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OPTIMIZATION OF REMAZOL RED DYE REMOVAL PERFORMANCE USING AEROBIC GRANULAR SLUDGE IN A SEQUENCING BATCH REACTOR

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Abstract

Textile wastewater pollution, mainly containing azo dyes such as Remazol Red, presents an environmental challenge in developing countries, including Indonesia. Although various wastewater treatment methods have been extensively studied, biological treatment efficiency at high dye concentrations remains challenging. In this context, aerobic granular sludge (AGS) technology in sequencing batch reactors (SBR) offers a potential solution. However, the existing knowledge gap lies in optimizing operating conditions for optimal dye degradation. This study demonstrates the use of response surface methodology (RSM) with a Box-Behnken design (BBD) to model the effects of independent variables such as aeration time, dye concentration, and COD on decolorization efficiency. Experimental results show that increasing aeration time and COD concentration significantly improve dye degradation, with an optimal decolorization value of 77% achieved at a COD concentration of 1000 mg/L and an aeration time of 24 hours. These findings imply that AGS-SBR technology can be further optimized for effective textile wastewater treatment on various industrial scales.

Keywords: Aerobic granular sludge; Decolorization; Remazol red; Sequencing batch reactor; Textile wastewater

Abstrak

Pencemaran air limbah industri tekstil, khususnya yang mengandung pewarna azo seperti Remazol Red, merupakan tantangan lingkungan di negara berkembang, termasuk Indonesia. Meskipun berbagai metode pengolahan air limbah telah banyak diteliti, efisiensi pengolahan secara biologis pada konsentrasi pewarna tinggi masih menjadi masalah. Dalam konteks ini, teknologi aerobic granular sludge *(AGS) dalam sequencing batch reactor (SBR) menawarkan solusi yang potensial. Namun, kesenjangan pengetahuan yang ada terletak pada optimasi kondisi operasi untuk mencapai degradasi pewarna yang maksimal. Penelitian ini menunjukkan penggunaan* response surface methodology *(RSM) dengan desain Box-Behnken (BBD) untuk memodelkan pengaruh variabel independen seperti waktu aerasi, konsentrasi pewarna, dan COD terhadap efisiensi dekolorisasi. Hasil eksperimen menunjukkan bahwa peningkatan waktu aerasi dan konsentrasi COD menghasilkan peningkatan signifikan dalam degradasi pewarna, dengan nilai optimal dekolorisasi 77% dicapai pada konsentrasi COD 1000 mg/L dan waktu aerasi 24 jam. Penemuan ini mengimplikasikan bahwa teknologi AGS-SBR dapat dioptimalkan lebih lanjut untuk pengolahan air limbah tekstil yang efektif di berbagai skala industri.*

***Kata Kunci**:* Aerobic granular sludge*; Dekolorisasi; Limbah tekstil; Remazol merah;* Sequencing batch reactor

1. INTRODUCTION

Textile wastewater is one of the primary sources of industrial water pollution in developing countries and often has high concentrations (around 20%) of dyes (Chang et al., 2009). In dyeing industries such as textiles, leather, paper, plastics, cosmetics, and others, dyes are used in their processes, yielding a substantial quantity of color wastewater (Roy & Saha, 2020). Industry is an important part of every country's economy, but faces significant environmental challenges. The World Health Organization (WHO) states that processing dye waste in several industries contributes 17-20 % of water pollution (Benkhaya et al., 2020a). The issue of textile waste is also relevant in Indonesia, as highlighted by the United Nations Educational, Scientific and Cultural Organization (UNESCO) (Birgani et al., 2016). The increasing popularity of Indonesian batik has led to significant growth in the number and diversity of batik industries (Rashidi et al., 2012). This has a detrimental impact on the environment, as many rivers surrounding batik factories are contaminated due to the improper disposal of batik industrial waste without proper treatment (Sharfan et al., 2018).

Based on data from the Indonesian Ministry of Trade, the average liquid waste produced by a micro, small, and medium-sized enterprise (MSME) engaged in batik production reaches 2,500 litres daily, with a COD concentration of 13,800 mg/L (Saksono et al., 2017). The waste disposed of has complex organic compounds in the form of dyes and textile dyes. The complexity and stability of these structures pose challenges in their degradation, making these components difficult to break down and inherently toxic (Buthelezi et al., 2012). Of the 10,000 available colors, azo textile dyes are the most commonly used in the batik industry, accounting for 60–70% (Benkhaya et al., 2020b).

Remazol red is a dye that is classified as a reactive dye (Costa & Paranhos, 2019). Chemicals with intricate and stable structures of aromatic and heteroatomic rings present challenges in degradation, making these components resistant to breakdown and inherently toxic (Szatylowicz et al., 2016). Due to its aromatic anthraquinone structure, which is highly stable through resonance, Remazol Red is resistant to chemical oxidation, non-biodegradable, and difficult to decolorize by ozonation (Chang et al., 2009). This waste contains color with varying degrees of COD 150-12,000 mg/l (Yaseen & Scholz, 2019). Simple biological and chemical treatments are inadequate for removing color and reducing organic matter (Kusumawati et al., 2020). Therefore, treatment is needed to remove constituents before discharge to process water. Biological degradation is the most cost-effective choice for organic-based wastewater if the contaminants are biodegradable (Show et al., 2020).

The water must not have dyes or toxic contaminants before being released into the environment (Syafiuddin et al., 2020). Therefore, textile industry waste must be processed effectively to absorb pollutants efficiently. Employed a series of chemical coagulation-flocculation and bio-oxidation procedures to treat mixed wastewater sourced from several stages of the textile industry in India, including spinning, sizing, scouring, kiering, bleaching, dyeing, and printing (Manekar et al., 2014). This combination yielded encouraging outcomes for detoxifying textile and utilized a pond system with duckweed to eliminate color from natural industrial wastewater in Turkey (Yaseen & Scholz, 2019).

In the last few decades, many academics have been interested in aerobic granular sludge (AGS) technology as a biological wastewater treatment (Truong et al., 2018). Aerobic granules sludge (AGS) is cultivated in sequencing batch reactors (SBR) (Geng et al., 2020). In comparison to conventional activated sludge and membrane technologies, the AGS-SBR approach has enhanced settling characteristics, increased resilience to toxic substances, and effective concurrent elimination of colors and organic contaminants, rendering it a more efficient option for dye wastewater treatment (Lawal et al., 2023). The principle of fill and discharge governs the function of SBR, which is divided into five stages: fill, idle, aeration, settle, and discharge (Sengupta, 2013). Their distinctive characteristics, including elevated settling velocities, biomass retention, significant activity, and resistance to high loading rates, have been extensively documented in the literature (Bisheh et al., 2021). From a technical and economic perspective, aerobic granulated mud is a promising method with the potential to lead the next generation in biological wastewater treatment technologies (Nancharaiah et al., 2019). The novelty of this study lies in investigating the effect of aeration time on biomass concentration, as well as dye and COD removal. Response surface methodology (RSM) may be the optimal selection for modelling and enhancing the wastewater treatment process in the SBR. Box–Behnken (BBD) model, as an optimization tool of RSM, helps build a quadratic model for the response variable (Bisheh et al., 2021). Integrating RSM with the BBD effectively minimises the necessity for redundant experimentation, particularly in experimental design; conversely, it assesses the interplay of elements influencing treatment efficacy and provides optimal conditions.

2. MATERIALS AND METHODS

2.1 Substrate and Inoculum

Inoculum refers to a population of cells or microbes introduced into a biological process medium, requiring optimization for improved performance (Sood et al., 2019). The activation form of the wastewater treatment plant (IPAL) phase II PTPN VII Way Berulu, South Lampung, will be used as an inoculum in this study.

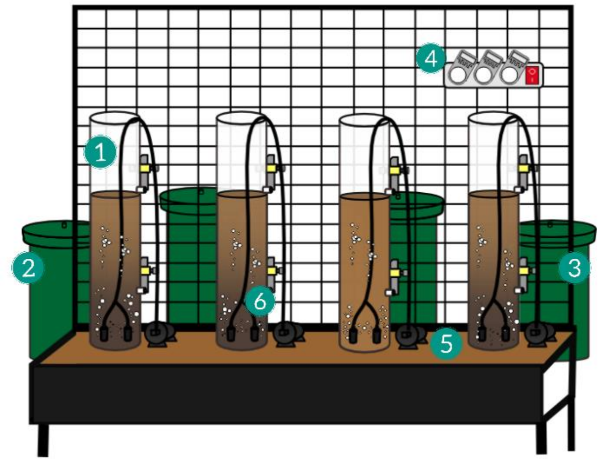
Table 1. Composition of artificial waste

Artificial Waste Content	Concentration of Organic Content
Na-acetate	1000 mg-COD/L
NH_4Cl	100 mg-N/L
$FeSO_4.7H_2O$	5 mg-Fe/L
KH_2PO_4	10 mg-P/L
$CaCl_2$	30 mg-Ca/L
$NaHCO_3$	300 mg-HCO_3/L

Table 1 shows the composition of synthetic wastewater fed to each reactor. This synthetic waste will be supplied to the reactor during the first four weeks of granular growth. Suppose the granules have started growing large enough to be observed macroscopically. In that case, they will be examined with the sludge settling volume (SSV) to determine whether the levels have persisted until they become stable.

2.2 Equipment

The equipment used in this research is shown in a front view (Figure 1). There are several main tools and indicator tools in each AGS-SBR. The buffered AGS-SBR reactor has dimensions of 120 cm in height and 10 cm in inner diameter. Apart from that, this series of tools is also equipped with 4 influent pump units and 4 effluent pump units with a power of 45 W, as well as 4 aerator units (2 units for each reactor) with a power of 45 W, a hose for sludge sampling, and a hose for influent flow.



Tool description:
1) Reactor tank, 2) Influent tank, 3) Effluent tank, (4) Timer, 5) pump, and 6) Aerator pump

Figure 1. Apparatus of a sequencing batch reactor

2.3 Aerobic Granulation Performance Test Against Remazol Red

The efficacy of biological wastewater treatment is contingent upon various operational and environmental parameters, including biomass content (Xs), temperature (Fernandes et al., 2013), pH (Aziz et al., 2011), aeration time (Alimohammadi et al., 2016), and COD influent (Fernandes et al., 2013). This study examined the biological treatment of municipal wastewater, which was influenced by three variables impacting the removal effectiveness of SBR and the SVI: COD influent, remazol red dye concentration, and aeration duration (t). Table 2 presents the experimental matrix utilized in this work.

Table 2. Conditions of the experimental matrix and the corresponding results

Run	Variable		
	Factor 1 Dye_{inf} (mg/L)	Factor 2 Aeration time (h)	Factor 3 COD_{inf} mg/L
1	30	6	750
2	20	6	1,000
3	20	6	500
4	10	15	500
5	10	6	750
6	10	15	1,000
7	30	24	750
8	30	15	1,000
9	10	24	750
10	20	24	500
11	30	15	500
12	20	15	750
13	20	24	1,000

2.4 Analysis Method

The analyzed parameters included soluble chemical oxygen demand (sCOD), granule diameter, sludge settling volume (SSV), sludge volume index (SVI), mixed liquor suspended solids (MLSS), and mixed liquor volatile suspended solids (MLVSS). pH was measured using a Lutron pH-208 meter calibrated with a pH 7.0 buffer solution. sCOD analyses were using the APHA 5200D method. SVI was regularly analyzed following the 2710d sludge volume index (SVI) method (2017). SSV, SVI, MLSS, and MLVSS were analyzed using the APHA 2540 method. Granule structure was observed using an image analysis system (Image Raster software and Optilab Viewer 2) with a Euromex 6803 ED microscope and OptiLab Advance digital camera. Granule size was measured using Image Raster software in the Petrology Lab at the Sumatra Institute of Technology. Color analyses of raw and treated dye wastewater were performed using spectrophotometry with a UV-visible spectrometer (Jenway 6405 UV/Vis).

After the studies, the quadratic polynomial equation (Eq. 1) was employed to forecast the response based on the independent variables, encompassing quadratic interactions and terms. The independent variables were encoded by Equation 2.

$$y = \beta_0 + \sum_{j=1}^{3} \beta_{ij}\, \beta_{jj} x_j^2 + \sum_{j<1}^{3} \sum_{j}^{3} \beta_{ij}\, X_i X_j + \cdots + e \quad (1)$$

$$X_i = \frac{X_i - X_0}{\Delta x_i}\, i = 1,2,3 \ldots\ldots, K \quad (2)$$

The regression equation coefficients were computed with Design Expert® V10 software by Stat-Ease. Multiple regression analysis, a response surface methodology (RSM) component, forecasted the model coefficients. The coefficient of determination (R^2) indicated the quality of the polynomial model fit, while statistical significance was assessed using Fisher's F-test. Model terms were accepted or dismissed depending on the P-value at a 95% confidence level. Results were evaluated with ANOVA in Design Expert software. Three-dimensional and contour plots were generated to examine the interaction effects among the three factors on two responses.

3. RESULTS AND DISCUSSION

3.1 AGS Growth Stages

Granule growth is a crucial parameter for assessing maturity, characterized by an increase in diameter. Moreover, granule growth must align with the sludge volume index (SVI) to accurately assess the quantity of granules within the reactor. The stages of granule growth are illustrated in Table 3.

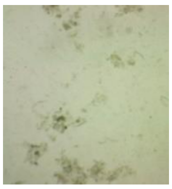
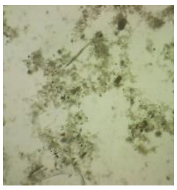
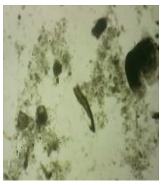
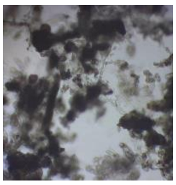
Based on microscopic observations at 100× magnification, as shown in Table 3, no granules were detected during the initial phase as bacteria were still adapting and responding to the nutrients provided. By day 5, although granule formation had not yet occurred, bacterial growth became more apparent. On day 12, filaments emerged, indicating increased bacterial growth and the early stage of granule formation. Granules began to appear on day 20. However, on day 28, a problem arose in the experiment; the aerator holes were too large, causing the production of larger air bubbles, which led to a decrease in granule diameter. In this study, the largest granules reached sizes between 251-1,000 µm at the end of the start-up phase, exceeding the minimum required size of 212-425 µm (Liu & Tay, 2015; Putri et al., 2019). This was achieved, as evidenced by the granule diameter distribution data shown in Figure 2a. Granules were first observed forming on day seven, with a maximum size of 1 mm, and a significant increase in size occurred on day 35. The granule growth process resulted from mass transfer through the diffusion of Ca^{2+} and Mg^{2+} metal ions from the cultivation medium, which in this case was synthetic wastewater, into the granules (Xu et al., 2021).

Based on the MLSS and MLVSS measurements during the start-up phase of the AGS-SBR system, as shown in Figure 2b, aerobic granules experienced significant fluctuations from the beginning of operation until day 20. This fluctuation is likely due to operational changes or microbial responses to new environmental conditions. After this fluctuation period, a steadily increasing trend in MLSS and MLVSS values was observed from day 35 to day 50. The observed increase and subsequent stabilization of MLSS and MLVSS values align with previous studies, which indicate that the granule maturation process is directly linked to a significant increase in biomass concentration, thereby potentially optimizing reactor performance (Mady et al., 2024). Granules in the AGS-SBR system can be considered stable when SVI values are consistent, as shown in Figure 2c. This stability reflects effective microbial adaptation to the reactor environment, where the granules maintain optimal density and settling performance. The declining trend in SVI as the granules mature mirrors findings from previous studies, where a gradual decrease in SVI values was observed as the aerobic granules reached a more stable and efficient state.

3.2 Decolorization Performance

The duration of aeration significantly affects the decolorization performance (Sathian et al., 2014). Figure 3 illustrates the performance of the AGS-SBR system in removing dye concentrations across varying aeration times and dye concentrations. As the aeration process continues, the dye removal efficiency increases steadily. By the 4-hour mark, the removal percentages have risen to between 47.28 and 61.27%. This upward trend continues through 6 and 8 hours of aeration,

Table 3. Granule Growth

Operational Day					
Initial day	**5th day**	**12th day**	**20th day**	**28th day (middle)**	**56th day (Last day)**
					

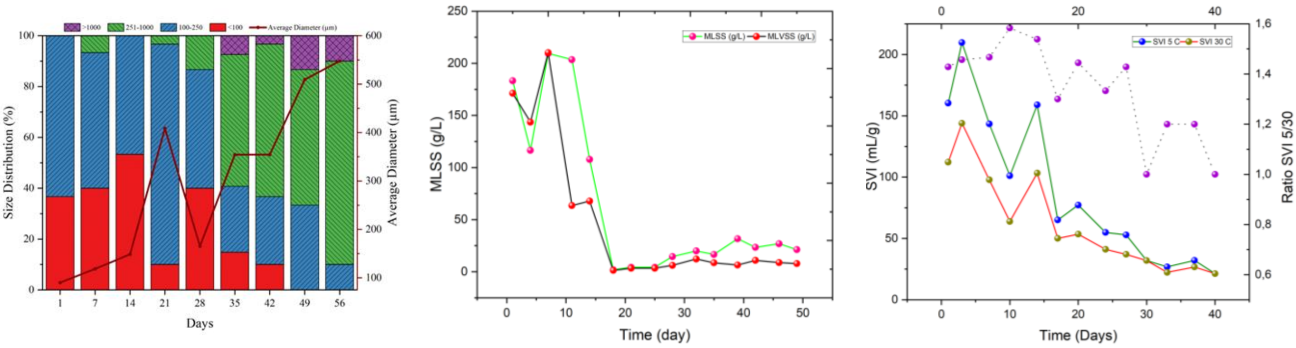
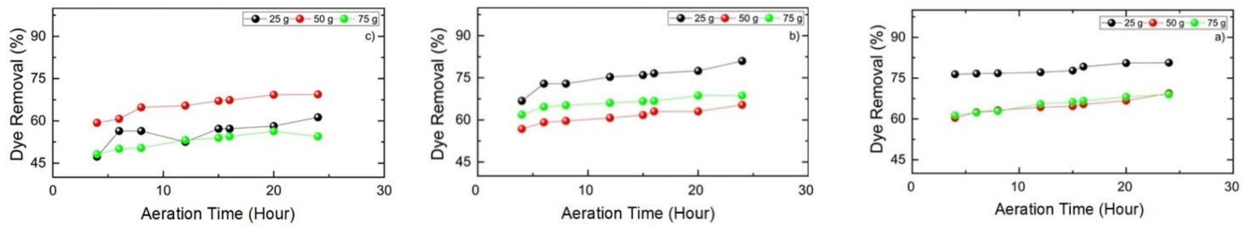


Figure 2. AGS stability a) size distribution, b) MLSS/MLVSS comparison, and c) Comparison chart of SVI5, SVI30 & SVI5/30



(a) (b) (c)

Figure 3. Performance test decolorization with variation concentration dyes and COD a) COD 500 mg/L, b) COD 750 mg/L, and c) COD 1000mg/L

where dye removal efficiencies improve to 56.42-62.32% and 56.42-62.89%, respectively. These increases highlight the effectiveness of extended aeration periods in enhancing the microbial degradation of the dye. At 12 and 15 hours, the dye removal efficiency significantly improved, reaching values between 52.53-65.49% and 57.20-66.20%, respectively. The data for 16 and 20 hours of aeration shows further incremental improvements, with removal efficiencies peaking at 57.20-66.62% and 58.17-68.10%, respectively. The highest dye removal efficiencies were observed at 24 hours, with 61.28-69.01% values. This result demonstrates that the AGS-SBR system can maintain high dye removal performance over prolonged operational periods, resulting in significantly cleaner effluent. Similar findings were reported by Lawal et al. (2023b), where increased dye concentrations led to decreased decolorization and COD removal in aerobic SBR systems. Compared to other studies using AGS-SBR for different dyes, such as the study by Xavier et al. (2023) on dyes reactive black (RB5), which achieved a maximum removal efficiency of 67.4%, the current results are comparable or slightly better. Meanwhile, conventional activated sludge systems treating the same dye typically report lower efficiencies, often below 54%, as shown by Mustafa et al. (2024). Therefore, the AGS-SBR method is more effective and reliable for dye wastewater treatment than traditional biological approaches.

3.3 Model Fitting and Analysis

This study employed RSM, specifically the BBD, to illustrate the impact of independent variables on response variables under experimental conditions. The experimental data were assessed, and the degradation percentages of dye, COD, and SVI were calculated using the following equation:

$$\text{Decolorization (\%)} = 115.99301 - 1.76406\,A + 0.887694\,B - 0.124208\,C - 0.002509\,A.B + 0.001045\,A.C - 0.000192\,B.C + 0.019839\,A^2 - 0.011802\,B^2 + 0.000076\,C^2 \quad (3)$$

where A is dyes concentration (mg/L), B is aeration time (hour), and C is COD Concentration (mg/L).

We performed a residual analysis to verify the model's fit and evaluate the adequacy of the developed quadratic model. Table 4 illustrates that the normal plot of externally studentized residuals (left panel) indicates a normal distribution, since the dots closely align with the red diagonal line. This signifies that the

Table 4. The experimental and predicted dye degradation, COD, and SVI values in the SBR system were evaluated

Run	*Actual values of the variable*			Dyes Degradation (%)		COD Degradation (%)		SVI (mg/L)	
	Dyes Concentration (mg/L) (A)	*Aeration Time* (Hour) (B)	COD Concentration (mg/L) (C)	*Observed (Y) (%)*	*Predicted (Y1) (%)*	*Observed (Y) (%)*	*Predicted (Y1) (%)*	*Observed (Y) (%)*	*Predicted (Y1) (%)*
1	10	6	750	56.42	61.71	100	98.05	19.57	16.08
2	30	24	750	68.62	63.33	89	90.95	48.39	51.87
3	30	15	500	53.94	61.61	98.67	99.94	40.40	49.06
4	20	24	1000	69.46	70.13	86.50	89.10	53.10	37.88
5	10	24	750	61.28	68.27	100	98.67	15.22	39.09
6	30	6	750	64.66	57.67	100	101.33	104.17	80.30
7	10	15	500	75.94	71.33	96.53	101.09	43.65	31.92
8	10	15	1000	77.75	70.09	100	98.73	19.34	10.68
9	30	15	1000	66.19	70.82	100	95.45	58.82	70.55
10	20	15	750	61.71	61.72	92.80	92.80	18.62	18.62
11	20	6	500	60.71	60.04	100	97.40	25.25	40.47
12	20	6	1000	62.51	64.89	95	98.23	16.35	28.49
13	20	24	500	69.38	67.02	100	96.78	37.79	25.65

Table 5. The ANOVA (analysis of variance) for textile wastewater treatment

Source	Dyes Degradation (%)		COD Degradation (%)		SVI (mg/L)	
	F value	***P value***	***F value***	***P value***	***F value***	***P value***
Model	0.2597	0.9496	0.6327	0.739	0.7174	0.6933
A-Dye Concentration	0.3716	0.5852	0.3268	0.6076	3.79	0.1467
B-Time	0.6872	0.4679	1.58	0.2976	0.0188	0.8997
C-COD	0.2919	0.6265	0.7803	0.4421	0	0.9953
AB	0.0019	0.9682	1.01	0.3898	0.8456	0.4256
AC	0.251	0.6508	0.0378	0.8582	0.5837	0.5004
BC	0.0069	0.9392	0.6007	0.4948	0.1874	0.6943
A^2	0.0828	0.7923	1.18	0.3571	0.9285	0.4063
B^2	0.0192	0.8985	0.02	0.8966	0.3157	0.6135
C^2	0.4758	0.5399	0.3234	0.6094	0.0494	0.8383

normalcy assumption is satisfied, corroborating the model's capacity to forecast the response variables.

From equation 3, the independent variables are identified as A, B, and C, while A^2, B^2, and C^2 represent their quadratic (non-linear) effects on the response. These squared terms help capture the curvature in the response surface, allowing for more accurate optimization. The t-test and p-value are used to determine the significance of each coefficient in Equation 3. Table 5 shows the ANOVA findings for this model, suggesting that it may be utilized to navigate the design space. The R^2 and adjusted R^2 values are near 1.0, indicating a significant connection between observed and predicted values. This suggests the regression model can explain the link between the independent variables (factors) and the response (color removal%).

To investigate the combined effects of dye concentration and aeration time, Response Surface Methodology (RSM) was applied, and the results are presented in the form of contour and 3D plots. Figure 4a shows the increase in dye degradation as a function of the interaction between dye concentration and aeration time. When aeration time is optimized and dye concentration is at its minimum, optimal dye degradation is achieved. As the dye concentration increases from 10 to 20 grams, the percentage of remazol red degradation decreases. These results indicate that the decolorization percentage declines as dye concentration increases due to a higher load on the removal process by aerobic granular sludge (AGS), reducing the decolorization rate.

Figure 4b illustrates the influence of aeration time and COD concentration on the decolorization percentage. The results show that the highest COD concentration combined with the longest aeration time produces the best decolorization outcomes. This is because COD provides essential support for microbial activity in the degradation process, and aeration time significantly impacts decolorization efficiency, as aerobic granular sludge (AGS) requires sufficient time for effective decolorization. Optimal results were achieved with increased COD concentration and extended aeration time, with the optimal

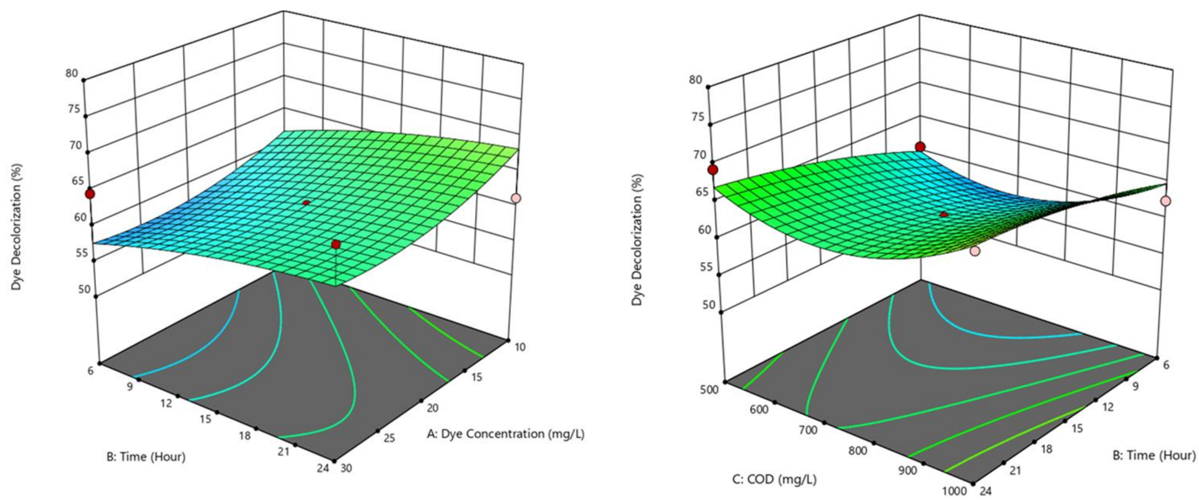


Figure 4. 3D Plots a) The influence of dye concentration and aeration time, b) The influence of COD concentration and aeration time

decolorization of 77% occurring at a COD concentration (X1) of 1,000 mg/L and system time (X2) of 24 hours for Remazol Red decolorization. This result is comparable to previous studies using AGS-SBR systems, such as the study by Xavier et al. (2023), which reported 67.7% decolorization of reactive black 5 under similar conditions. In contrast, conventional activated sludge systems often report lower decolorization efficiencies (below 54%) for similar dye types (Mustafa et al., 2024), highlighting the superior performance of AGS-SBR for azo dye removal.

4. CONCLUSION

This study demonstrates that aerobic granular sludge (AGS) technology in sequencing batch reactors (SBR) effectively removes remazol red dye from textile wastewater. Response surface methodology (RSM) successfully modelled and optimized operational variables, such as COD concentration, aeration time, and dye concentration, to maximize decolorization. The results indicate that higher COD concentrations and extended aeration times yield the best outcomes, with a decolorization efficiency of 77% achieved under optimal conditions. These findings endorse the use of AGS-SBR as an efficient technology for textile wastewater treatment and provide further opportunities for industrial-scale application.

5. ACKNOWLEDGMENT

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