation*, 117:16-27.
- [27] M. S. M. Annuar, I. K. P. Tan, Ibrahim and K. B. Ramachandran (2008), A kinetic model for growth and biosynthesis of medium-chain-length poly- (3-hydroxyalkanoates) in pseudomonas putida, *Brazilian Journal of Chemical Engineering*, 25(2): 217 – 228.
- [28] Moser, A. H.J., Reed, (1985), Kinetics of batch fermentations, in Rehm, Biotechnology Fundamentals of Biochemical Engineering, *Verlaag Chemie*, Weinheim, 243-283.
- [29] Carrera, Julián (2014), Kinetic models for nitrification inhibition by ammonium and nitrite in a suspended and an immobilized biomass systems, *Process Biochemistry*, 39(9): 1159-1165.
- [30] Agarry, S. E., and B. O. Solomon (2008), Kinetics of batch microbial degradation of phenols by indigenous Pseudomonas fluorescence, *International Journal of Environmental Science & Technology*, 5(2): 223-232.
- [31] Saravanan, Pichiah, K. Pakshirajan, and Prabirkumar Saha (2008), Growth kinetics of an indigenous mixed microbial consortium during phenol degradation in a batch reactor, *Bioresource Technology*, 99(1): 205-209.
- [32] Dey, Sudipta, and Somnath Mukherjee (2010), Performance and kinetic evaluation of phenol biodegradation by mixed microbial culture in a batch reactor, *International Journal of Water Resources and Environmental Engineering*, 3(2): 40-49.
- [33] Pramparo, Laura (2012), Kinetics of aerobic biodegradation of dihydroxybenzenes by a p-nitrophenoldegrading activated sludge, *Bioresource technology*, 110: 57-62.
- [34] Tazdait, Djaber (2014), Comparison of different models of substrate inhibition in aerobic batch biodegradation of malathion, *Turkish Journal of Engineering and Environmental Sciences*, 37(3): 221-230.
- [35] Tziotzios, G., S. Michailakis, and D. V. Vayenas (2007), Aerobic biological treatment of olive mill wastewater by olive pulp bacteria, *International Biodeterioration & Biodegradation*, 60(4): 209-214.
- [36] Hao, Oliver J. (2002), Kinetics of phenol and chlorophenol utilization by Acinetobacter species, *Chemosphere*, 46(6): 797-807.
- [37] Han, Keehyun, and Octave Levenspiel (1988), Extended Monod kinetics for substrate, product, and cell inhibition, *Biotechnology & Bioengineering*, 32(4): 430-447.