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Batch Kinetics and Effects of Process Parameters for Biodegradation of Reactive Black 5 in an Aerobic Mixed Microbial Culture

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ABSTRACT

Mixed microbial culture used in this study was developed from sludge that was taken from local textile wastewater treatment tank. Acclimatization process was performed before starting the biodegradation experiment to obtain a microbial culture with high degradation properties. Kinetic studies by the mixed microbial culture were determined quantitatively for the model pollutant, Reactive Black 5 (RB 5). By using Michaelis-Menten model, the constants were found to be 11.15 mg l^{-1} h^{-1} and 29.18 mg l^{-1} for V_m and K_m respectively. The values of kinetic constants for Monod model were found to be 33.11 mg l^{-1} cell h^{-1} for the maximum specific microbial growth rate, μ_m and 86.62 mg l^{-1} for Monod constant, K_s. The effects of process parameters such as pH, inoculum size and initial dye concentration on the biodegradation of azo dye, RB 5 were systematically investigated. Maximum removal efficiencies observed in this study were 75% for pH 6, 100% for 15% inoculum concentration and 75% for 20 ppm of initial dye concentration.

Keywords: *Kinetic study, Mixed microbial culture, Reactive Black 5, Biodegradation.*

INTRODUCTION

Dyes in wastewater are one of the main sources of pollutants. It is estimated that about 10% to 15% of unused dyestuff is discharged into the wastewater directly [1]. Most dyes are designed to withstand extreme conditions and are stable against photodegradation, biodegradation, and oxidizing agents [2]. Synthetic dyes are able to resist fading upon exposure to oxidizing agents, sweat, water, light, chemicals, and microbial attack [3]. There are three most common groups of dyes namely, azo dyes, anthraquinone dyes and phthalein dyes [4]. These dyes are toxicant and carcinogenic [5]. These dyes can cause serious damage to the environment since they may directly affect the photosynthetic activity that reduce the penetration of light [6] and toxicant towards certain aquatic life due to their hydrolysis products.

Synthetic dyes contribute to about 70% of the textile dyestuffs produced. According to Pallavi *et al.* (2010), textile dyes are highly reactive and it is difficult to treat [7]. Wastewater containing dyes are one of the most problematic effluents to treat due to their high biological and chemical oxygen demand, suspended solids, toxic compounds and aesthetic issues by their colour and odour [8]. The most commonly used azo dyes are usually stable, hard to be degraded, resistant to the microbial, physical and chemical attack, and therefore, difficult to eliminate.

Effluents containing dyes can be treated by using numerous methods such as physical, chemical and biological treatment processes [9]. Each of these methods has their own advantages and disadvantages. But most of the biological treatment methods are environmental friendly, require low investment, produce less sludge with non-toxic end products and require less water consumption compared to that of other physio-chemical methods [3, 10].

The decolourization and degradation of azo dyes through biological methods can be achieved by using fungi, yeast, plants (phytoremediation) and bacteria. Many microorganisms that belong to the different taxonomic groups of fungi, bacteria, actinomycetes and algae have been proven for their ability to degrade azo dyes [11]. Pure algae cultures have been developed, but the performance of decolourization is limited due to their long growth cycle and their scanty properties. On the other hand, it is known that bacteria

are able to decolourize and degrade many reactive dyes completely and mineralize under certain conditions [11- 14].

The use of mixed cultures became a great potential for large-scale application in industrial wastewater treatment due to their synergistic metabolic activities and their ability to degrade azo dyes with complete mineralization. Based on a research made by Xu (2011), mixed cultures were able to degrade azo dyes completely within 32 hours in a continuous shaking condition [15]. In another study, Kumar *et al.* 2009 [16] achieved 98% decolourization for reactive azo dyes in 18 hours time. Whereas single cultures require long time for adaptation procedures and also for isolation from dye-containing wastewater samples. According to Kumar *et al.* (2009), for single cultures, it is not easy to maintain aseptic conditions and to fully degrade all different dyes present in the real effluents. Thus, it is not practically suitable for commercial application [16]. Many researchers have reported that high biodegradation can be achieved from mixed culture due to co-metabolic activities within the microorganism when different species with different capabilities are mixed together [16-19]. Single bacterial strains may produce intermediates that are carcinogenic such as aromatic amines and hence require further treatment systems. However, in mixed cultures, aromatic amines are degraded in an efficient way which is a distinct beneficial feature in using mixed microbial culture in the wastewater treatment.

RB 5 was used in this study as a model pollutant due to its predominant use among synthetic dyes, representing almost 70% of the textile dyestuffs produced. The chemical structure of RB 5 is shown in Figure 1. This challenge initiated the investigation using mixed microbial culture instead of pure cultures. The effect of pH, initial concentration of dyes and the concentration of inoculums on the biodegradation of RB 5 were carried out. The kinetic parameters related to Michaelis-Menten and Monod models were also evaluated for the biodegradation of RB 5.

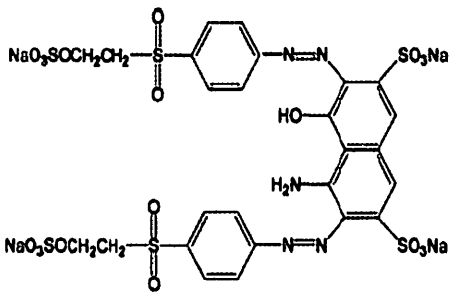

Figure 1: Chemical Structure of RB 5

MATERIALS AND METHODS

Preparation of Dye Samples

A stock solution of 100 ppm was initially prepared in a standard flask by dissolving the required amount of RB 5 dye (Saujanya Dye Chem, India) in distilled water. The solutions with desired concentrations for the various experiments were obtained by several successive dilutions.

Inoculum Source and Acclimatization

Dye-resistant microorganisms were collected from the sludge sample found in the wastewater storage tank from MasterWan Batik, Kajang. The sludge enriched with mixed culture was grown aerobically in a medium containing 1% (w/v) glucose (Jaya Glucose (M) Sdn. Bhd) as the carbon and energy source with RB 5. This technique is used for the enrichment of culture in heterogeneous population.

The amount of glucose was regularly checked and maintained at 1% during the acclimatization process which was for about 45 days. The composition of the nutrient medium (Becton Dickinson and Company, USA) used for degradation studies was: Peptone, 5 g l^{-1}; sodium chloride, 5 g l^{-1}; yeast extract, 2 g l^{-1} and beef extract, 1 g l^{-1}. The concentrations of dyes were gradually increased periodically upon observing the decolourization of dyes during the acclimatization period of microbial culture [20]. Successive transfers of culture into fresh glucose medium containing higher concentrations of the dyes up to 100 ppm were done at room temperature.

After 45 days of acclimatization process, microorganisms with high-degradation property were obtained and had working limitations of a maximum of 100 ppm initial concentration of RB 5 and at room temperature.

Kinetic Studies

The kinetic constants of mixed microbial culture were determined by Monod and Michaelis-Menten equations through equations (1) and (2) respectively, as given below.

$$\mu = \frac{\mu_m S}{K_s + S} \qquad \mu = \frac{\mu_m S}{K_s + S} \tag{1}$$

$$v = \frac{V_m S}{K_m + S} \qquad v = \frac{V_m S}{K_m + S} \tag{2}$$

Where μ_m, μ and K_s are the maximum specific growth of micro organism (h^{-1}), the specific growth rate (h^{-1}) and the saturation constant (mg l^{-1}) respectively, for the Monod equation. While v is the observed velocity of the reaction at a given substrate concentration (mg $l^{-1} h^{-1}$), V_m is the maximum velocity at a saturating concentration of substrate (mg $l^{-1} h^{-1}$) and K_m is the Michaelis constant (mg l^{-1}) for Michaelis-Menten equation. S denotes the substrate concentration (mg l^{-1}) for both the equations. These kinetic models can be linearized using double-reciprocal form as follows.

$$\frac{1}{\mu} = \frac{K_s}{\mu_m S} + \frac{1}{\mu_m} \tag{3}$$

$$\frac{1}{v} = \frac{K_m}{V_m S} + \frac{1}{V_m} \tag{4}$$

From the equation (3), K_s and μ_m values can be determined by plotting $1/\mu$ versus $1/S$ From the plot, linear line was obtained with a slope of K_s/μ_m and $1/\mu_m$ as the intercept.

Similarly from equation (4), K_m and V_m values can be determined by plotting $1/V$ versus $1/S$ From the plot, linear line was obtained with a slope of K_m/V_m and $1/V_m$ as an intercept.

To carry out kinetic study, a 20 ppm of dye solution was added into a 250 ml Erlenmeyer flask containing the 10% acclimatized inoculum. A total working volume of 200 ml was maintained by mixing 180 ml of media, 10 ml of dye solution and 10 ml of acclimatized inoculum. After adjusting the pH to 7 using hydrochloric acid and sodium hydroxide, the flask was covered with a cotton plug and kept in the incubator shaker set at 180 rpm and 30°C. For every 4 hours, aliquots of about 2 ml samples were withdrawn and centrifuged at 5,000 rpm for 10 minutes. The supernatant was taken out to measure the absorbance and the sediment was dried for several hours to get its dry weight to study the growth of the bacteria and its enzymatic reactions towards the whole process in their optimum conditions.

Effect of Different Parameters on Removal of Dye

250 ml Erlenmeyer flasks were used to carry out this experiment in batches. The acclimatized mixed microbial culture was used as an inoculum where nutrient media and dye were added to the flasks under the constant operating concentrations. Effects of various parameters such as pH (5.0, 6.0, 7.0 and 9.0), inoculum concentration (5%, 10%, 15% and 20%) and initial dye concentration (20 ppm, 50 ppm, 75 ppm and 100 ppm), on the removal of RB 5 were investigated. The samples were drawn at regular intervals of time and were analyzed by UV-Vis spectrophotometer for absorbance calculations.

Analytical Methods

The samples were centrifuged at 5,000 rpm for 10 minutes to precipitate suspended biomass. The concentration of RB 5 in the supernatant was determined by measuring the absorbance at 597 nm. Removal efficiency was calculated according to the formulation below:

$$\text{Removal efficiency (\%)} = (C_i - C_r)/C_i \times 100\% \quad (5)$$

where C_i is the initial concentration and C_r refers to the residual concentration of dye samples.

RESULTS AND DISCUSSION

Microbial Growth Curve

RB 5 was utilized as the substrate intake for the microbes as their nutritional values. The observed growth profile for the mixed microbial consortia is shown in Figure 2. The result showed that the microbial culture did not undergo the lag phase as they had been acclimatized prior to the biodegradation experiment. This result revealed that acclimatized period had contributed to the deletion of a poor growth cycle of bacteria, lag phase thereby favouring very efficient removal activity quickly.

Log phase or exponential growth phase is very important in a biological process. During this phase, the microbes are growing and dividing rapidly as their metabolic activity increases throughout time. From 0 to 11 hours, the bacterial consortia were found to be in log phase where they begin to multiply and reach their maximum growth rate during a specific time period. From 11 to 18 hours, stationary phase was observed. The dry cell weight did not increase as the number of cell remains constant throughout time. The reproduction of the mixed bacterial culture was slowed down from about 18 to 32 hours of incubation and there was a steep decrease in bacterial growth during which time they undergo death phase. This might be due to nutrients depletion (dye) with an accumulation of metabolic waste products. The microbes were unable to reproduce and the number of dead cells exceeded the number of live cells. Only some organisms were able to resist this condition and stay alive by producing endospores. Thus, it can be seen that RB 5 obviously influences the mixed microbial growth for their sustainable of life.

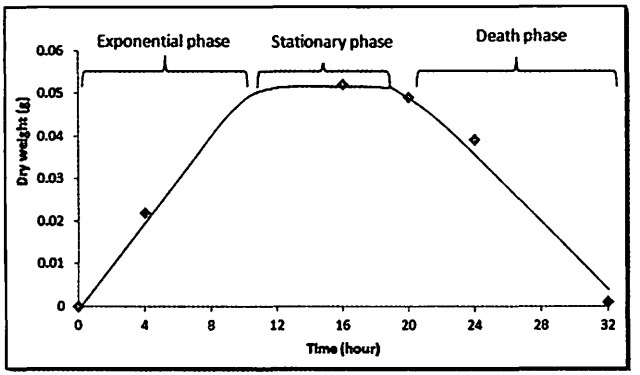

Figure 2: Growth Profile for the Mixed Bacterial Culture

Kinetic Studies on the Biodegradation of Reactive Black 5

In order to evaluate the kinetic constants by Monod model, batch biodegradation experiments were carried out at the process conditions of pH 7, 10% (w/v) inoculum concentration, 20 ppm dye concentration, 30^{0}C and 180 rpm. The increase in microbial growth rate and decrease in substrate concentration (dye) were observed. These could be due to the growing mechanisms of bacteria by consuming dyes as their carbon source that has been acclimatized before. From the result obtained, data were fitted for Monod's model through Figures 3 and 4 and the kinetic constants estimated were given in Table1.

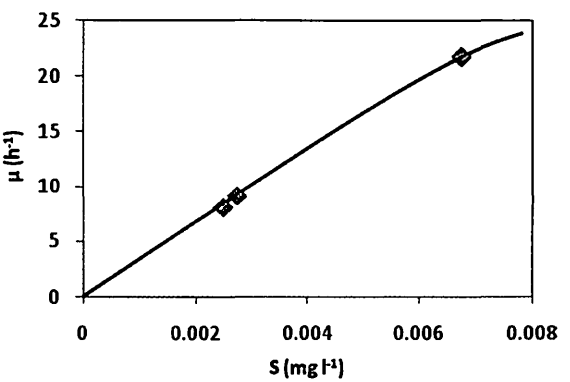

Figure 3: Specific Growth Rate VS Substrate Concentration for Monod Model

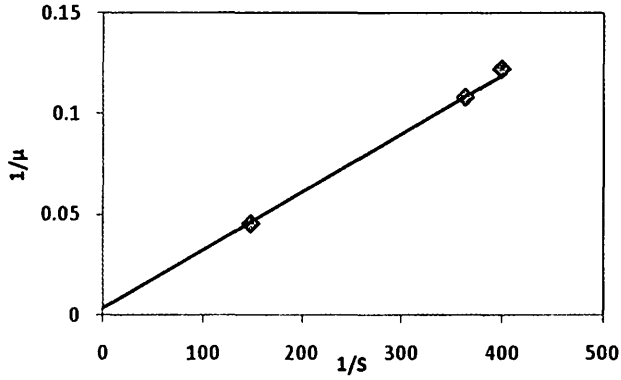

Figure 4: Line Weaver Burk Plot for the Monod Model

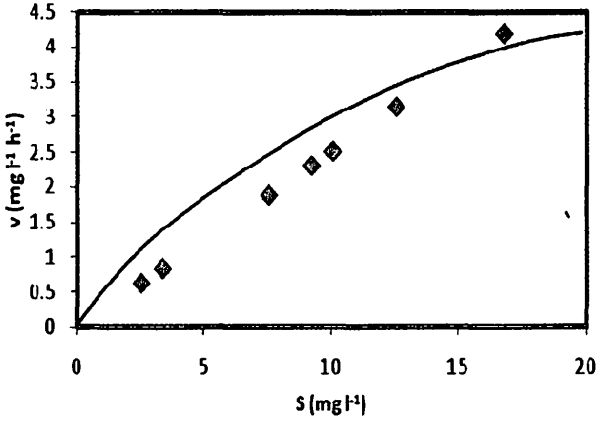

Figure 5: Velocity of the Reaction Vs Specific Dye Uptake for Michaelis-Menten Model

Telke *et al.* (2008) reported that the kinetic constants obtained from their experimental data were 1.26 h^{-1} and 50 mg l^{-1} for μ_m and K_s respectively by a bacterium identified as *Rhizobium radiobacter* MTCC 8161 [21]. Therefore, it is important to note that mixed bacterial culture can yield higher rate of reaction compared to using pure bacterial culture. This is due to the fact that mixed bacterial culture can achieve a higher degree of biodegradation and mineralization due to their synergistic metabolic activities of the microbial community [9].

For the kinetic study by Michaelis-Menten model, the concentration of dyes, inoculum concentration, pH, temperature and the agitation speed were kept constant throughout the experiment (20 ppm, 10% (w/v), pH 7, 30°C and 180 rpm). Figure 5 shows the observed velocity of the reaction and the specific dye uptake attained by the microbial cultures for Michaelis-Menten model. Double reciprocal plot was constructed as shown in Figure 6 and the kinetic constants evaluated are shown in Table 1.

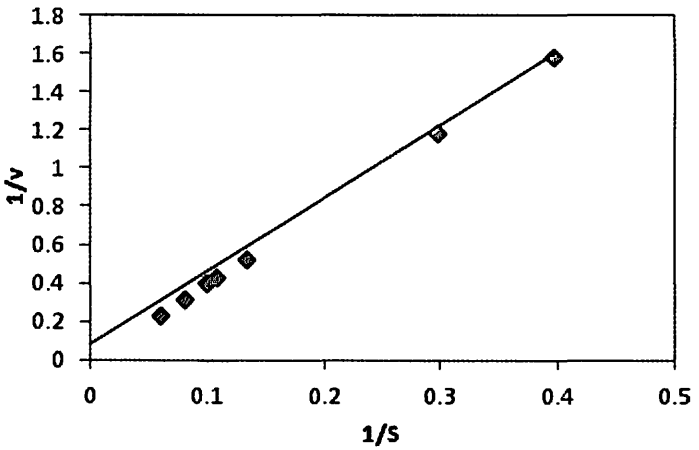

Figure 6: Double Reciprocal Plot for for Michaelis-Menten Model

Parin and Rao (2011) reported that the values of V_m and K_m obtained during degradation of Reactive Azo dye using novel bacteria strain, *Alcaligenes faecalis* were 27.1 mg l^{-1} h^{-1} and 105.0 mg l^{-1} respectively [22]. Similar results were reported by Chen *et al.* (2011) during batch decolorization of RB 5 by using bacterial strain named *Enterobacter* sp. [23] and they stated that enzyme that has a low value of K_m would reach its maximum catalytic efficiency where the affinity of the enzyme to the substrate was stronger.

Relatively a lower value of K_m obtained in this study therefore shows that the mixed microbial culture had reached their maximum catalytic efficiency, thus enhancing the biodegradation of RB 5. From these it is evident that the use of mixed microbial culture has a significant effect on the enzyme-substrate complex in the biodegradation of RB 5.

Table 1: Kinetic Constant for the Biodegradation of RB 5

(a) Monod model	
μ_m	33.11 h^{-1}
K_s	86.62 mg l^{-1}
(b) Michaelis-Menten model	
V_m	11.15 mg l^{-1} h^{-1}
K_m	29.18 mg l^{-1}

Effect of pH on Removal of RB 5

The effect of pH on the dye removal of RB 5 was carried out by varying pH from 5 to 8 and maintaining other conditions constant at 20 ppm dye concentration and 10% inoculum concentrations. From Figure 7, it was observed that the best removal was achieved at pH 6.0 with 76% removal efficiency. With a further increase in pH, the dye removal activity of mixed bacterial culture was found decreased. This is due to the effect of pH on dye transport molecules across the cell membrane that is considered as the rate-limiting step. This finding is in accordance with Kumar *et al.*, (2009) where the optimal pH for dye removal was at pH 6.0 for most of the dyes [16]. The effect on different pH is significant because reactive azo dyes bound to cotton fibers under alkaline conditions by substitution or addition mechanisms.

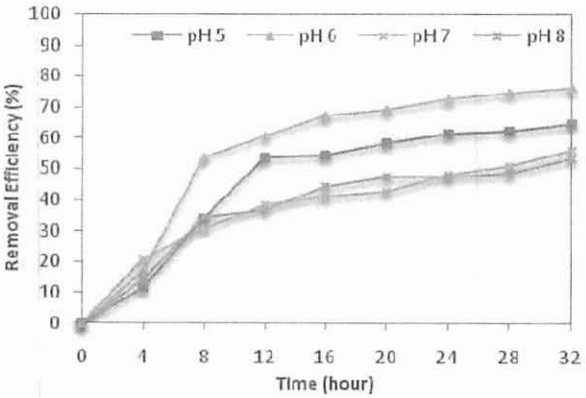

Figure 7: Effect of pH on Removal of RB 5

Effect of Inoculum Size on Removal of RB 5

The effect of inoculum concentration ranging from 5% to 20% (w/v) was studied on the removal of RB 5 with an initial dye concentration of 20 ppm and at pH 6. There was an increase in removal of RB 5 with increase in the inoculum concentration. The maximum removal activity was recorded at 10% inoculum concentration with 100% percentage removal within 32-hour incubation. From Figure 8, it is clear that percentage removal of dye increased with increase in time for all concentrations of inoculum. After 32 hours, the percentage removal of RB 5 was recorded to be 79, 100, 89 and 85% at inoculum concentration of 5, 10, 15 and 20%, respectively. Kumar *et al.* (2009) also reported the similar observations [16]. Another study made by Telke *et al.* (2008) on reactive red using Rhizobium radiobactor could yield maximum of 90% decolourization with a 20% inoculum concentration which is comparable with the present study [21]. However, excess inoculum concentration might inhibit the bacterial growth as their survival competitiveness might have increased with the increase in the inoculum concentrations.

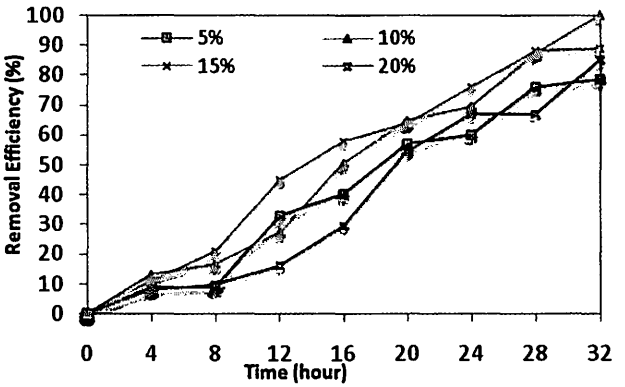

Figure 8: Effect of Inoculum Size on Removal of RB 5

Effect of Initial Dye Concentration on Removal of RB 5

The effect of initial dye concentrations on the removal of RB 5 was studied by varying the dye concentrations from 20 to 100 ppm and maintaining other conditions of pH at 6 and inoculum concentrations of 10%. The removal efficiency was observed in the increasing trend as shown in Figure 9. Higher the initial concentration, lower was the

removal efficiency during the experimentation as a general observation, but however, complete removal was observed for all concentrations at 32 hours. Furthermore, 20 ppm indicated the highest removal rate compared to other dye concentrations.

However, this mixed culture was able to fully utilize the dye with 100% removal efficiency within the incubation time. The result indicated that mixed bacterial culture under aerobic condition had high removal capabilities at relatively higher initial dye concentrations.

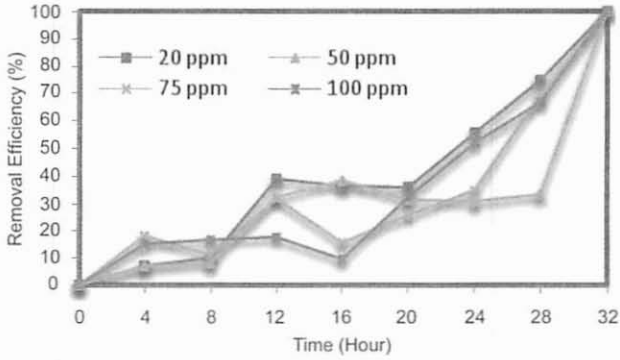

Figure 9: Effect of Initial Dye Concentration on Removal of RB 5

CONCLUSIONS

A mixed bacterial culture, isolated from sludge that was taken from local textile wastewater treatment tank showed promising results in the removal of RB 5 in aerobic batch biodegradation experiments. Within 32 hours, the bacterial consortia managed to remove about 76-100% removal of dyes. The maximum values of removal efficiency were found to be 76% removal for pH 6, 100% removal for 15% inoculum concentration and 75% for 20 ppm of initial dye concentration. However, at 32 hours, a complete removal of dyes was observed irrespective of the initial dye concentrations. The batch biodegradation kinetics was also evaluated for the removal of RB 5 at the optimum conditions using Monod and Michaelis-Menten models. Using Michaelis-Menten model the values were found to be as 11.15 mg l^{-1} h^{-1} and 29.18 mg l^{-1} for V_m and K_m respectively, whereas the value of kinetic constants for Monod model was found to be 33.11 h^{-1} for the maximum

specific microbial growth rate, μ_m and 86.62 mg l^{-1} for Monod constant, K_s. Thus, the results obtained support that the mixed microbial consortia developed in this study have a potential application in treating the effluents from textile industries containing reactive azo dyes at a lower cost.

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