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BIOREMEDIATION OF RESTAURANT WASTEWATER USING IMMOBILIZED GREEN MICROALGAE, (*BOTRYOCOCCUS* SP.) ON SEQUENCE BATCH REACTOR STORAGE DESIGN

Wastewater is produced by natural biological processes and a variety of human activities. It is unfit to use directly as the wastewater contains variety of chemicals, contaminants, and pollutants. Wastewater is categorized into two groups of sources which are greywater and blackwater as they differ in contamination levels. Greywater are from showers, baths, whirlpool tubs, washing machines, dishwashers and sinks except for the kitchen sink while the sources of black water are from toilets and kitchen sinks. This study aims to design a Sequence Batch Reactor (SBR) equipped with microalgae microbeads (*Botryococcus* sp.), to examine the applicability of SBR technology in greywater treatment specifically evaluating the efficiency of SBR performance and to measure the efficiency in removing pollutant greywater of microalgae beads. This was done with the application of SBR to ensure the greywater are properly treated before being discharged.

Keyword: Wastewater; microalgae; *Botryococcus* sp.; Bioremediation

1. Introduction

The essential element of living is water. The rapid expansion of industrial society caused a notable rise in water demand [1]. In this era of globalization, the world has been exposed to several pollutions, which is mainly water pollution. Activities that caused water pollution were waste from the industrial sector, sewage and waste water, mining activities, pesticides and chemical fertilizers, radioactive waste, domestic wastewater, and urban development.

According to [2], in 2022, 42.2% of household wastewater flow was not properly treated on a global scale. Globally, there are significant regional differences in wastewater treatment. In North America and Europe, 86.5 percent of the wastewater flow from households is safely treated; in Sub-Saharan Africa and Central and Southern Asia, the percentage is less than 25 percent. Fig. 1 has depicted one of the wastewater proportions which is domestic wastewater flow that has been safely treated worldwide in 2022, by region.

When water was contaminated and decontamination process is required, the best purification strategy should be selected to meet the decontamination goals. A blend of physical, chemical, and/or biological procedures and processes were used in conventional wastewater treatment to eliminate solids from effluents, including colloids, organic matter, nutrients, and soluble pollutants. The primary sources of wastewater were from residential usage, industrial activities, establishments of commercial, runoff of stormwater, and also from agriculture activities [3].

Wastewater from residential usage is known as domestic wastewater too. It is accumulated when water has been used in a home for activities like drinking, bathing, washing dishes, flushing toilets, and doing laundry. Water is used by industries in their operations, and the water that is discharged often contained different chemicals, heavy metals, and pollutants, adding to industrial wastewater. Wastewater also got produced when commercial facilities like hotels, restaurants, and offices used water. Stormwater runoff, rainwater that traveled over cities and picked up debris and pollutants along the way, was frequently regarded as a form of wastewater.

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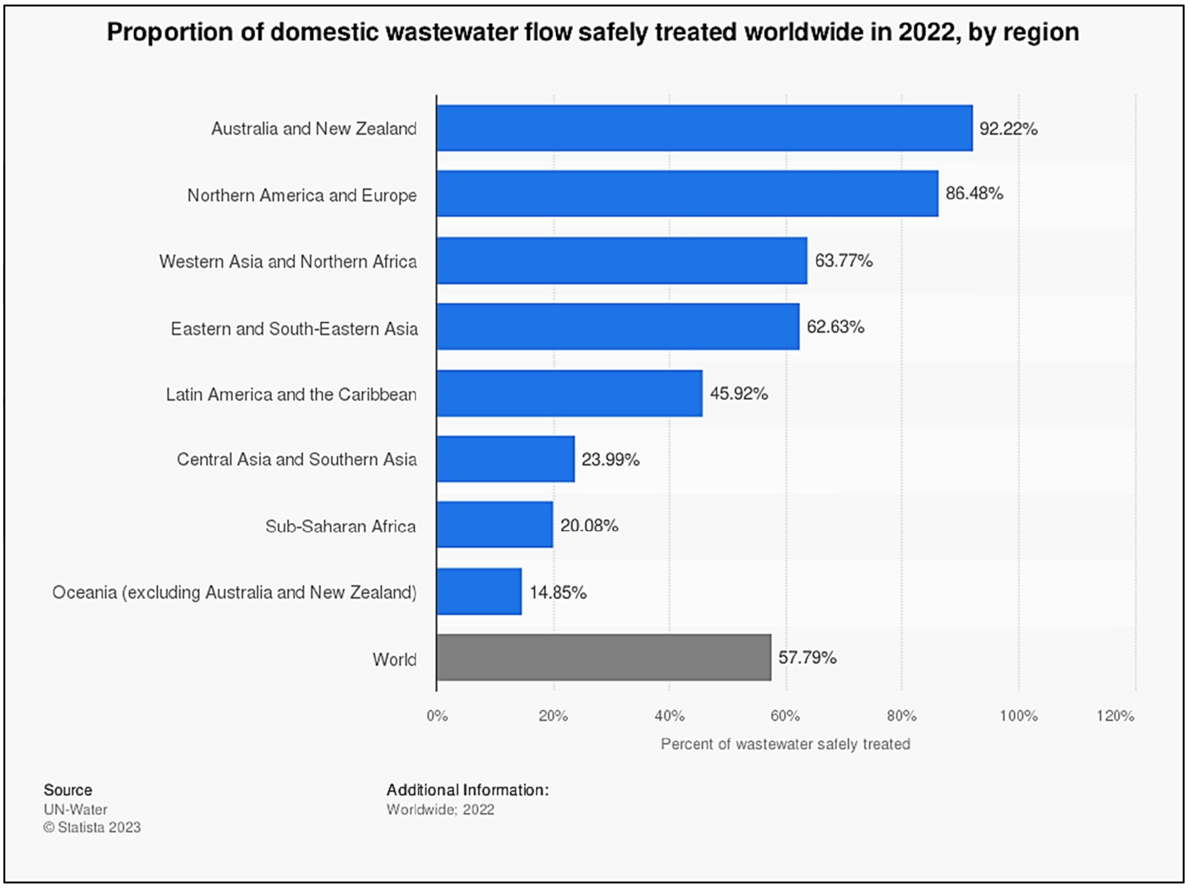


Fig. 1. Proportion of domestic wastewater flow safely treated worldwide in 2022, by region

TABLE 1

Physiochemicaldata for restaurant wastewater characteristic

Parameters	Characteristics	Initial value of water quality (n =)	National Water Quality Standards for Malaysia (Class IIA)	Environmental Quality (Sewage) Regulations (Standard A)
pH	Physical	8.2	6.0-9.0	6.0-9.0
Turbidity	Physical	37.48 NTU	50	—
Chemical Oxygen Demand (COD)	Chemical	868.2 mg/L	25	120

The goal of improving water purification techniques while keeping costs low has been worked on by researchers around the world. The researchers started to create technologies that enable products made from wastewater to be both economically and socially viable [4-5]. Microalgae has been one of the bright lights of this new paradigm. It was also able to decrease the concentration of toxic substances like heavy metals or pharmaceuticals, as well as nitrogen and phosphate [6-7]. Additionally, nutrient recovery, CO2 capture, and microalgal biomass production can all benefit from using microalgae-based wastewater treatment and flue gas absorption [8-9].

Botryococcus sp. has been proven effective for wastewater treatment. According to Gani et al. 2023 [6], due to their high binding affinity, large surface area, and abundance of binding sites, microalgae are increasingly being used in the phycoremediation of toxic heavy metals. It was revealed that Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD) have been significantly reduced by the usage of *Botryococcus* sp. [9]. Besides, *Botryococcus* sp., is a nutrient-removal-beneficial organism, could grow sustainably in secondary-treated sewage [11].

The objective of the present study is to assess the effectiveness of the immobilized *Botryococcus* sp. in removing pollutants, particularly organic matter and nutrients, from restaurant wastewater. These pollutants were typically present in high concentrations in restaurant effluents and can pose environmental risks if not adequately treated before discharge. Consequently, by employing the immobilized microalgae within the SBR storage design, the present study has aimed to demonstrate the capability of *Botryococcus* sp. in efficiently and sustainably treating restaurant wastewater (RW). The microalgae's ability to absorb and convert nutrients, such as nitrogen and phosphorus, and break down organic matter through photosynthesis is expected to play a vital role in the bioremediation process.

The research of the immobilized green microalgae, specifically, *Botryococcus* sp. usage within the SBR has not been studied by other researchers yet. Moreover, the parameters involved, which were pH, turbidity of waters, and (COD) for the immobilized green microalgae, *Botryococcus* sp. did not analysed elsewhere and the contribution of this study is also known as evidence gap. Previous researchers had only scrutinized about using other types of microalgae with other parameters [12], excluding the analysis of different concentration of microalgae beads too. Hence, using the correct number of parameters with the right concentration of microalgae beads definitely has given the best result in this study, where the SBR tank's performance has been improved.

Besides, the application of SBR in this study has demonstrated the technology's potential for treating wastewater that is complex and variable, like that found in restaurants. Traditional wastewater treatment methods might not be as effective or adaptable in handling the kind of wastewater generated by restaurants, which can have high levels of grease, food waste, and cleaning chemicals. The use of SBR and *Botryococcus* sp. offers a more tailored and efficient solution. Overall, this approach to wastewater treatment not only helps in achieving better water quality but also aligns with sustainable and environmentally friendly practices. The adaptability and efficiency of SBR systems, combined with the natural bioremediation capabilities of *Botryococcus* sp., present a compelling solution for managing the complex wastewater from restaurant environments.

2. Materials and method

2.1. Restaurant Wastewater Sampling

Restaurant wastewater (RW), have been collected from SS Curry House Pagoh Jay, Pagoh, Johor and transferred into a 20L bottle. The samples were kept in a sterilized container and was stored at 4°C at the laboratory prior to the experiment. Parameters such as pH, turbidity of waters, and (COD) samples were tested as described by APHA (2012) [13].

2.2. Preparation of the *Botryococcus* Sp. beads

In this study, freshwater microalgae *Botryococcu*s sp. sourced from the tropical rainforests in southern Malaysia were utilized. To prepare the microalgae for experimentation, they were cultured in Bold Basal Medium (BBM) to create an inoculum solution as detailed by Gani et al. 2023 [7].

The microalgae cells were then harvested using the flocculation method, where alum was used as a coagulant and stirred at 80 rpm for 3 minutes, followed by a reduction to 30 rpm for 20 minutes. The cell density was determined using a spectrophotometer at 600 nm. To create the immobilized microalgae beads, a specific volume of the concentrated *Botryococcus* sp. cell suspension was thoroughly mixed with an alginate solution of 50 g L^{-1}. This suspension was then carefully dripped into a 500 mL solution of $CaCl_2$ (4%). The number of cells in the alginate solution was counted using a Neubauer improved hemocytometer, with the assistance of a compound microscope.

2.3. Sequencing Batch Reactor Set-Up

The primary step in the design of an SBR is to identify the influent wastewater characteristics, design flow, and effluent specifications for the proposed system. The indicator of influent wastewater is pH, ammonia nitrogen, BOD, COD, TSS, alkalinity, temperature, and total phosphorus. For the treatment of domestic and commercial wastewater, additional precise parameters might be required. The important SBR design parameters were identified after learning about the system's influent and effluent characteristics. Fig. 2 below shows the pre-setup of SBR.

3. Results and discussion

3.1. Characteristic of Restaurant Wastewater

The parameters tested were pH, turbidity of waters, and (COD). The tested parameters have been discussed with the

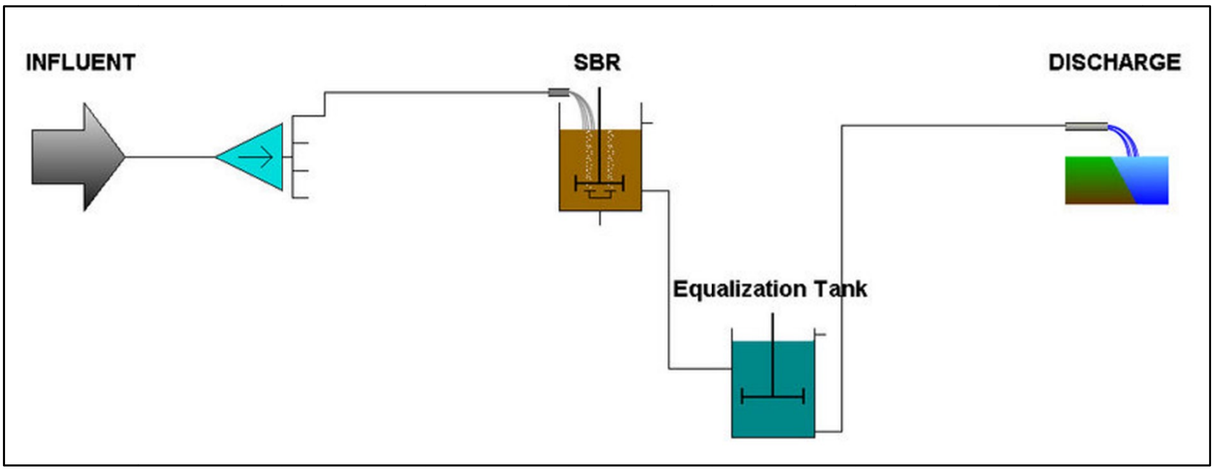


Fig. 2. Pre-setup of Sequencing Batch Reactor (SBR)

comparison to effluent standard limits set by the National Water Quality Standards for Malaysia (Class IIA) and Environmental Quality (Sewage) Regulations (Standard A) discharge limit.

3.2. Bioremediation of Restaurant Wastewater Using Immobilized by *Botryococcus* sp. in SBR

Three parameters were analyzed in this study; pH, turbidity and COD. The graphs of different volume of microalgae *Botryococcus* sp. in SBR for RW bioremediation are shown in Figs. 3-4.

3.2.1. Analysis of pH

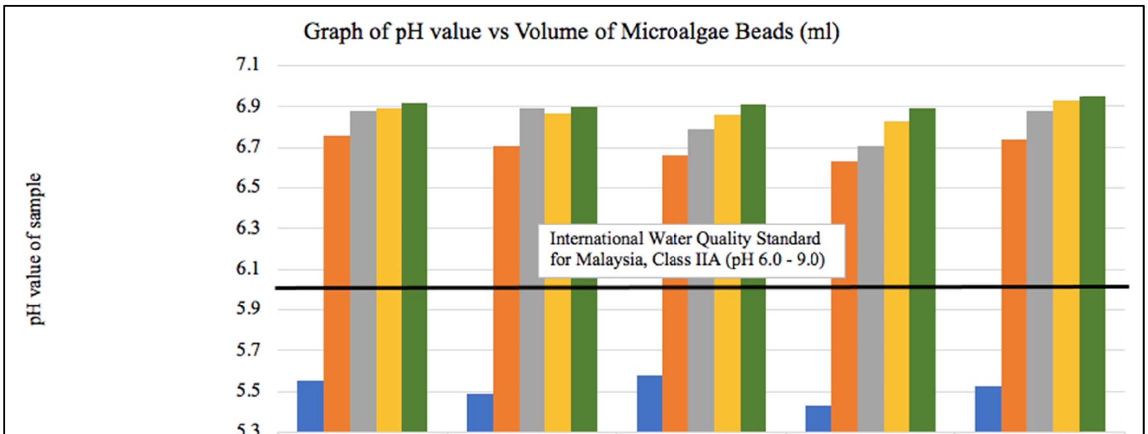
The pH scale measures the negative logarithm of the hydrogen ion concentration (pH = –log10 [H+]). For both natural water and wastewater, the hydrogen-ion concentration is a critical quality indicator. In order to protect organisms, wastewater must have a pH of 6 to 9 [14]. The process of treatment can be stopped by acids and other things that change pH. Three components consist of a typical pH measuring system: a pH electrode, a temperature compensation element, and a pH meter or controller. The process of taking a pH reading involved by rinsing the electrode tips until a click is heard. When the reading stabilized, the information was recorded.

It was important to observe the acidity, neutral and alkalinity of the water sample to measure the pH level. Hence, the pH level of the greywater sample was calculated in 100 ml, 150 ml, 200 ml and 300 ml of microalgae beads (*Botryococcus* sp.). The reading was collected at an immediate time to prevent any technical error and were repeated 5 times with 5 water samples taken from the same study area. It was to detect the changes in pH values when the microalgae beads of different concentration were placed in the SBR tank. The SBR should design such a way that pH should not fall below 6.0 in the reactor. Here, in our experiments pretreated greywater was used and the average pH level of the water sample was 6.57. The measurement obtained are shown in the chart below.

According to the Fig. 3, the pH reading for all 5 water samples in 5 tests conducted in SBR tank has shown an increase reading in pH value respectively in according to different concentrations of microalgae beads introduced to them for 100 ml, 150 ml, 200 ml and 300 ml of the microalgae beads (*Botryococcus* sp.) treatment. As for the 100 ml of microalgae beads treatment, the highest pH reading was observed at 6.76 and the lowest was at 6.63 with an average value of 6.70. Meanwhile, for the concentration of 150 ml microalgae beads, the highest pH reading was observed at 6.89 and the lowest was at 6.71 with an average value of 6.83. For the concentration of 200 ml microalgae beads, the highest pH reading was observed at 6.93 and the lowest was at 6.83 with an average value of 6.88. For the concentration of 300ml of microalgae beads, the highest pH was observed at 6.95 and the lowest was 6.89 with average of 6.91. The pH value of the blank sample has the highest number at 5.58 while the lowest is at 5.43.

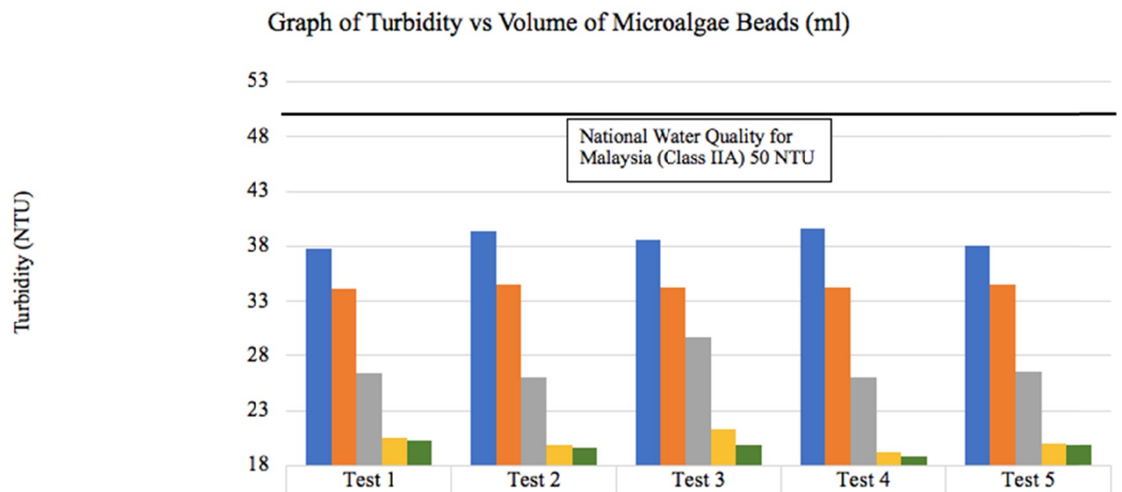
3.1.2. Analysis of Turbidity

Turbidity is measure of clarity and transparency of water [15]. Although it is a measurement of the amount of light that is either absorbed or scattered by suspended material in water, suspended solids were not directly quantified by turbidity. Absorption and scattering were influenced by the suspended material's size and surface properties. The turbidity meter was used to indicate the dispersion degree of lights produced by insoluble grain matter which was suspended in water. It can characterize the quality of these suspended grain matter. The determination of turbidity required the preparation of standard vials where



	Test 1	Test 2	Test 3	Test 4	Test 5
Blank Sample	5.55	5.49	5.58	5.43	5.53
100ml of Microalgae beads	6.76	6.71	6.66	6.63	6.74
150ml of Microalgae beads	6.88	6.89	6.79	6.71	6.88
200ml of Microalgae beads	6.89	6.87	6.86	6.83	6.93
300ml of Microalgae beads	6.92	6.9	6.91	6.89	6.95

Fig. 3. The Graph pH value versus Volume of Microalgae Beads (ml)



	Test 1	Test 2	Test 3	Test 4	Test 5
Blank Sample	37.8	39.4	38.6	39.6	38.1
100ml of Microalgae beads	34.1	34.5	34.3	34.3	34.5
150ml of Microalgae beads	26.4	26.1	29.7	26.1	26.6
200ml of Microalgae beads	20.6	19.9	21.3	19.2	20.1
300ml of Microalgae beads	20.3	19.6	19.9	18.9	19.9

Fig. 4. The graph of Turbidity (NTU) rate versus Volume of Microalgae beads (ml)

at first the vials has been observed to detect any scratches or marks on the glass vial. The fresh vial was rinsed 3 times with approximately 2 ml to 3 ml of distilled water. The container then was filled with the water sample until it reached the reference line. Afterward, the surface of the vial was washed using desired cloth to get rid of all traces and dust caused by fingerprints. After completing the filling process, the vial then was inserted into the turbidity meter at the holder of the vial. The reading and the result can be observed on the turbidity screen. The results are reported in Nephelometric Turbidity Unit (NTU).

In this study, turbidity was measured using turbidity meter (Model Eutec TN-100) in a laboratory. The unit of measurement is called NTU. Low turbidity values indicated high water clarity; high values indicated low water clarity. This analysis also has evaluated the turbidity level of the water samples post treatment in designated SBR tank with different variables of microalgae beads concentrations ranging from 100 ml, 150 ml, 200 ml, 300 ml and a blank sample.

The effects of microalgae beads on reduction of turbidity at varying concentrations is shown in Fig. 4. Overall, turbidity reduction for the water samples differed significantly by the number of microalgae beads added into the SBR tank. In general, higher microalgae beads concentrations produced greater turbidity reductions, with poor reductions at a lower concentration. However, even at lower microalgae concentrations, of 100 g, 150 g and 200 g, there appeared to be dose effects. When dose was increased to 300 g of microalgae beads, turbidity reduction also decreased.

All water samples have shown the same reading pattern, which shown a reduction in turbidity over the increasing in microalgae beads concentrations for all water samples in 5 tests conducted. The graph above shows the percentage of turbidity removal based on different concentrations of microalgae beads (*Botryococcus* sp.).

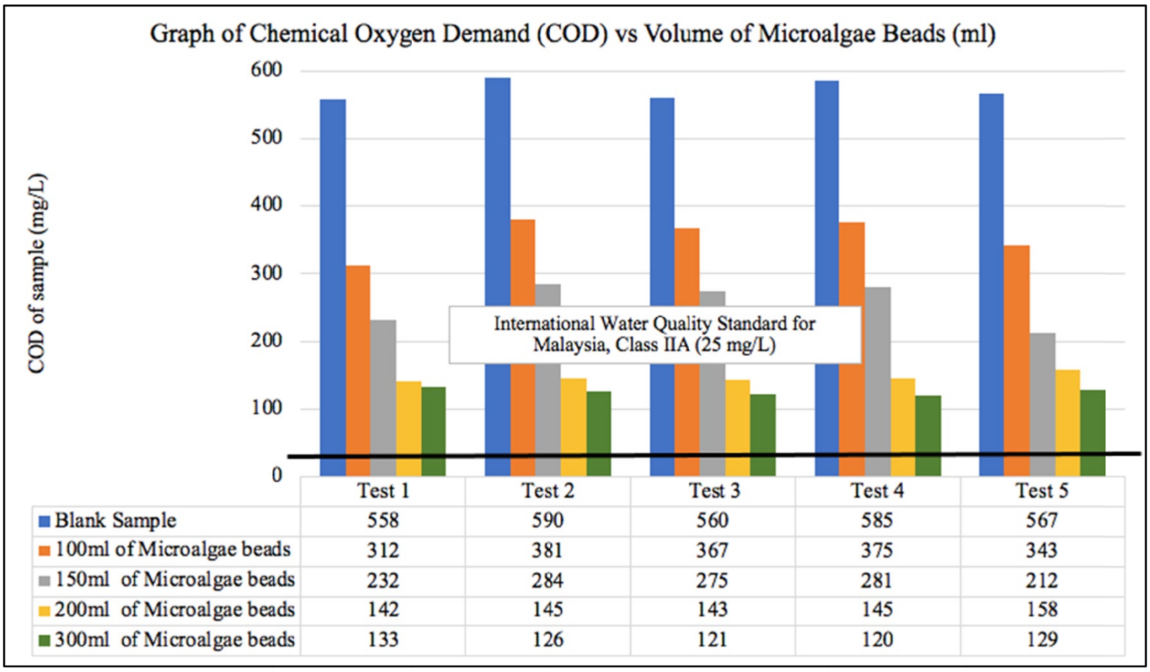
3.1.3. Analysis of Chemical Oxygen Demand (COD)

One of the most common research methods used to determine the characteristics of sewage is the COD [16]. The equivalent quantity of oxygen needed to completely oxidize the organic material in a water sample using a potent chemical oxidizer is known as COD.

COD also has the potential to be thought of as a sum parameter that is used to determine how much organic matter is present in wastewater. The oxygen demand produced by both biodegradable and non-biodegradable materials is taken into account in the COD values. Therefore, COD values are higher than BOD values. The sample needs to be prepared with a blank and zero in absorbance mode where 5 ml deionized water in a vial is used. With this, a measurement of the blank's absorbance is made.

The COD test was carried out to observe the capacity of water consuming oxygen during the composition of organic matter. This study measured COD readings of the water samples in variable concentrations of microalgae beads (*Botryococcus* sp.) range from 100 ml, 150 ml, 200 ml and 300 ml. The test was also conducted for 5 times with 5 water samples taken from the same study area for a more precise data.

According to Fig. 5, COD value of the water samples with variable volume of microalgae beads (*Botryococcus* sp.) range from 100 g, 150 g, 200 g and 300 g in 5 tests that have been observed. The blank sample had a high initial COD reading range from 555 ml-595 ml with an average of 572 ml. Prior to treatment with 100 ml of microalgae beads (*Botryococcus* sp.) volume, the COD reading of the water samples decreases to 310 ml-390 ml respectively with an average of 355.6 ml. Meanwhile, for volume of 150 ml microalgae beads (*Botryococcus* sp.), the COD reading was between 210 ml-290 ml with an average of 256.8 ml and 140 ml-1600 ml with an average of



	Test 1	Test 2	Test 3	Test 4	Test 5
Blank Sample	558	590	560	585	567
100ml of Microalgae beads	312	381	367	375	343
150ml of Microalgae beads	232	284	275	281	212
200ml of Microalgae beads	142	145	143	145	158
300ml of Microalgae beads	133	126	121	120	129

Fig. 5. The graph of COD (mg/L) versus microalgae beads concentrations (ml) for 5 tests

146.6 ml for volume of 200 g. Overall, the lowest COD reading for the greywater samples obtained was with the 300 g volume of microalgae beads (*Botryococcus* sp.) treatment which was 120 ml-140 ml with an average of 125.8 ml.

3.2. Summary of the analysis

This study was carried out to determine the performance and effectiveness of the designated SBR tank equipped with variable concentrations of microalgae beads (*Botryococcus* sp.) greywater treatment. The concentrations that were used as a measurement tool in this study were 100 ml, 150 ml, 200 ml and 300 ml of microalgae beads (*Botryococcus* sp.). The test was conducted for 5 times to observe the average data for a more precise result to investigate the most effective concentration for greywater treatment in SBR tank in prior to meet the quality standard and guideline.

According to the results, microalgae beads were proven to be effective as an effort to remove pollutants of the water sample taken from SS Curry House Pagoh Jaya. Each water sample were treated with variable concentrations of microalgae beads (*Botryococcus* sp.) in the designated SBR tank for 5 times and the average data was analyzed. The significant result in decreasing the value of turbidity and COD in water sample reading, as well as the improvement in pH level after undergoing the treatment was compared to the initial value of the mentioned parameters. It was done as evidence that microalgae beads (*Botryococcus* sp.) in conjunction with the designated SBR tank are efficient to treat greywater. Additionally, the greywater discharge quality is merely depending on the activities, and variations are likely to occur over different circumstances. Untreated greywater discharge adds oxygen-intensive substances, nutrients, and toxic elements (AN) to water bodies. Hence, this study aimed to ensure that the sullage (greywater) is properly treated before being discharged to water bodies such as rivers and drainage. There were a few limitations occurred throughout the study. The SBR tank was difficult to implement and maintain as well due to the design and operation.

Therefore, as a future research, researchers may study and plan out to design a bigger SBR tank, so that it could scale up to the industrial level that have more challenges. Due to the globalization, this has to be given a great importance in future. Thus, thorough consideration is required for matters such as sustaining the effectiveness of microalgae immobilization, supplying nutrients, and enhancing system performance on a larger scale. Also, more research is needed to determine the best operating parameters which are aeration, cycle length, and nutrient supply for the SBR design in order to maximize the effectiveness of pollutant removal and the growth of *Botryococcus* sp. This optimization could improve the system's overall performance as the *Botryococcus* sp. has a number of practical implications and potential application area which are the industrial wastewater treatment, for a sustainable treatment, aquaculture, and fish farming. Applying this technology to use across multiple sectors could be a useful resource for farming, minimizing the need for freshwater and lowering pollution levels in the environment.

5. Conclusions

This research has demonstrated the significant potential of Sequential Batch Reactors (SBR) integrated with *Botryococcus* sp. in addressing the complex and variable nature of wastewater generated in restaurant environments. Our findings reveal that this innovative approach outperforms traditional wastewater treatment methods, particularly in efficiency and adaptability. The study shows that choosing the right concentration of microalgae beads improved the SBR tank's performance. Advantages of the treatment include high pollutants removal efficiency with the conjunction of microalgae *Botryococcus* sp. beads being inserted into the SBR tank. Besides, it is a high-performance treatment system that is easy to design and operate, and the small portable size of designated SBR tank makes it environmentally friendly and more sustainable. It portrays that microalgae *Botryococcus* sp. beads can assimilate pollutants effectively. However, the sensitivity towards the environmental conditions needs to be taken into account as *Botryococcus* sp. is sensitive with the light, temperature, and also pH value. Thus, future researchers may consider of adding the feature of controlling this aspect on the SBR tank. In conclusion, the successful application of the SBR system coupled with *Botryococcus* sp. in our study paves the way for broader adoption of this technology in the restaurant industry and potentially in other sectors with similar wastewater characteristics. This research not only contributes to the field of wastewater treatment but also underscores the importance of innovative approaches in tackling environmental challenges associated with commercial activities.

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