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PAPER

Performance Evaluation of a Moving Bed Biofilm Reactor and Its Microbial Diversity for Wastewater Treatment of a Desiccated Coconut Manufacturing Plant

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ABSTRACT

Desiccated coconut (DC) wastewater contains a high load of organic compound, which needs a series of unit operations. Most DC plants use anaerobic and aerobic biological treatments to remove organic compounds. This produces methane gas, a greenhouse gas (GHG), during anaerobic digestion, and a larger aeration tank to house more aerobic bacteria. The application of biofilm-carrying bacteria was studied to address these concerns. Moving bed biofilm reactor (MBBR) worked efficiently as the general biological treatment, even eliminating the anaerobic treatment. This study aimed to evaluate the performance of MBBR. Specifically, it aimed to determine the profile of wastewater, the microbial diversity of MBBR, and the optimized operation of MBBR using the central composite design (CCD) of response surface methodology (RSM). The wastewater profile showed high biological oxygen demand (BOD), oil and grease, total suspended solids (TSS), and low pH. 1.7 x 106 CFU/mL of heterotrophic bacteria was found in the MBBR tank with identified *Aeromonas veronii* using BLASTn. The bacteria produced enzymes crucial in breaking down complex organic compounds, thus employing a bioremediation process. The maximum removal of 98.67% was noted on a neutral pH of 7.5 and 160 minutes HRT, indicating an optimal condition, thus a potential technology in treating wastewater of DC plants.

KEYWORDS

MBBR, BOD, bioremediation, response surface methodology, microbial diversity

1 INTRODUCTION

With its tropical climate and fertile soil, the Philippines is one of the world's major exporters of desiccated coconut (DC) due to its abundance of coconut trees [1]. The desiccated Coconut plant generates huge amounts of wastewater – mainly from coconut water from matured coconut and processed water. Its wastewater has a high loading of organic compounds (i.e., lipids, suspended solids, and volatile fatty acids), which can hinder the efficiency of anaerobic and aerobic digestion processes. Thus, it requires a series of unit operations to attain the standard set of effluent [1], [2]. Existing wastewater treatment methods for desiccated coconut plants face several challenges, primarily due to the high organic load and inhibitory substances present in the effluent [3]. Wastewater generation from industries continuously increases globally, with only 55.5% currently being treated [4]. Discharging untreated wastewater added to water bodies has been a challenge to waste management, affecting the water bodies' physiochemical quality, resulting in degradation and the worst fatality of marine animals [5][6]. In the Philippines, statistics show that only 42.9% of effluent is treated, and 22,000 tons of untreated wastewater are discharged annually in Metro Manila alone [3]. Based on sampling results, 13% of the 126 freshwater bodies assessed exhibited poor water quality. Consequently, by 2025, many major cities will likely face water shortages, particularly affecting 8 of the 19 major river basins in the Philippines [5]. This entails that improper discharge due to the unavailability of a treatment plant or inadequate design can pollute the waterbodies (i.e., rivers, lakes, seas) and the groundwater – the source of potable water [7].

A study was conducted to improve the anaerobic digestion of wastewater generated in a Desiccated Coconut plant by adding an enzyme. Due to the high oil and grease concentration, an enzymatic hydrolysis pre-treatment is introduced. As a result, lipase pre-treatment increases biogas production, exhibiting lipids' biochemical degradation [1]. While enzymatic hydrolysis can enhance biogas production, it requires effective management of pH and substrate ratios [3]. In addition, a microbial fuel cell demonstrated 51.85% and 62.72% Chemical Oxygen Demand (COD) removal efficiencies when KMNO4 and carbon cloth electrodes in anolyte were used, respectively. Despite the advantages of this technology, there are still practical barriers to consider, including low electricity production, current instability, high internal resistance, and costly materials used [2].

Wastewater with high BOD is usually treated with a series of biological treatments, anaerobic, followed by aerobic treatment. Recent studies showed that the Moving Bed Biofilm Reactor (MBBR) works as effectively as conventional biological treatments. Hence, a study evaluated the MBBR using polyethylene as biofilm in treating terephthalic acid (TPA) wastewater. Using a lab-scale MBBR filled with Delfitia sp. WL-3, a 68% and 76% removal of COD and TPA, respectively, were observed in changing the organic load rate (ORT) and hydraulic retention time (HRT) [8]. Moreover, pharmaceutical pollutants are considered emerging contaminants. Thus, a study was conducted using sponge-based MBBR. A 96.7% chemical oxygen demand (COD) and 92.7% Ibuprofen (IBU) removal efficiency with a 10-hour HRT and 20% filling ratio is considered optimal. With this, MBBR demonstrated a promising technology [9]. MBBR has proven to increase the efficiency of wastewater treatment by innovating materials, optimizing operational parameters, and improving microbial dynamics. The major advantage is the use of biocarriers, such as high-density polyethylene (HPDE), which help the growth of bacteria and nutrient removal compared to traditional materials [10]. An example of bacterial integration is Rhodococcis sp. CPZ24. The biofilter shows improved membrane fouling and enhanced treatment performance [11]. Conditions for optimizing in terms of Hydraulic Retention Time (HRT), filling ratios, and microbial diversity could lead to improved nitrification and denitrification [12], [13]. However, the challenge is to scale up such technology and to be further studied by optimizing in varying conditions.

Wastewater effluents contain microorganisms that are beneficial to the ecosystems [14]. However, vast amounts of nutrients, like nitrogen and phosphorus, are critical and can result in eutrophication [14], [15], [16]. These nutrients must be removed to decrease the production of waterborne pathogens and toxin-producing bacteria [14].

The primary objective of this research is to evaluate the performance of the MBBR tank. It specifically aims to (1) determine the influent profile of wastewater, (2) determine the microbial diversity of the MBBR tank, and (3) create a mathematical model through optimization using the Central Composite Design (CCD) of Response Surface Methodology (RSM).

This research utilizes the newly built Wastewater Treatment Plant of Agri Exim Global Philippines Inc. The use of an MBBR is introduced by the University of Science and Technology of Norway under the corporation Kaldnes Miljoteknologi [17]. Its main objective is to address issues faced in other biological wastewater treatment methods [12]. Beneficial to companies, MBBR has been observed to have (1) a smaller tank volume compared to a clarified activated sludge but the same treatment efficiency in removing nitrogen and carbon-oxidation, (2) improved biomass retention, and high surface area for microbial growth, (3) eliminated the requirement in performing backwashing for active filter due to clogging [17], [18]. The success of this research provides baseline data for other Desiccated Coconut plants and other industrial plants with high concentrations of BOD.

This research focuses on the performance of the MBBR tank. It is limited to the BOD removal, influent, and effluent wastewater of the MBBR tank, with varying pH and HRT. The efficiency of the entire Wastewater Treatment Plant is not included in the research.

2 MATERIALS AND METHODS

2.1 Sampling site

The Wastewater Treatment Plant of AGRI EXIM Global Philippines Inc., a Desiccated Coconut plant, was located at Upper Quinocol, Barangay Darong, Sta Cruz, Davao del Sur, Philippines, with coordinates of (6.94559, 125.47373). It was designed to treat 200 - 240 m3 of wastewater per day.

2.2 Profiling of Wastewater Influent

Wastewater from the production line gravitationally went to the WWTP pre-treatment area. Wastewater was screened in a Rotary Screen to remove coarse particles and was pumped to a Vibrating Screen to remove fine particles. After being screened, wastewater was pumped into an Equalization Tank (EQ Tank) before Primary Treatment. The sample was taken using different containers in line with the parameter to be analyzed. A 4-liter plastic gallon was used for BOD, pH, and TSS. A glass bottle covered with foil before screw capped was used for Oil and Grease. The samples were transported to SGS Laboratory – Davao for analysis.

2.3 Assessment of Microbial Diversity in MBBR Tank Systems

The MBBR tank was filled with suspended Polyethylene carriers (biochips), supplied by Nam Trung Viet Environment Company, and comprised 40% of the reactor's volume. Table 1 and Fig. 1 show the physical and operational characteristics of the MBBR treatment system. A grab sample of mixed liquor was obtained from the MBBR tank, as shown in Fig. 2. It was sent to SGS Laboratory – Davao for Heterotopic Plate Count using the Pour Plate Method for microbial load determination. The agar plate, where the pure culture of bacteria, was sent to Omics Laboratory Services under the Philippine Genome Center Mindanao – University of the Philippines Mindanao for Capillary Sequencing. The cultured bacteria were subjected to DNA extraction and purification. The purified extracted DNA underwent amplicon sequencing for molecular identification using the Nucleotide Basic Local Alignment Search Tool (BLASTn) [38], [39].

Table 1. MBBR Treatment System.

Particular	Description
Diameter of chip, mm	30
Thickness of chip, mm	1
Surface contact area, m^2/m^3	≥ 4500
Density, kg/L	0.95 – 0.98
Dimension of reactor, m	4.5*4.5
Volume, m^3	93

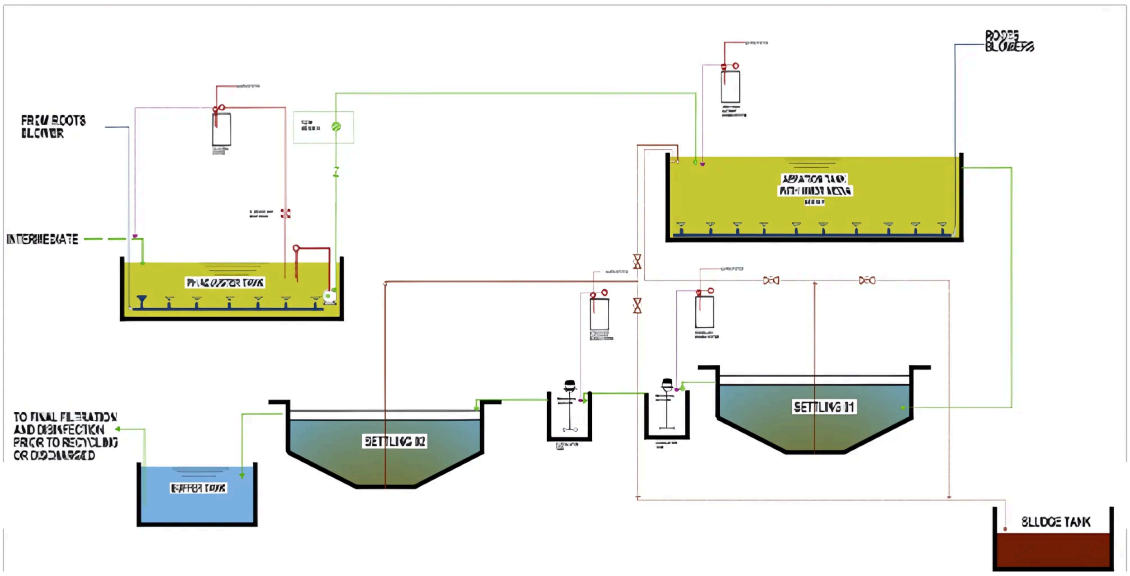

Fig. 1 Schematic diagram of newly built MBBR System.

2.4 Mathematical Modelling

Central Composite Design (CCD) from Response Surface Methodology (RSM) using free-trial Stat-Ease 360 Software was used to determine the optimum condition of the MBBR Tank. The method used mathematical and statistical methods to assess the significance of the set operating factors in BOD removal. Analysis of variance (ANOVA) with a 95% confidence interval was used to evaluate the validity of the proposed model. Correlation coefficients (r^2) and tests for lack-of-fit were also determined to confirm the fit quality of the response surface models. The optimized response surface plot was adopted to show the best process parameters [9]. Two parameters were set as independent variables: pH and Hydraulic Retention Time (HRT). Table 2 shows the experimental conditions based on the factorial design. The pH was set to 6.5 – 8.5, as optimal for biological activity [19]. The HRT was calculated using Equation 1 with Influent Flow Rates (IFR) of 7 m^3/hr, 8 m^3/hr, and 9 m^3/hr and Return Activated Sludge (RAS) flowrate of 27 m^3/hr. These flow rates were used based on the designed treatment capacity.

Fig. 2 a) MBBR Reactor, b) Biochips with attached biofilm.

The chemically treated wastewater was pumped to the pH tank and adjusted the pH by adding Sodium Hydroxide (aqueous solution) with a concentration of 3.0625 M, prepared from dissolving 25 kg of 98% purity Caustic Soda Flakes in 200 L of water. A sample was obtained in the Diffused Air Flotation (DAF) tank discharge, stored in a plastic gallon, and labeled as initial BOD (BODi). This was treated as the control and was repeatedly obtained together with the different runs. The wastewater was pumped to the Intermediate Tank and was regulated based on the desired HRT. The discharge from the Intermediate Tank gravitationally entered the MBBR tank. Biologically treated wastewater sample was taken in the Settling Tank 1 and followed the Design of Experiment (DOE) as shown in Table 5 with 13 experimental runs. The sampling was conducted for two weeks; two days were needed each week to obtain samples per run and control. The obtained samples were labeled as final BOD (BODe). The BOD removal (%) was calculated using Equation 2. The empirical formula (Eq. (3)) for each response (Y = pH, HRT) was correlated to the intercept (β_o), linear (β_1,β_2), interaction (β_{12}), and quadratic (β_{11},β_{12}) regression.

$$HRT, min = \frac{Volume\ of\ reactor}{IFR\ + RAS}\ X\ 60 \qquad (1)$$

$$BOD\ removal\ (\%) = \frac{BOD_i - BOD_e}{BOD_i}\ X\ 100. \qquad (2)$$

$$Y = \beta_o + \beta_1 x_1 + \beta_2 x_2 + \beta_{12} x_1 x_2 + \beta_{11} x_1^2 + \beta_{22} x_2^2 \qquad (3)$$

Table 2. Independent variables in the RSM.

Factors	Symbol	Coded level		
		-1	0	1
pH	A	6.5	7.5	8.5
HRT (min)	B	155	160	165

3 RESULTS AND DISCUSSION

3.1 Wastewater Influent Profile

The wastewater generated in the Desiccated Coconut plant consists of high concentrations of medium-chain saturated triglycerides. It came from washed coconut meat, coconut water, and by-products of coconut milk during extraction. Table 3 shows a high oil and grease concentration was detected in the influent, with a lower pH due to excess coconut water [20], [3]. The profile was aligned with the typical wastewater quality in Dessicated Coconut Manufacturing Plant, as shown in Table 4 [21].

Table 3. Wastewater Influent Profile of Desiccated Coconut plant.

Parameter	Result	UoM
BOD	9,569	mg/L
TSS	3,600	mg/L
Oil and Grease	2,247	mg/L
pH	5.4	

Table 4. Typical wastewater quality of some industries in the Philippines – Manufacturing of Desiccated Coconut.

Parameter	Result	UoM
BOD	6,000 – 10,000	mg/L
COD	17,000 – 20,000	mg/L
TSS	2,400 – 4,000	mg/L
pH	5.0 – 6.3	

3.2 Microbial Diversity of MBBR Tank

Microbial count in the MBBR tank showed an abundance of Heterotrophic bacteria with a count reaching 1.7 x 106 CFU/ml. This showed that a huge population of microorganisms was present in the nutrient cycling, as shown in Fig. 3. Using the BLASTn tool for microbial identification, the species *Aeromonas veronii* strain ANYA 18661 chromosome was identified. And another possible strain of *Aeromonas allosaccharophila* strain CB-15 with partial sequence.

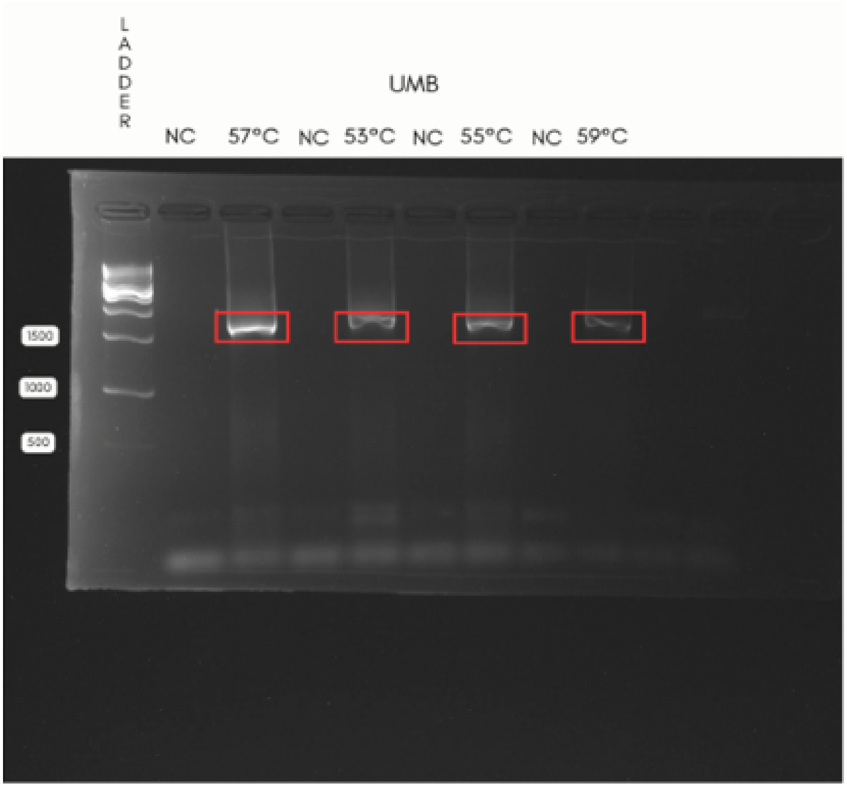

Fig. 3 Gel image of amplified DNA using 16S primer. Samples were run at different temperatures with the ideal annealing temperature at 53°C, 55°C, 57°C, and 59°C.

Aeromonas veronii was known to produce enzymes that could degrade organic material in wastewater [22]. The identified enzymes were cellulase, pectinase, protease, and amylase [23], which contributed to the hydrolysis of organic matter and breaking it down employing a bioremediation process – removal of contaminants using microbes and bacteria [24]. Hence, it could also resist heavy metals and hemolytic properties, which can potentially detoxify polluted sites, resulting in a valuable organism for environmental management, making it a significant candidate for further research and industrial use [22][24].

3.3 Mathematical Modelling

Stat-Ease 360 Software was used to analyze variance (ANOVA) to determine the optimum condition of the MBBR Tank by using the experimental results for BOD removal under various conditions, as shown in Table 5. The CCD model was used to evaluate the factors for optimizing the BOD removal from the wastewater of the Desiccated Coconut plant. The established model was determined, and the optimal conditions were identified. The relationship between the response variable and factor variables has been expressed as an equation (Eq. 4).

$$BOD\ removal\ (\%) = -1150.43692 + 86.41586*pH + 11.30001*HRT - 0.258500*pH*HRT - 2.85750*pH^2 - 0.0288*HRT^2 \quad (4)$$

The quadratic model was statistically significant with a p-value of 0.0315, less than 0.05, at the confidence level of 95%. The Lack of Fit has a p-value of 0.1978, greater than 0.05, indicating a non-significant, which is desirable for the obtained model. This indicated that the independent variables used and set have a smaller difference in % BOD removal. According to Table 6, model terms of A and A^2, have p-value <0.05, contributing positively to the model. Conversely, model terms of B, AB, and B^2, with p-value > 0.05, could negatively impact the model and must be further investigated. Increasing the sampling size and adding another variable (e.g., BOD influent) could be considered. Additionally, the model F-value of 4.82 implied that the model was significant, and there was only a 3.15% chance that this large model could occur due to noise. Correlation coefficients ($r^2 = 0.7748$), are a good indicator of the model's fit.

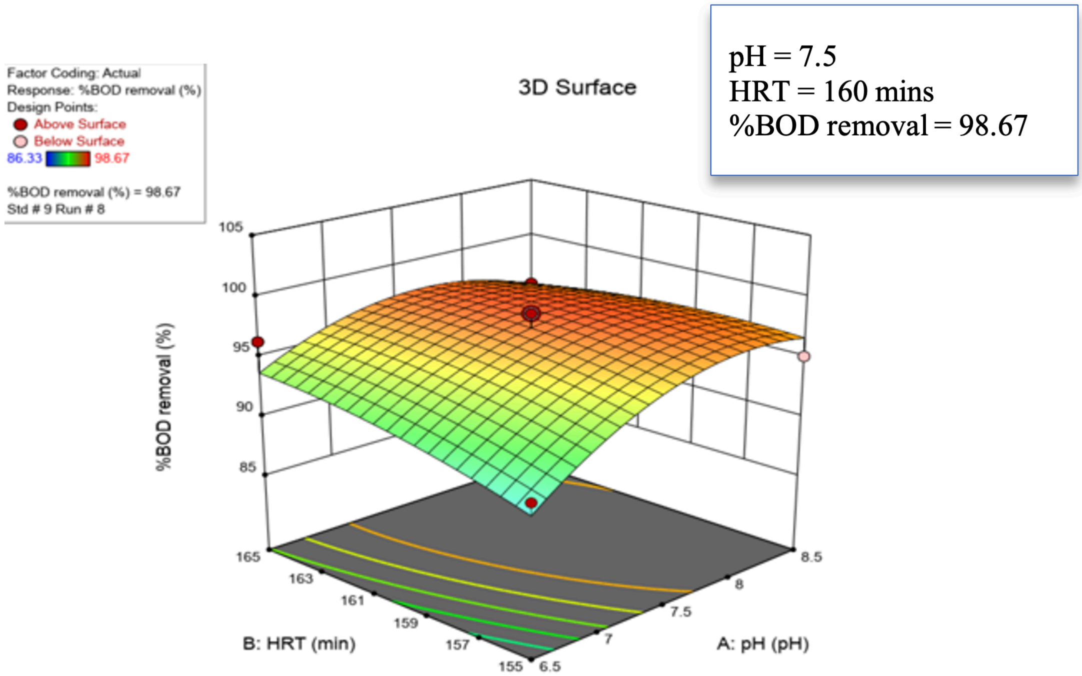

Fig. 4 Response surface plot of the effects of pH and HRT on % BOD removal.

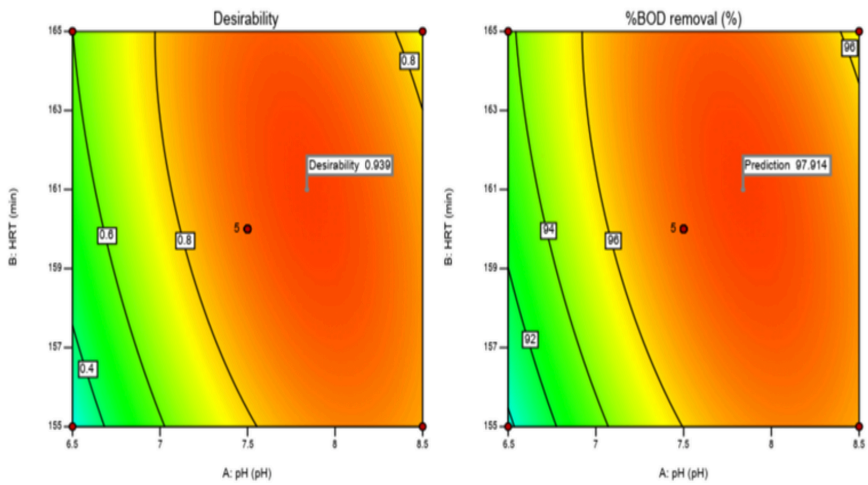

Fig. 5 Relationship between pH and HRT to Desirability and BOD removal efficiency.

12 out of 13 runs exhibited favorable results in the effluent BOD. The constructed experimental design demonstrated an average of 94.86% BOD removal. The maximum removal of 98.67% was noted on a neutral pH of 7.5 and 160 minutes HRT. The pH of entering the MBBR tank significantly affects its treatment efficiency, showing a p-value of 0.0353, less than 0.5, based on Table 6. This coincided with the findings on achieving neutral pH conditions; it promoted the highest biofilm concentration and removal rates, underscoring the critical role of pH in shaping microbial communities [25]. Maintaining an optimal pH can maximize the treatment efficiency

of the MBBR tank [26]. Hence, adjusting pH does not directly stabilize carbonaceous matter; it creates optimal conditions for the growth of microorganisms, highlighting the indirect role of pH in biological treatment [27]. In contrast, the 6.1 pH did not pass the effluent standard, indicating that the acidic conditions in aerobic biological wastewater treatment can disrupt microbial activity, leading to reduced treatment efficiency, as evident in the results obtained with only 90.67 and 86.33 % removal in pH of 6.5 and 6.1 respectively [28], [29].

Table 5. Design of Experiment through RSM.

Run	pH	HRT (min)	BOD < 30 (mg/L)	BOD removal (%)
1	8.5	165	18	95.61
2	6.5	165	11	96.33
3	7.5	160	20	98.67
4	7.5	160	20	98.67
5	8.5	155	20	95.12
6	7.5	160	13	95.67
7	7.5	160	14	95.33
8	6.5	155	28	90.67
9	7.5	160	26	93.66
10	7.5	152.929	18	95.61
11	8.91421	160	16	96.10
12	6.08579	160	41	86.33
13	7.5	167.071	19	95.37

Table 6. ANOVA table of the response surface quadratic model

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	107.04	5	21.41	4.82	0.0315	significant
A-pH	38.49	1	38.49	8.66	0.0216	
B-HRT	4.22	1	4.22	0.9495	0.3623	
AB-[pH][HRT]	6.68	1	6.68	1.50	0.2598	
A^2-pH^2	56.80	1	56.80	12.78	0.0090	
B^2-HRT^2	3.61	1	3.61	0.8133	0.3977	
Residual	31.11	7	4.44			
Lack of Fit	20.31	3	6.77	2.51	0.1978	not significant
Pure Error	10.80	4	2.70			
Cor Total	138.16	12				

On the other hand, a 0.3623 p-value of HRT was obtained, greater than 0.05, indicating that HRT was statistically insignificant. In a recent study, the impact of HRT in MBBR was statistically insignificant due to a stable microbial community from the addition of biofilm [30]. The biofilm characteristics and media properties can lead to better treatment efficiency. An example of this was the high-density propylene (HDPE) media, which has greater stability in performance under varying operational conditions than PPE carriers. The HDPE media enhanced the biofilm growth and overall reactor performance [31]. Moreover, this showed that higher or lower HRT would yield a high % BOD removal. HRT significantly affects MBBR performance; longer HRT improves the removal efficiencies of organic pollutants and nutrients, while shorter times increase biofilm thickness, affecting overall reactor performance and effluent quality [32]. This is true for MBBR, which allows microorganisms to attach to the media, enhancing treatment efficiency and reducing sludge return, which minimizes the sludge management challenge [33]. A balanced biofilm accumulation and suspended biomass known as the mixed liquor can be referred to as the biosolids dynamics, an essential in optimizing MBBR performance [34]. Lower HRTs can achieve high removal efficiency using MBBR. A study showed that one (1) hour HRT can remove over 90% of BOD in saline-produced water. Thus, the statistical insignificance of HRT can still demonstrate a robust performance of MBBR and may not be the sole determinant of system performance across varying HRTs, maintaining high removal efficiencies and operational stability with varying feed composition [35], [36].

The response surface plot (Fig. 5) showed the combined effects of pH and HRT on BOD removal. The plot reveals % BOD removal = 83.33-98.67% within the tested conditions. The response surface plot reveals adjustments on the response % BOD. It is recommended to have an operating condition of pH from 6.92 to 8.08 and HRT from 156.58 to 163.42 minutes to have a % BOD removal of greater than 95%. The parameters were optimized by using the optimization function of Stat-Ease 360 software. The main objective is to maximize the BOD removal and minimize the HRT. According to the CCD design, the optimum conditions were pH = 7.839, HRT = 160.996 minutes, pH = 7.836, HRT = 161.042 minutes, and pH = 7.840, HRT = 160.956 minutes for a maximum BOD removal of 97.914% with a desirability score of 0.939 as shown in figure 6, a high level of desirability nearing 1. Based on the experimental setting, the peak BOD removal of 98.67% was achieved under pH = 7.5 and HRT = 160 minutes, closely resembling the results from the CCD design. This coincides with the study of treating municipal wastewater using MBBR with a 77% and 80% removal efficiency using an HDPE biofilm carrier [10], [37].

4 CONCLUSION AND RECOMMENDATION

This study evaluated the performance of MBBR and its microbial diversity in Desiccated Coconut plant wastewater treatment. The MBBR tank was filled with HDPE carriers and aerobic bacteria and was investigated with varying pH and HRT. The Desiccated Coconut plant wastewater contained high fats, BOD, and low pH. This was due to a high concentration of medium-chain saturated triglycerides. With a million aerobic bacteria present in the MBBR tank, one bacterium was identified as Aeromonas veronii, an enzyme-producing bacteria that helped break down complex organic compounds by employing a bioremediation process. Moreover, the result showed that at a neutral pH of 7.5, the system obtained a higher % BOD removal in lower and higher HRT. The maximum % BOD removal, reaching 98.67%, was attained under a pH of 7.5 and HRT of 160 minutes. However, reducing the pH to lower than neutral resulted in a decrease in % BOD removal and a failed BOD of 41 ppm, higher than standard. Creating a neutral environment for the bacteria generally improved the removal of BOD, and it did not significantly affect the treatment process in terms of decreasing and increasing the HRT. Thus, the MBBR system could be a promising technology used in Desiccated Coconut plants to address problems, particularly BOD removal.

Furthermore, further research is recommended to optimize the primary treatment (chemical treatment) and determine the BOD concentration that will enter the MBBR tank. Also, it is recommended to identify other microbial species and their ability to degrade BOD. Lastly, the treatment cost could be identified as a result of optimizing the entire system for better revenue.

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