Optimization of Rotary Algae Biofilm Reactor (RABR) Using *Ulva sp.* for Batik Liquid Waste Management: Effects of Algal Suspension Concentration, CO_2 Tablet Mass, and Rotation Speed

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ABSTRACT

The batik industry is a vital part of Indonesia's cultural heritage, but generates significant liquid waste containing high levels of pollutants. Batik wastewater often exhibits excessive suspended solids, Chemical Oxygen Demand (COD), and Biological Oxygen Demand (BOD), which surpass permissible discharge standards. For instance, the batik industry in Ambulu produced effluent with a COD of 1,011 mg/L and a BOD of 511.79 mg/L, far exceeding environmental limits. Conventional treatment methods are generally costly, energy-intensive, and often generate secondary waste, making them less suitable for small-scale industries. Therefore, sustainable and affordable alternatives are urgently needed. This study investigates the use of a Rotary Algae Biofilm Reactor (RABR) integrated with Ulva sp. macroalgae as a natural biofilter for batik wastewater treatment. Optimization was conducted using Response Surface Methodology with Central Composite Design (RSM-CCD), focusing on three operational parameters: Ulva sp. suspension concentration (4–6%), CO_2 tablet mass (1–3 tablets), and rotation speed (40–60 rpm), with a contact time of 1 day. The optimal condition was identified at a 5.991% Ulva sp. suspension, 1 CO_2 tablet, and a rotation speed of 60 rpm. Under these conditions, the COD was reduced to 42.923 mg/L (96% removal) and the BOD to 55.300 mg/L (89% removal), both of which meet the Indonesian wastewater discharge standards (COD < 100 mg/L, BOD < 100 mg/L). The findings confirm that the RABR-Ulva sp. system provides a cost-effective, eco-friendly, and scalable solution for batik wastewater treatment. This approach minimizes operational costs, avoids secondary waste, and supports the principles of sustainable development, particularly SDG 6 (Clean Water and Sanitation) and SDG 12 (Responsible Consumption and Production).

1. Introduction

Batik has been recognized by UNESCO as an Intangible Cultural Heritage since 2009, highlighting its cultural significance and economic contribution to Indonesia [1]. Batik production is widespread, ranging from home industries to large-scale manufacturers. One of the active batik producers is Rezti's Batik, located in Tegalsari Village, Ambulu District, Jember Regency. While supporting local economic growth, the batik production process also generates liquid waste, particularly from dyeing, washing, and wax removal processes [2], [3]. This wastewater contains pollutants that, if not adequately treated, can harm the surrounding environment. Complex physical, chemical, and biological parameters characterize batik wastewater. Physically, it contains suspended solids, color, odor, and elevated temperature. Chemically, it has high acidity (pH), conductivity, COD, and hardness. Biologically, it contains high BOD and microbial loads [2]. Based on Indonesia's Regulation of the Minister of Environment and Forestry No. 16/2019, the wastewater quality standard for textile industries (with a discharge limit of ≤100 m³/day) requires BOD ≤ 60 mg/L, COD ≤ 150 mg/L, and pH between 6–9 [4]. However, measurements from Rezti's Batik wastewater show COD levels of 1011 mg/L and BOD of 511.79 mg/L, which far exceed the regulatory limits, indicating a critical need for effective treatment solutions. Conventional wastewater treatments such as sedimentation, filtration, and coagulation are commonly applied but are inadequate for removing dissolved dyes and complex organic compounds typical of batik wastewater [5], [6]. Advanced technologies, such as membrane filtration, electrocoagulation, and advanced oxidation, offer higher efficiency but are often costly, energy-intensive, and produce secondary waste [7]. As a sustainable alternative, algae-based treatments are gaining attention due to their natural ability to absorb nutrients and pollutants, their low operational costs, and minimal generation of secondary waste.

One promising system is the Rotary Algae Biofilm Reactor (RABR), a photobioreactor designed with rotating disk media that supports the growth of biofilms from algae [8], [9]. RABR integrates biological treatment with photosynthesis, providing enhanced oxygenation, nutrient uptake, and biomass productivity. The rotation alternately exposes algae to air (for CO_2 uptake) and wastewater (for nutrient absorption), making it highly effective even in wastewater with high turbidity [10], [11]. Key operational parameters affecting RABR performance include rotation speed, which influences shear force, oxygen transfer, and nutrient delivery, as well as CO_2 supplementation, which supports optimal algal growth and biofilm development [8]. Analysis of prior studies (Table 1) shows that higher rotation speeds (5–7 rpm) coupled with longer contact times (3–5 days) generally yield better COD and BOD removal efficiencies. However, limited research has been conducted on applying RABR with macroalgae, particularly for batik wastewater.

Table 1. Previous Research

Material	Method	Operating Conditions	Result	Ref.
Algae	*Rotary Algae Biofilm Reactor* (RABR)	Variation of rotation speed: 3, 5, and 7 rpm. Variation of contact time: 0, 1, 3, and 5 days.	BOD removal efficiency of 73.42% and COD of 70.64% at a disk rotation speed of 7 rpm on the 5th day.	[16]
Chlorella sp	*Rotary Algae Biofilm Reactor* (RABR)	Rotation speed variations: 2, 4, and 6 rpm.	The highest COD removal efficiency (73.08%) was achieved at a rotation speed of 4 rpm.	[17]
Microalgae bacteria	*High Rate Algae Reactor* (HRAR)	Variation of microalgae suspension concentration: 0, 10, 15, 20, and 25 (% v/v).	The best COD removal efficiency is 78.79%.	[18]
Algae	*High Rate Algae Pound* (HRAP)	Variables of K element addition: 0%, 1% and 3%, C concentration (0 and 29.41 mg/L), and time: 0, 3, 6, 9, 11, 13, 16, and 18 days.	On the 6th day, with the addition of 3% K element and 29.41 mg/L C element, the COD decreased by 46%.	[19]
Chorella sp.	*Rotary Algae Biofilm Reactor (RABR)*	Tofu liquid waste concentration: 40%, 60%, 80%, and 100%, and contact duration (0, 1, 3, and 5 days)	The COD reduction efficiency was 75.88% at a tofu liquid waste concentration of 40% on the 5th day.	[20]
Chlorella vulgaris	*Biofilm*	Time variation: 0, 12, 24 hours.	BOD reduction 58.68%, COD 60.77%, TSS 68.36%, Nitrate 67.80%, Ammonia 80%, and Phosphate 60.38%	[21]
Algae	*Rotating Algae Contactors* (RACs)	Time variations: 20, 30, and 45 days	Waste reduction efficiency of 63%	[22]
Logan Lagoons Cyanobacteria selection 2 (LLC2)	*Rotary Algae Biofilm Reactor* (RABR)	Condition variations: low and high light	Waste reduction efficiency 87.6%	[10]

Unlike microalgae such as *Chlorella sp.,* commonly used in prior studies, *Ulva sp.,* a green macroalgae, is abundant in Indonesian waters [12], [13], and offers several advantages, including higher nutrient uptake capacity, faster biomass growth, and resistance to wastewater conditions [14], [15]. Moreover, *Ulva sp.* aligns with eco-friendly principles, producing no secondary sludge and operating efficiently in saline or brackish wastewater, which is typical of some textile processes. Some previous studies related to algae, along with the methods and operating conditions listed in Table 1.

As shown in Table 1, variations in disk rotation speeds between 4–7 rpm tend to achieve higher COD and BOD removal efficiencies, while algal biomass concentrations in the range of 3–7% support optimal biofilm formation without hindering oxygen transfer. The addition of CO_2 also plays a vital role in enhancing algal photosynthetic activity, particularly in alkaline wastewater conditions. These trends support the selection of parameters in this study for further optimization using RSM. The volume of wastewater produced by Rezti's batik is 125 liters/day, and if discharged into the environment, this volume will have a negative impact. Batik wastewater contains synthetic dyes, complex chemicals, and heavy metals such as Cr, Pb, and Cu, which are difficult to degrade and highly polluting when discharged without proper treatment. Studies show that this wastewater can reduce dissolved oxygen and kill up to 100% of fish within 96 hours [23], [24]. In addition to damaging aquatic ecosystems, batik wastewater also poses risks to human health and increases environmental remediation costs [25], [26]. Therefore, proper treatment of batik wastewater is crucial to prevent broader ecological and social impacts.

This study explores a novel application of the Rotary Algae Biofilm Reactor (RABR) integrated with Ulva sp. for treating batik wastewater. The research focuses on optimizing three key parameters: Ulva sp. suspension concentration (4–6%), CO_2 tablet mass (1–3 tablets), and rotation speed (40–60 rpm) to maximize pollutant removal. These ranges were selected based on prior studies [16], [19-24] Preliminary tests were conducted to tailor the characteristics of batik wastewater. Previous findings indicate that Ulva sp. concentrations of 3–7% provide sufficient biomass for biofilm formation without shading or oxygen transfer limitations, while rotation speeds of 40–60 rpm enhance aeration and nutrient uptake without causing detachment. Preliminary tests also showed that moderate CO_2 supplementation (1–3 tablets/day) supports Ulva sp. photosynthesis in alkaline conditions without acidification or oversaturation. The hypothesis is that higher biomass and rotation speed, combined with adequate CO_2 supply, improve COD and BOD removal. Optimization was conducted using Response Surface Methodology with Central Composite Design.

2. Research Methodology

2.1. Tools and Materials

The equipment used in this study included a RABR, glassware, a COD thermoreactor (brand VELP Scientifica, model F101A0127 ECO8), a wastewater treatment photometer (brand HANNA Instrument, model HI-83214), an analytical balance, and a stand & clamp. The disk used in this study has a diameter of 20 cm with an active surface area of approximately 0.125 m². It is made of transparent acrylic, which is corrosion-resistant and supports biofilm growth [27], [28], [29]. The motor used is a 12 V DC motor with a power of approximately 10 W, and this setup provides a stable rotational speed in the range of 40–60 rpm, which is effective for biofilm development without causing detachment due to excessive shear force [30], [31], [32]. The light intensity used in this study ranged from 100 to 300 μmol photons/m²/s, which is considered optimal for macroalgae growth and pigment production [33], [34]. The primary material used in this study was *Ulva sp.* obtained from Sanur Beach, Denpasar City, Bali Island. Other materials used include liquid batik waste from Rezti's Batik located in Tegalsari Village, Ambulu District, Jember Regency, 1N alkali iodide azide, sulfuric acid (H_2SO_4), 1 N starch indicator, 1N potassium dichromate ($K_2Cr_2O_7$), 1N potassium iodide (KI), 1N manganese sulfate ($MnSO_4$), 0.025N sodium thiosulfate ($Na_2S_2O_3$), effervescent CO_2 tablets, distilled water (H_2O), and COD reagent brand HANNA High Range 93754C-25. The use of effervescent CO_2 tablets is more practical and economical than pressurized gas injection systems, which require special equipment such as gas cylinders, regulators, and distribution systems. CO_2 tablets gradually release carbon into the medium, increasing carbon availability for photosynthesis without the need for complex gas pressure controls. Increasing carbon availability through CO_2 supplementation enhances algal growth rate and the efficiency of removing organic pollutants, such as COD and BOD, making the tablet form highly suitable for laboratory-scale and small-scale industrial applications [35]. The number of samples required for the data collection process is 17.

2.2. Research Variabel

The RSM approach was used in conjunction with an experimental CCD treatment to optimize wastewater treatment. The study employed a combination of three treatment factors: CO_2 tablet mass, *Ulva sp.* suspension, and rotation speed. The independent variables used in this study included the mass of 1, 2, and 3 CO_2 tablets [36]; rotation speeds of 40, 50, and 60 rpm, and the concentration of *Ulva sp.* suspension used was 4%, 5%, and 6% [18]. The control variables used in this study included contact time for 1 day [18] And the addition of 12 grams of salt. The dependent variables included BOD and COD. The alpha (α) used was face-centered with an alpha value of one (1). Design Expert 13 software was used to determine the treatment formulation through CCD. Based on the software settings, 17 runs were conducted in this study.

2.3 Research Stages

The outermost part of the RABR (shown in Fig. 1.) is made of aquarium glass with a volume of 20 liters. Twelve liters of batik waste were processed in the RABR, along with Ulva sp. The stirrer was connected to an adapter and turned on to initiate the waste processing process. CO_2 tablets were then placed into the RABR to supply CO_2 to *Ulva sp.* The waste processing process was conducted over 24 hours. Waste processing was conducted in the Chemical Engineering Department Laboratory at the Universitas Jember, utilizing *Ulva sp.* algae in the RABR.

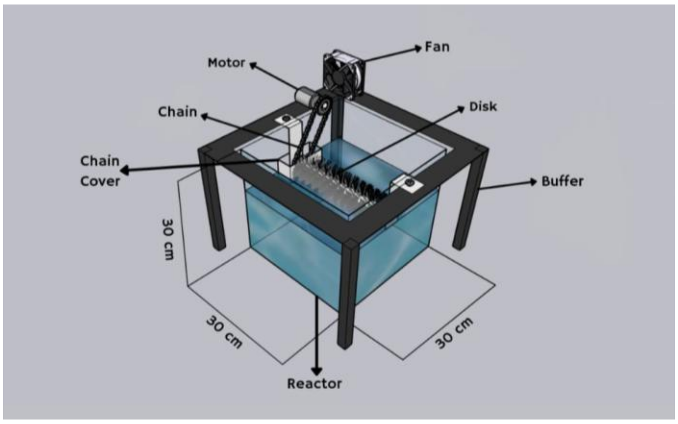

Fig. 1. Design RABR

According to the Standard Methods for the Examination of Water and Wastewater, the sample volume for COD and BOD tests should be at least 500 mL to 1 liter to allow for duplicates, blanks, and spike controls. In several small-scale laboratory studies, samples can be stored in sealed containers at a stable room temperature (around 20–25 °C) and protected from direct sunlight, to prevent photolysis activity and microbial growth before analysis [24],[37],[34]. This approach is commonly used when analysis is conducted within a short time period (less than 24 hours) after sample collection. The process of taking and testing samples is carried out in a single run. The runs carried out in this study consisted of 17 runs, resulting in 17 samples to be observed. Sampling was carried out when the run had finished according to its contact time. The samples were then tested for COD and BOD. For optimization analysis using RSM, statistical model validation is essential. The quadratic model in CCD must be validated using ANOVA analysis, reporting values such as the F-statistic, p-value ($p < 0.05$), R^2 ($R^2 > 0.90$), and a lack-of-fit test to confirm the model's adequacy [38]. Additionally, the relative contribution of each factor to the response should be presented to identify the most influential variables in the system [23].

1) COD Level Testing

The COD value testing method is carried out in accordance with SNI-6989.15.2019. The COD value test stage begins with the preparation of waste samples and blanks, each in the form of distilled water, in quantities of 0.2 mL, which are then inserted into the COD reagent vial. The COD thermoreactor is heated to 150°C, and the reagent vial containing the sample and blank is inserted into it. The reagent vial is heated to 150°C for 2 hours. After being heated, the thermoreactor is turned off and allowed to cool for 20 minutes, until the vial reaches a temperature of 120°C. The vial is turned over several times while still warm and moved to the vial rack until it reaches room temperature. When the vial has reached room temperature, the COD value of each vial can be measured using a wastewater treatment photometer with a high-range setting. In COD testing, quality control and assurance (QC/QA) steps, such as instrument calibration and the inclusion of blank, duplicate, and spike samples, should be conducted at least once per batch to ensure the accuracy and precision of the results [39].

2) BOD Level Testing

The BOD value does not indicate the actual amount of organic matter, but only measures the relative amount of oxygen needed to oxidize the waste material [40]. The principle of BOD measurement involves two main stages, namely measuring the initial dissolved oxygen levels (DO1) in the sample immediately after collection, and measuring the dissolved oxygen levels after 5 days of incubation (DO5). For DO5 measurement, the sample is incubated in an incubator at 20 ± 1 °C for 5 days. The difference between DO1 and DO5 is the BOD value, which is expressed in milligrams of oxygen per liter (mg/L). Incubation is carried out in dark conditions to prevent the process of photosynthesis, which can produce oxygen, allowing only the decomposition of organic matter by microorganisms to occur. The remaining oxygen after incubation is measured as DO5, which reflects the amount of oxygen used by microorganisms to decompose organic matter in the sample. The calculation in finding the BOD level can be determined by equations 1 and 2 [41]:

$$OT = \frac{a \times N \times 8000}{V - n} \quad (1)$$

$$BOD = \frac{(X_0 - X_5) - (B_0 - B_5)(1 - P)}{P} \quad (2)$$

Where OT is dissolved oxygen (mg O_2/L), a is the volume of titrant (mL), N is the normality of the solution (eq/L), V is the volume of the Winkler bottle (mL), n is the added solution (mL), X is the OT of the sample (mg O_2/L), B is the OT of the blank (mg O_2/L), and P is the degree of dilution.

3. Results and Discussion

3.1 Initial Waste Characterization

Table 2. Initial characteristics of the homemade batik industry's liquid waste

Parameter	Batik Liquid Waste	Textile Industry Waste Quality Standards
COD (mg/L)	1011	150
BOD (mg/L)	511.8	60

Initial wastewater characterization (Table 2) showed that batik wastewater from Rezti's Batik contained extremely high levels of COD value on 1011 mg/L and BOD value on 511.79 mg/L. These values exceed the national discharge standards as regulated by the Indonesian Ministry of Environment and Forestry Regulation No. P.16/2019. Elevated COD and BOD levels result in decreased dissolved oxygen (DO), which can lead to the death of aquatic organisms in a short time, as also reported by [23], [24] in their studies on RABR systems.

3.2 RSM-CCD Optimization

Optimization using RSM with a CCD (Table 3) revealed that rotation speed (C) was the most dominant factor in reducing COD and BOD levels. Based on the ANOVA results (Tables 4 and 5), a speed of 60 rpm significantly improved pollutant removal efficiency by enhancing oxygen transfer and promoting biofilm circulation. Jonathan [24] noted that increasing the speed from 40 to 60 rpm improved the oxygen transfer coefficient (kLa) by 2.3 times. In the quadratic model developed, the rotation speed factor contributed to over 58% of the COD removal efficiency. Furthermore, the desirability function yielded a value of 0.988, indicating that the optimal combination of parameters (5.991% *Ulva sp*., 1 CO_2 tablet, and 60 rpm) is highly applicable to small-scale industries. The final COD and BOD levels of 42.923 mg/L and 55.3 mg/L, respectively, demonstrate the effectiveness of the RABR system in reducing pollutants below regulatory discharge thresholds [11], [25].

Table 3. Response data for Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD)

Run	Variable			Response	
	Concentration Suspension (%)	*Rotation Speed (rpm)*	*Tablet Mass CO_2 (tablet)*	*COD Value (mg/L)*	*BOD Value (mg/L)*
1	6	40	1	292	215.3
2	4	50	2	255	112.4
3	6	50	2	172	176.9
4	5	60	2	44	53.7
5	5	50	2	186	157.4
6	4	60	1	95	50.3
7	6	60	1	42	56.2
8	5	50	3	178	110.2
9	4	40	3	328	246.2
10	5	50	1	194	125.7
11	5	50	2	186	134.6
12	6	40	3	283	265.4
13	6	60	3	45	58.6
14	4	60	3	78	54.5
15	4	40	1	345	198.5
16	5	40	2	294	208.8
17	5	50	2	180	143.9

3.3 Mechanistic Insights

1) The Influence of Independent Variables on the Response of COD Values

The results of the COD value test after waste processing are presented in Table 4. The ANOVA results indicate that the model is highly significant with a p-value of < 0.0001, confirming that the variables in this study have a substantial effect on COD reduction. Among the three variables, rotation speed (C) is the most dominant factor, with a p-value of 0.0001 (12981.87), as it plays a crucial role in enhancing oxygen transfer and improving biofilm efficiency in pollutant removal [42]. Furthermore, the suspension concentration (B) also has a significant impact, with a p-value of 0.0001 (475.77), indicating that increasing the concentration of *Ulva sp.* enhances the pollutant absorption capacity up to an optimum point. Meanwhile, CO_2 tablet mass (A) shows a more minor but still significant effect (p = 0.0001 (26.56)) by serving as a carbon source to support the photosynthetic activity of *Ulva sp.*

Table 4. Results of ANOVA of COD value responses

Source	Sum of Squares	df	Mean Square	p-value	
Model	16.03E+05	9	17808.54	1508.43	*significant*
A-CO_2 Tablet Mass	313.60	1	313.60	26.56	
B-Suspension Concentration	5616.90	1	5616.90	475.77	
C-Rotation Speed	1.533E+05	1	1.533E+05	12981.87	
AB	98.00	1	98.00	8.30	
AC	18.00	1	18.00	1.52	
BC	18.00	1	18.00	1.52	
A^2	27.15	1	27.15	2.30	
B^2	658.99	1	658.99	55.82	
C^2	511.49	1	511.49	43.32	
Residual	82.64	10	11.81		
Lack of fit	58.64	5	11.73	0.9774	*not significant*
Pure Error	24.00	5	12.00		
Cor Total	1.604E+05	19			

* Note = Text in bold indicates significant results

Based on the results of ANOVA using Design Expert 13 software, the recommended COD value response model is quadratic. A summary of the results of the ANOVA statistical analysis, indicating the adequacy of the model with a p-value of <0.05, can be seen in Table 4. The results of the model testing are used to test each component of the model at a certain probability level (α) defined by the term *p-value*. The probability level in this study is 5%, so that variables with a *p-value*<0.05 are considered significant and have a substantial impact on the model [43]. Based on Table 4, the COD value response was significantly influenced by the mass of the CO_2 tablet, rotation speed, and suspension concentration variables, as indicated by the presence of a *p-value* < 0.05.

Lack of fit is a term used to describe a model's inability to represent the data accurately. If the model's inaccuracy is not statistically significantly different from the observed inaccuracy, then the model is considered suitable to show the problem in the system being studied. If the *p-value* is more than 0.05 or the *lack of fit* is not statistically significant, this model choice is considered adequate. If the *p-value* is less than 0.05, the choice is deemed inadequate. The p-value of 0.5758 exceeds 0.05, indicating that this COD response model is not appropriate or suitable. Based on the ANOVA analysis results for the COD value response, the lack-of-fit value does not yield *significant information*. This indicates that the model and test accuracy are suitable and can effectively explain the problems in the analyzed study [44].

The desirability value, also known as R2, is the value of the optimization objective function that describes how well the program performs based on the final set of criteria, with values ranging from zero (0) to one (1). If the desirability value approaches one (1), it indicates that the program is getting better at achieving its optimization goals [44]. The stronger the greater desirability value indicates the influence of the independent variable on the fixed variable, which also shows that the correlation between the observation results and the resulting model is perfect [43]. The results are presented in Fig. 2.

The experimental data values are spread around the line in Fig. 2, indicating the suitability of the regression model in relation to the experimental data. Therefore, a good regression model, with regularly distributed values, can be applied. The desirability value obtained from the ANOVA analysis results for the COD value response variable is 0.9692. This indicates that the factors studied account for 96.92% of the variation in the COD value response. The COD value response included in the model has a correlation of 96.20% based on actual data, as indicated by the adjusted R-squared value of 0.9620. The predicted data, indicated by a predicted R-square value of 0.9491, is 94.91%. Therefore, it can be concluded that the concentration factor of Ulva sp. suspension, rotation speed, and mass of CO_2 tablets have a significant effect on the COD value response. The results of the RSM analysis indicate the impact of independent variables on each response. This effect is indicated by the presence of model equation coefficients, which are expressed in both coded and natural (actual) forms presented in Equation 3.

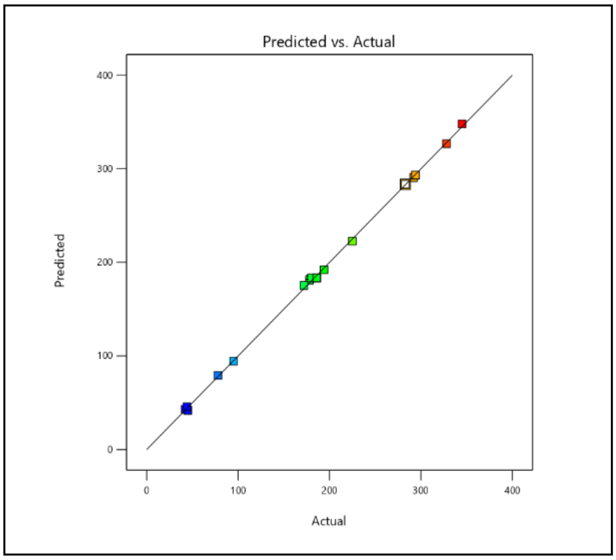

Fig. 2. Graph of actual values against predicted values for each COD value response

$$COD = 183{,}32 - 5{,}6A - 23{,}70B - 123{,}80C + 3{,}50AB + 1{,}50AC + 1.50BC + 3.18A^2 + 15.68B^2 + -13.82C^2 \quad (3)$$

Where A is the mass concentration of CO_2 tablets (tablets), B is the suspension concentration (%), and C is the rotation speed (rpm).

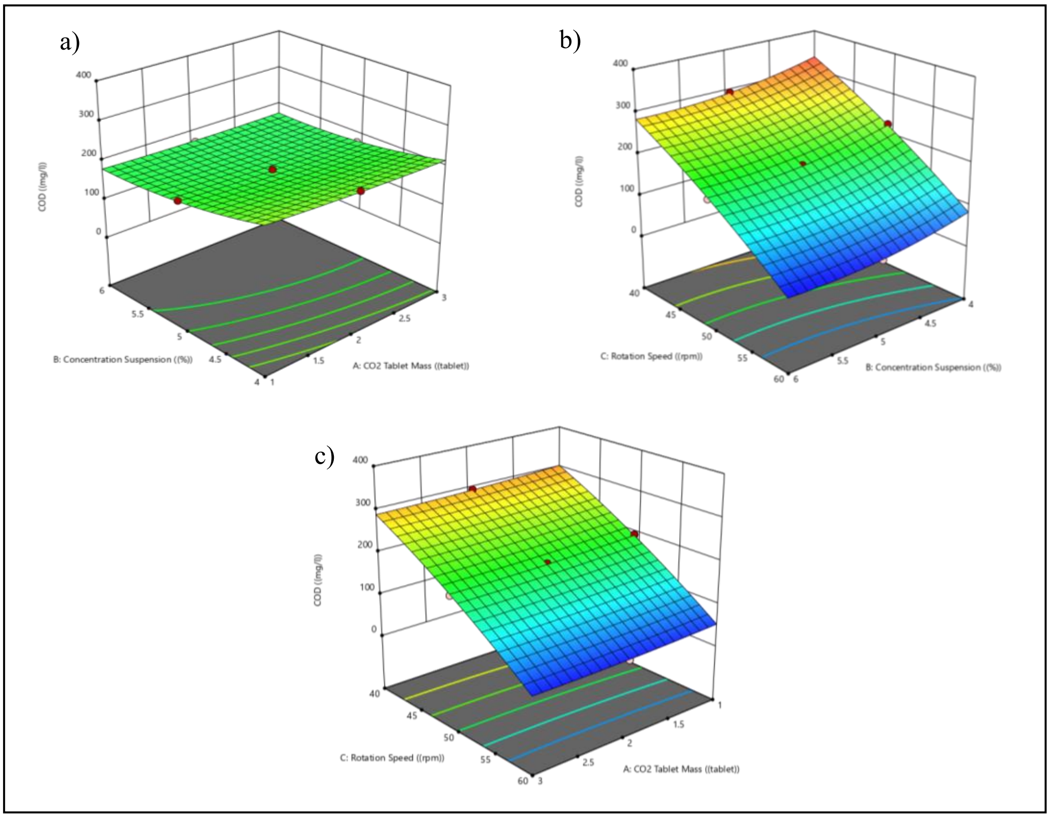

Fig. 3. Three-dimensional graph (a) of the effect of suspension concentration and mass of CO_2 tablets (b) of the effect of rotation speed and mass of CO_2 tablets (c) of the effect of rotation speed and suspension concentration on the COD test.

Based on Fig. 3 (a), COD tends to decrease with increasing concentration of *Ulva sp*. suspension and does not decrease significantly with increasing CO_2 mass. Fig. 3. (b) COD tends to decrease with increasing rotation speed, and the addition of CO_2 tablet mass does not decrease significantly. Fig. 3(c) shows that COD tends to decrease with increasing rotation speed and concentration of the *Ulva sp*. suspension. The highest COD was obtained in the sample with a variable CO_2 tablet mass of 1 tablet, an Ulva sp. suspension concentration of 4%, and a rotation time of 40 rpm, yielding a COD of 345 mg/L. The lowest COD was obtained in the sample with a variable CO_2 tablet mass of 1 tablet, an Ulva sp. suspension concentration of 6%, and a rotation time of 60 rpm, yielding a COD of 42 mg/L and a removal efficiency of 95.84%.

The concentration of *Ulva* suspension has a significant effect on COD values in this study. Increasing the concentration of macroalgae suspension accelerates the exponential phase of growth in macroalgae [45]. With a higher concentration of macroalgae suspension, the exponential phase can be reached more quickly, making the wastewater treatment process more efficient. This finding is also supported by previous research [46], which shows that a higher concentration of macroalgae suspension can increase oxygen supply, accelerate organic matter degradation, and reduce COD values. Bacteria play a role in decomposing complex organic compounds in waste into simple compounds, producing CO_2 through metabolic processes [47]. The synergistic interaction between algae and bacteria strongly supports the effectiveness of the RABR system in removing organic pollutants. *Ulva sp*. releases extracellular polymeric substances (EPS), particularly polysaccharides, that enhance biofilm adhesion on the surface of the rotating disks [26]. These EPS compounds provide a structural matrix that supports stable colonization and protects microbial communities [24]. At the same time, heterotrophic bacteria within the biofilm degrade complex organic compounds and textile dyes into simpler substrates that the algae can assimilate as nutrients. This algae–bacteria synergy allows for the breakdown and assimilation of otherwise recalcitrant compounds found in batik wastewater [48], [49]. Macroalgae then utilize these simple organic compounds for cell growth and use CO_2 for photosynthesis, producing oxygen that supports bacterial activity in the decomposition of organic matter. With increasing concentration of macroalgae suspension in RABR, the efficiency of COD reduction in this study also increased.

The rotation speed in the RABR system has a significant effect on COD removal. The study revealed that a rotation speed of 60 rpm yielded the highest COD removal efficiency of 70.64%, with an initial COD concentration of 1,011 mg/L decreasing to 288 mg/L. Higher rotation speeds increase oxygen transfer and even distribution of algae, thus supporting biofilm growth in the exponential phase longer [50]. This accelerates the degradation process of organic matter in wastewater [10]. Thus, the optimal rotation speed in RABR is crucial for enhancing the efficiency of COD processing in the batik industry's liquid waste.

Research shows that the use of CO_2 tablets in wastewater treatment can increase the solubility of carbon dioxide in wastewater, thereby helping to reduce COD. Additional CO_2 supports the growth of aerobic and anaerobic microorganisms that accelerate the decomposition of organic matter, thereby increasing the efficiency of wastewater treatment [51]. This is also supported by research [52], which shows that the addition of CO_2 can reduce COD values by increasing algae activity. Algae utilize CO_2 contained in wastewater as a source of carbon for the photosynthesis process. Therefore, the optimal arrangement of CO_2 addition can accelerate the decomposition process of organic materials in liquid waste.

2) The Influence of Independent Variables on BOD Value Response

The results of the BOD test, conducted for waste treatment, are presented in Table 5. Based on the results of the ANOVA in Design Expert 13 software, the recommended BOD value response model is linear. A summary of the results of the ANOVA statistical analysis indicating the adequacy of the model with a *p-value*<0.05 can be seen in Table 5.

The ANOVA results indicate that the model is statistically significant with a p-value of 70.02, demonstrating that the combination of variables has a substantial effect on BOD reduction. Among these variables, rotation speed (C) is the most dominant factor, with a highly significant p-value of 0.20452, indicating that increasing the rotation speed greatly enhances oxygen transfer and promotes biofilm formation of Ulva sp., thereby accelerating the degradation of organic matter. [50]. Meanwhile, suspension concentration (B) also shows a significant effect with a p-value of 3.37, as higher biomass concentrations contribute to greater pollutant absorption. On the other hand, CO_2 tablet mass (A) presents a lower p-value of 2.18, indicating a minor but relevant contribution by providing a carbon source to support *Ulva sp.* photosynthesis and biofilm growth.

Table 5. Results of ANOVA of BOD value responses

Source	Sum of Squares	df	Mean Square	p-value	
Model	76126.23	3	25375.41	70.02	*significant*
A-CO_2 Tablet Mass	790.32	1	790.32	2.18	
B-Suspension Concentration	1221.02	1	1221.02	3.37	
C-Rotation Speed	74114.88	1	74114.88	204.52	
Residual	4710.97	13	363.38		
Lack of fit	4448.11	11	404.37	3.08	*not significant*
Pure Error	262.86	2	131.43		
Cor Total	80837.20	16			

*Note = Text in bold indicates significant results

The results of the model testing are used to test each component of the existing model. Based on Table 5, the BOD value response is significantly influenced by the rotation speed, as indicated by the *p-value* <0.05. The BOD response value is not significantly influenced by the CO_2 tablet mass variable and suspension concentration, as indicated by a p-value greater than 0.05. The *lack-of-fit value of this BOD response model is 0.2708, which means that the p-value is greater than* 0.05 (not significant). There is a fit between the models as shown in Fig. 4.

According to the results of the ANOVA analysis, the choice of this model is considered adequate. A good fit between the regression model and the experimental data is evident in Fig. 4, where the experimental data values are spread around the line. Based on the desirability value for the BOD value response variable of 0.9417, which indicates that the studied factors have an influence of 94.17% on the BOD value response. The respondents' actual BOD value included in the model shows a value of 92.83%, as indicated by the adjusted R-squared value of 0.9283, and a data prediction value of 89.82%, as indicated by the predicted R-squared value of 0.8982. These results suggest that the concentration of *Ulva sp*. suspension, rotation speed, and mass of CO_2 tablets affect the BOD value response. The results of the RSM analysis indicate the influence of independent variables on each response. This influence is indicated by the presence of model equation coefficients, which are expressed in both coded and natural (actual) forms. The coded equation for the response obtained from the BOD value response analysis results is presented in Equation 4.

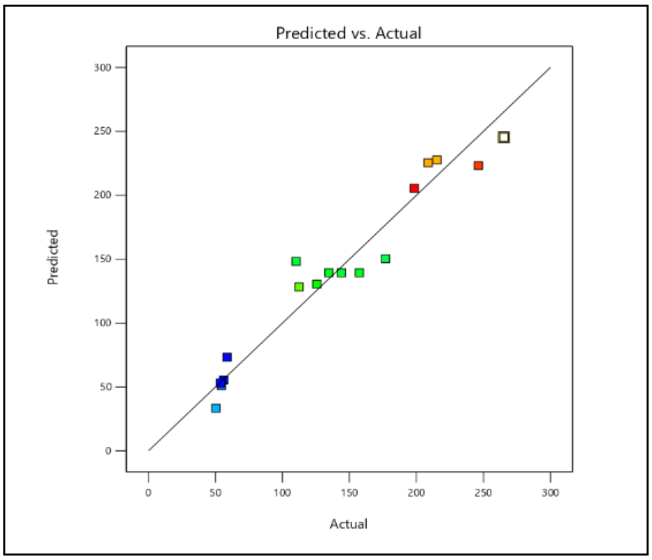

Fig. 4. Graph of actual values against predicted values for each BOD value response

$$BOD = 139{,}33 + 8{,}89A + 11{,}05B - 86{,}09C \quad (4)$$

Where A is the mass concentration of CO_2 tablets (tablets), B is the suspension concentration (%), and C is the rotation speed (rpm).

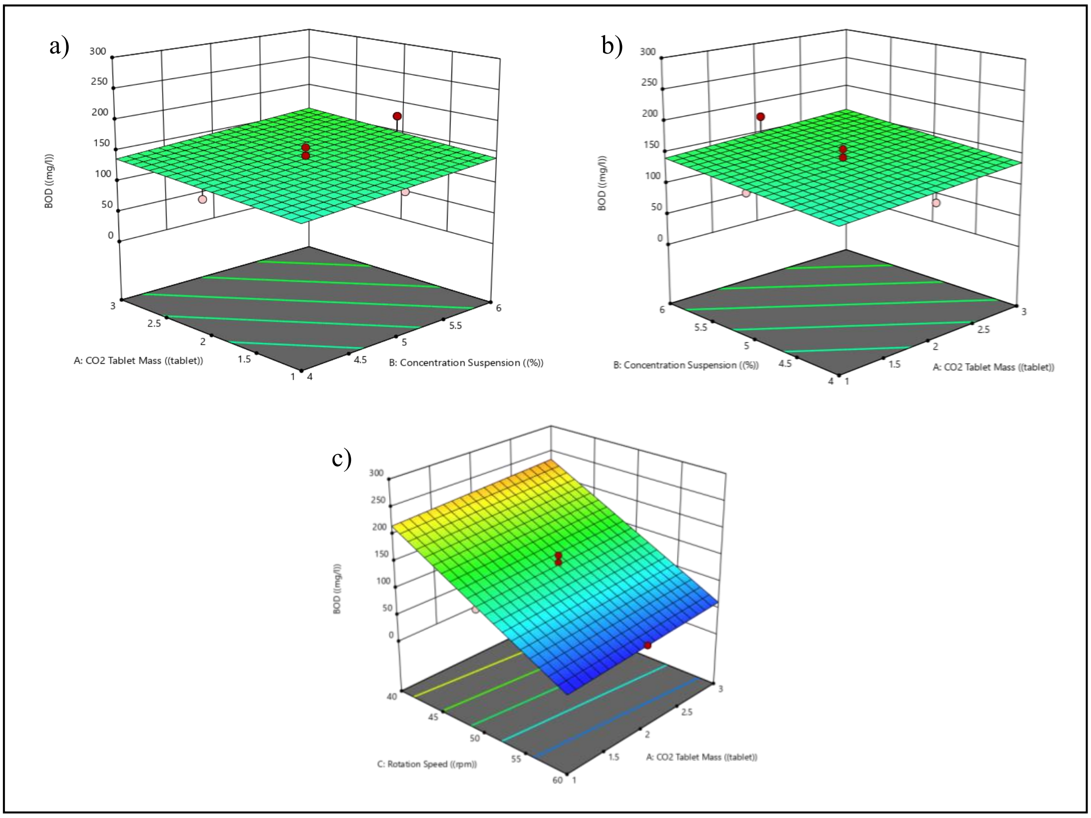

Fig. 5. Three-dimensional graph: (a) the effect of CO_2 tablet mass and suspension concentration, (b) the effect of suspension concentration and CO_2 tablet mass, and (c) the effect of rotation speed and CO_2 tablet mass on the BOD test

Based on Fig. 5 (a), BOD tends to increase with increasing mass of CO_2 tablets and suspension concentration. Fig. 5. (b) BOD tends to increase with growing suspension concentration and mass of CO_2 tablets. Fig. 5. (c) shows that BOD tends to decrease with increasing rotation speed and increases with increasing mass of CO_2 tablets. The highest BOD was obtained in the sample with a variable mass of CO_2 tablets (3 tablets), a concentration of Ulva sp. suspension of 6%, and a rotation time of 40 rpm, yielding a BOD of 265.4 mg/L. The lowest BOD was obtained in the sample with a variable mass of CO_2 tablets (1 tablet), a concentration of Ulva sp. suspension of 4%, and a rotation time of 60 rpm, resulting in a BOD of 50.3 mg/L and a removal efficiency of 90.17%.

The BOD content in batik wastewater is caused by the high levels of organic compounds such as protein, carbohydrates, oil, and fat, which require dissolved oxygen for the decomposition process. [53]. A speed of 60 rpm is considered to be the optimal speed for removing BOD content in this study. According to [54] Higher rotation speed increases oxygen transfer from the air into the wastewater. Liquid batik waste media is a liquid with a high concentration and high organic content. Hence, an appropriate rotation speed is necessary to optimize the metabolism of microorganisms through turbulence caused by rotation. This is also supported by research stating that one of the key factors in biofilm formation is turbulence caused by the rotation speed of the disc, which affects the direct transfer of oxygen from air to water [55]. Moreover, the rotation speed of the RABR plays a critical role in oxygen transfer. Operating the system at 60 rpm significantly enhances the oxygen transfer coefficient (kLa), with studies showing up to a 2.3-fold increase compared to 40 rpm [23]. Higher kLa values ensure sufficient dissolved oxygen supply, which is vital for aerobic bacterial activity in degrading BOD causing substances. Thus, optimizing hydrodynamic conditions such as rotation speed not only improves biofilm integrity but also enhances metabolic efficiency within the RABR system [26].

In contrast, the concentration of Ulva sp. suspension and the mass of CO_2 tablets were not significant in reducing BOD levels. Increasing the concentration of *Ulva sp.* is actually not good for reducing BOD because the high density of *Ulva sp*. can inhibit oxygen diffusion into wastewater, especially if there is decomposition of dead biomass, which actually increases BOD [56]. Similarly, the addition of CO_2 tablets in the processing of batik liquid waste. The addition of CO_2 tablets can provide additional carbon to support algae photosynthesis. However, in conditions where carbon is already sufficiently available (for example, through aeration or natural gas transfer mechanisms in the system), the addition of CO_2 tablets does not show a significant effect on reducing BOD. In some studies, the algae system has reached saturation in absorbing carbon, so that additional carbon no longer increases the efficiency of photosynthesis [51].

3.4 Validation & Comparison

1) Optimization of Independent Variables on the Response of Batik Waste Quality

The purpose of optimization is to obtain the optimum response value through analysis and calculation. If the mathematical model of each response is found, then optimization can be carried out [57]. Response measurements and independent variable data are used by Design Expert 13 software to perform optimization. The formula with the highest desirability value is the most optimal. The approach using the desirability function is employed to produce the optimum response, utilizing an empirical equation that involves more than one response [10]. Table 6 presents the independent variables and their corresponding optimum reactions in the study.

Table 6. Batik waste processing optimization solution

Independent Variable			**Response**		***Desirability***
Suspension Concentration (%)	***Rotation Speed (rpm)***	***CO_2 Tablet Mass (tablet)***	***COD Value (mg/L)***	***BOD Value (mg/L)***	
5.991	60	1	42.923	55.300	0.988

Based on the validation results, the optimal conditions were a rotation speed of 60 rpm**,** *Ulva sp.* concentration of 5.991%**,** and 1 CO_2 tablet**,** which produced a COD of 42 mg/L and a BOD of 56.2 mg/L**,** close to the predicted values of COD 42.923 mg/L and BOD 55.3 mg/L**,** with a desirability of 98.8%**.** The rotation speed of 60 rpm was optimal because it enhanced oxygen transfer and nutrient exchange without damaging the biofilm. The *Ulva sp.* concentration of 5.991% provided enough biomass for maximum pollutant absorption without causing overcrowding. Meanwhile, one CO_2 tablet supplied sufficient carbon for photosynthesis, supporting biofilm growth without excessively lowering the pH. This indicates that the model accurately represents the real system, and that biofilm dynamics and CO_2 supplementation play crucial roles in enhancing wastewater treatment efficiency.

2) Comparison with Previous Research

A comparison of the results of Ulva sp. cultivation conducted in this study with those of previous studies is presented in Table 7 and Fig. 6.

Table 7. Comparison of research results with previous research

Method	Types of Algae	Rotation Speed (rpm)	COD Value Decrease	BOD Value Decrease	Reference
Rotary Algae Biofilm Reactor (RABR)	*Chlorella sp.*	7	70.64%	73.42%	[16]
Rotary Algae Biofilm Reactor (RABR)	*Ulva sp.*	60	90.17%	65.9%	(This research)

Although the RABR system using *Ulva sp.* has demonstrated high efficiency in reducing COD and BOD levels, several limitations must be considered for its large-scale implementation. One of the main limitations is that the availability of *Ulva sp.* biomass is seasonal and highly dependent on environmental conditions such as salinity and water temperature. This may pose a challenge for continuous operations, thus requiring a consistent cultivation system or a reliable biomass supply throughout the year [25]. The results of this study are highly relevant to the SDGs, particularly SDG 6.3, which targets improved water quality through reduced pollution, elimination of hazardous chemicals, and increased recycling and safe reuse of wastewater [42]. The RABR system holds great potential for application in small and medium-sized enterprises (SMEs) such as the batik industry due to its energy efficiency, minimal secondary waste, and low chemical input requirements.

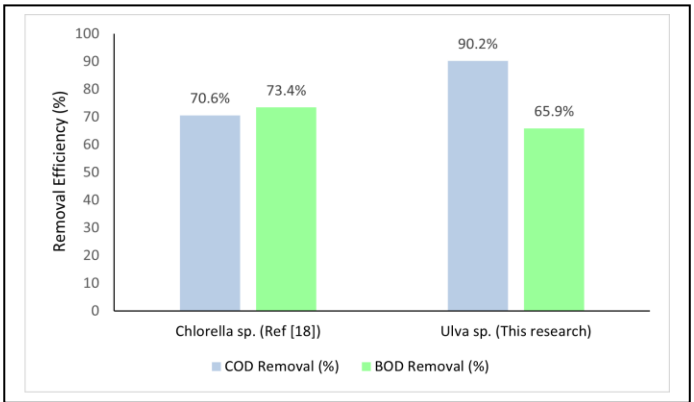

Fig. 6. Side-by-side bar charts comparing COD/BOD removal across studies

Further research is recommended to test the system in a continuous-flow mode to evaluate its long-term performance, resistance to fluctuations in pollutant loads, and sustained biofilm development. Continuous-mode studies are also essential to assess operational efficiency, energy costs, fouling potential, and overall system stability under real-world industrial conditions [23], [26].

4. Conclusion

This study demonstrates that the mass of CO_2 tablets, rotation speed, and the concentration of Ulva sp. suspension influence the treatment of 12 liters of batik wastewater in a 24-liter RABR. Optimal conditions were achieved at 5.991% Ulva sp., 60 rpm, and 1 CO_2 tablet, resulting in a reduction of COD to 42.923 mg/L and BOD to 55.300 mg/L, both of which are within national standards. The 60 rpm rotation enhanced oxygen transfer and reduced shear forces, facilitating efficient biofilm growth and degradation, while 5.991% Ulva sp. ensured sufficient biomass without overcrowding. A single CO_2 tablet supplies inorganic carbon, boosting photosynthesis and pollutant removal. The RABR-*Ulva sp*. system thus offers a low-cost, eco-friendly, and scalable solution for batik wastewater treatment, aligning with SDG 6 and SDG 12.

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