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EFFECTS OF DIFFERENT MICROALGAE *BOTRYCOCCUS* SP. BEADS CONCENTRATIONS ON THE GROWTH AND NUTRIENTS UPTAKE IN KITCHEN WASTEWATER

The present study is aimed to access the growth rates, biomass productivity and nutrient removal in different concentrations of microalgae *Botryococcus* sp. beads using kitchen wastewater as a media. Verhulst logistic kinetic model was used to measure the optimal concentrations of microalgae *Botryococcus* sp. in kitchen wastewater in terms of cell growth rate kinetics and biomass productivity. The study verified that the maximum productivity was recorded with 1×10^6 cell/ml of the initial concentration of *Botryococcus* sp. with 42.64 mg/l/day and the highest removal of TP and ammonia was obtained (78.14% and 60.53% respectively). The highest specific growth rate of biomass at 0.2896 μ max/d compare to other concentrations, while the lowest occurred at concentrations of 10^5 cells/ml at 0.0412 µmax/d. The present study shows the different concentrations of *Botryococcus* sp. in alginate beads culturing in kitchen wastewater influence the cells growth of biomass and nutrient uptake with optimum concentration (10^6 cells/ml) of *Botryococcus* sp. which is suggested for wastewater treatment purposes. The result of scanning electron microscopy (sem) shows differences in morphology in terms of surface; smoother and cleaner (before the experiment), cracks and rough surface with black/white spots (after the experiment). These findings seemly can be applied efficiently in kitchen wastewater treatment as well as a production medium for microalgae biomass.

Keywords: Microalgae; immobilized; *Botryococcus* sp.; kitchen wastewater

1. Introduction

The generation of considerable amounts of wastewater from different types of industrial processes represents a major challenge to most societies, as the rise in demand and shortage in supply of fresh water become more prevalent. Nowadays, wastewater is of great concern in modern societies and since our food, environment and health are greatly affected by soil and water quality, the final disposal of wastewater calls for the integrated understanding of the possible consequences of the environment, public health and also the direct or indirect used for wastewater from the kitchen outlet plays a crucial role in the production of large amounts of organic waste, oil/fat and soap/detergents [1]. Kitchen wastewater (KW) is left over by organic compounds, washing soap and detergent from restaurants, hotels and households whereby restaurants play a critical part in discharging kitchen waste into the environment [1]. These contaminants could negatively affect the environment, including fouling and clogging in drainage and sewer systems, causing unpleasant odors, and contributing to the municipal wastewater collection system's work performance [2]. One of the newest areas of research is the development of eco-friendly and efficient wastewater treatment technology. Phycoremediation is thought to be a better green remediation technology for the removal of pollutants found in wastewater [3].

Microalgae are unicellular photosynthetic organisms with a variety of beneficial properties that could help to solve some of today's issues [4]. They can serve as water bioremediation agents, as feed in aquaculture, as food for humans and animals, and in agriculture [5]. Microalgae can assimilate nutrients such as

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phosphorus and nitrogen which can be provided from municipal wastewater, making them a suitable option for wastewater treatment. Furthermore, microalgae are capable of producing a variety of valuable products that can be used in a variety of industries such as food, cosmetics, pharmaceuticals, etc. The immobilization of microalgae is a current topic in biotechnology. According to Abirama et al. [6], *Botryococcus* sp. appears to have a high potential for removing nutrients from industrial wastewater due to the characteristics of industrial wastewater in terms of nitrogen and phosphorus compounds. Among the different immobilization techniques, the calcium alginate matrix is one of the most used. The transparency of small calcium alginate beads is enough to permit the growth of immobilized microalgae. The major goal of this study was to determine the growth rates, biomass productivity and nutrient removal in different concentrations of microalgae *Botryococcus* sp. beads using kitchen wastewater as a medium.

2. Materials and methods

2.1. Microalgae *Botryococcus* sp. culturing

Microalgae *Botryococcus* sp. was obtained from microbiology laboratory FTK, Universiti Tun Hussein Onn Malaysia. Prior to the experiment, *Botryococcus* sp. was culture for 14 days in 1 liter Bold's Basal Medium and placed under outdoor sunlight. Microalgae cells were harvested with the flocculation method by using alum as a coagulant and stirred at 80 rpm for 3 minutes, then reduced to 30 rpm for 20 minutes. The cell density was determined by a spectrophotometer at 600 nm. For immobilized microalgae beads, a selected volume of the concentrated *Botryococcus* sp. cell suspension was thoroughly mixed with 50 g l^{-1} alginate solution. Since many years ago, alginate has been employed as an immune barrier for cell transplantation, shielding the transplant from the host immune system [7]. This indicates that throughout the solubilization of microbial cells, the alginate matrix protects immobilized cells against significant chemical and physical condition changes. The alginate with microalgae *Botryococcus* sp. was dripped into a 500 ml solution of $CaCl_2$ (4%). The number of cells in the alginate solution was counted by a Neubaeur improved haemocytometer with the aid of a compound microscope.

2.2. Sampling and characteristic of KW

Kitchen wastewater (KW) samples was collected from a cafeteria at UTHM, Pagoh. The sample was collected using a plastic container (rinsed before use) and was transferred to the lab and preserved in the laboratory chiller room at a temperature of 4°C until the experiment was conducted. The investigated characteristic of kitchen wastewater (KW) included chemical oxygen demand (COD), total phosphorus (TP) and ammonia were determined as described by APHA (2012).

2.3. Factor effecting *Botryococcus* sp. beads growth

The factors affecting immobilized *Botryococcus* sp. were in different initial cell concentrations (1×10^4, 1×10^5, 1×10^6 and 1×10^7 cells/ml). The experiments were carried out in KW and incubated at room temperature for 21 days. The final concentration of *Botryococcus* sp. beads was determined.

2.4. Growth rate and productivity kinetic model

The verhulst logistic kinetic model was used to forecast the evolution of the experimental *Botryococcus* sp. biomass concentration growth. The microalgae beads growth was expressed as a sinusoidal curve (Eq. (1))

$$\frac{dx}{dt} = \mu \max\left(1-\frac{x}{xm}\right)x \tag{1}$$

Where $\frac{dx}{dt}$ represent the microalgae growth rate and x is the microalgae biomass dry weight.

Eq. (1) is integrated to Eq. (2)

$$x = \frac{X_0 X_{\max} e^{u_{\max} t}}{X_{\max} - X_0 + X_0 e^{u_{\max} t}} \tag{2}$$

Where, $\mu_{\max}$ is the maximum specific growth rate (day^{-1}), $x_{\max}$, x_0 and x are the concentration of biomass (mg/l) at an operating time equal to infinity, zero and t respectively.

The kinetic growth profiles of *Botryococcus* sp. was calculated based on the maximum growth rate (day^{-1}), according to Eq. (3) for the division per day (dd) and Eq. (4) doubling time (td) respectively. The slope of the exponential stage of the growth curve was obtained to determine the maximum growth rate ($\mu_{\max}$) [8]. This mathematical model is estimated to determine the growth of the microalgae, as previously presented by [8]. The part of the curve is approximately linear and the slope of this curve section is sprightly taken by linear regression. The number of cells (n) or the number of logarithms of cells [log (n)] is a function of time.

$$\text{division per day}\left(dd\right) = \frac{\mu_{\max}}{\ln 2} \tag{3}$$

$$\text{doubling time}\left(td\right) = \frac{1}{Dd} \tag{4}$$

2.5. Scanning electron microscopy (sem)

The morphology structure of microalgae beads before and after nutrient removal was analyzed using Scanning Electron Microscopy (SEM). Each sample took several processes prior to microscopic examination, including fixation, washing, and dehydration

3. Results and discussions

3.1. Physical and chemical characteristic of kitchen wastewater

One of the major elements of water pollution in Malaysia and other developing countries is the discharge of kitchen wastewater into the environment [2]. KW is the world's most widely produced organic wastewater, and its treatment has become a social issue because it is abundant in nutrients like nitrogen, phosphorus, and total organic compound [9]. Furthermore, the unrestricted release of kitchen wastewater produces eutrophication due to the presence of nitrogenous substances and significant ammonium convergence due to the decomposition of nutrition protein. Various methods for treating KW have been tried, including typical grease traps, adsorption, coagulation, biosorption, and more. In this study, kitchen wastewater samples were collected after the wastewater passed through the pre-treatment by using a grease trap system and the characteristic of KW can vary depending on water usage and product used such as soaps, oil and other related product. Therefore, experimental procedures were performed immediately after sampling. The physiochemical parameters of kitchen wastewater are discussed in relation to the effluent standard limits established by the Malaysian Environmental Quality Act (1974) under the Sewage and Industrial Effluent Regulation, DOE (2009) discharge limit. This result shows the characteristics of kitchen wastewater are highly variable in the regulation.

The direct discharge of kitchen wastewater into the environment and water system represented source pollution due to high contents of nutrients such as COD (319-787 mg/l), TP (1.32-3.66 mg/l) and pH (5.10-6.90) in these wastes which could possibly be derived from the kitchen activities. The effluent discharge is also highly polluted in terms of BOD and COD with values of 787 mg/l which are high compared to the allowable effluent in Standard B 200 mg/l. The substantial contributions from blood residues from meat cutting, poultry carcasses and other sources can explain the high COD concentration. These COD concentrations are slightly low than the 851 mg/l values COD reported by [2]. Meanwhile, the pH levels compared to the effluent standard were acceptable, indicating that the KW was neutral to alkaline and entirely suitable for microalgae. The kitchen wastewater also contained 3.90 mg/l TP, which was below standard A and this parameter was compared to a study conducted by [10], who obtained TP was 3.7 mg/l. Therefore, the highest pollutant for ammonia nitrogen in this study was 14.7 mg/l and the lowest was 9.4 mg/l, both of which were above the acceptable level. The result obtained from the analysis of the raw KW in this study was a clear indication and in fact evident that KW coming from domestic usage needs to be properly treated to discharge into the environment. A few researchers [11-13] has shown the potential immobilized microalgae in wastewater treatment.

3.2. Cell growth of immobilizes *Botryococcus* sp. and biomass productivity

The mathematical model for the growth profiles of *Botryococcus* sp. beads in kitchen wastewater as a response to different initial concentrations observed at different cell concentrations within 21 days is presented in Fig. 1. These figures illustrate the growth pattern of *Botryococcus* sp. beads and the best initial cell concentration was found to be in 1×10^6 cell/ml with biomass production of 42.64 mg/l. The summary of the growth kinetic data, maximum growth rate, division per day, doubling time and biomass productivity are illustrated in TABLE 2. The findings obtained in this study were similar to those reported by Gani et al. [15] who indicated that the initial cell concentration of 1×10^6 achieved high biomass productivity of *Botryococcus* sp. in domestic wastewater.

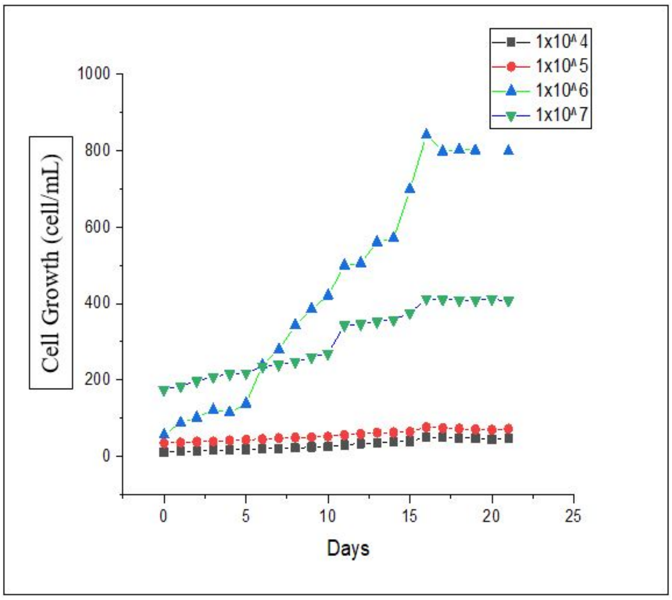

Fig. 1. The growth of *Botryococcus* sp. beads

TABLE 1

Characteristic of kitchen wastewater

Parameter	Concentration (mg/l)	Effluent standard (mg/l) EQA 1974	
		A	B
pH	5.10-6.90	6.0-9.0	5.5-9.0
Chemical Oxygen Demand, COD	319-787	120	200
Total Phosphorus, TP (mg/l)	1.32-3.66	5	10
Ammonia Nitrogen (mg/l)	2.57-6.52	5.0	5.0

TABLE 2

Growth kinetic and biomass production in different initial bead concentration

Kitchen wastewater						
Initial cell concentration (cell/ml)	**X_{max}(mg/l)**	**X_0 (mg/l)**	**Maximumgrowth rate (μ_{max}/d)**	**Division per day (Dd)**	**DoublingTime (td)**	**Biomass Productivity (mg/l/d)**
1×10^4	50.025	10.390	0.2580	0.3722	2.687	2.540
1×10^5	76.669	35.233	0.0412	0.0594	16.824	0.573
1×10^6	841.203	57.041	0.2896	0.4178	2.3935	42.643
1×10^7	393.842	174.998	0.0420	0.0602	16.622	3.0338

Apandi et al. [14] claimed that the growth rate and biomass productivity of microalgae depend on the available nutrients and initial cell concentration. Moreover, the findings obtained in this study were similar to those reported by Banerjee et al. [15] who indicated that the municipal wastewater achieved the growth of microalgae beads from an initial 7×10^6 cell/bead to approximately 90×10^6 cell/bead. It is clear that the immobilized microalgae cells were able to undergo cell division and photosynthesis in the microenvironment inside the sodium alginate. Emparan et al. [12] stated that it may be inferred that the immobilized cell demonstrated better catalytic activity, viability and growth rate than the free cell microalgae. Moreover, microalgae cells concentration of 1×10^4, 1×10^5 and 1×10^7 cell/ml for immobilized microalgae *Botryococcus* sp. are also capable to accommodate this kitchen wastewater but led to few productions of biomass. Meanwhile, for the initial cell of 1×10^7 cell/ml the growth rate and biomass productivity were lower than the concentration of 1×10^6 cell/ml. This situation might possibly be because of the inability of microalgae cells in the beads to receive enough light intensity [16].

In this study, it seemed that the concentration of KW determined the overall capability of immobilized *Botryococcus* sp. to survive or not. Thus, an appropriate microalgae concentration within a single bead can optimize biomass production and nutrient removal performances. According to cao et al. [13], once an optimized high microalgae concentration was determined, nutrient removal could be further through the increasing of number beads. With the initial concentration of 1×10^6 cell/ml, higher biomass productivity and shorter doubling times of *Botryococcus* sp. was successfully achieved compared to other initial concentration.

3.3. Nutrients removal

The changes in TP (total phosphorus) with time in different beads concentrations of *Botryococcus* sp. for 20 days of phycoremediation in comparison to the pseudo-first-order kinetic model are presented in Fig. 2. According to the figures, the microalgae beads concentration showed the ability of beads to reduce the value of nutrients at 1×10^4, 1×10^5, 1×10^6 and 1×10^7 cell/ml. The experimental data first-order kinetic coefficient of TP for all samples was obtained by linear regression. The kinetic model pattern for all concentrations was in the line with the phycoremediation increasing time in kitchen wastewater. Fig. 2 shows the highest TP removal was noted with 1×10^6 cell/ml, the total removal was 78.14% (from kitchen wastewater sample with the removal rates ranging from 12.3% to 78.14%. The previous researcher also reported that a number of immobilized microalgae capable of rapidly absorbing TP successfully reduced more than 70% of TP in kitchen wastewater. This indicates that immobilized facilitated rapid cell production and alginate-matrix did not limit the rates of transfer of the nutrient into the microalgae cells. According to Katam et al. [17], he reported that the highest TP removal in domestic wastewater was 80% and stated that in the immobilized system, the nutrients get adsorbed on the surface of the beads, then penetrate through the matrix and are continually absorbed by the culture cells.

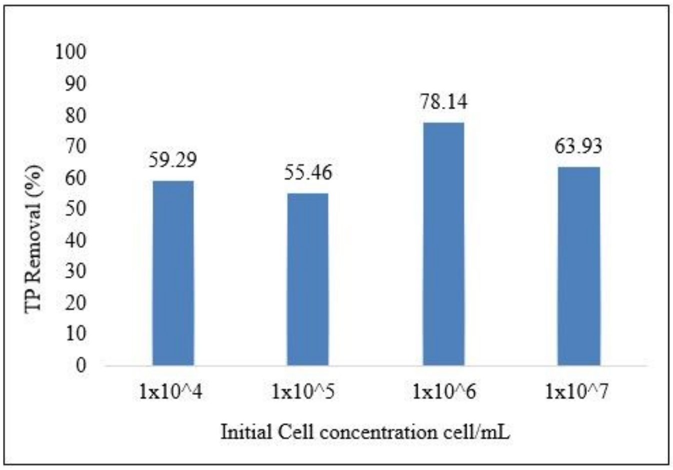

Fig. 2. Percentage of TP removal (%)

According to Fig. 3, the removal of TP in the present study in KW for in $1\times1\times10^5$ cell/ml was low compare to 1×10^4 cell/ml concentration. Tp was an essential nutrient for microalgae and it was quite limited when dealing with wastewater treatment [18]. Additionally, Fig. 3 demonstrated that all microalgae *Botryococcus* sp. beads concentrations experience a reduction in TP concentrations after 21 days. During the following days 18-20 days, the impact of initial total microalgae biomass on nutrient removal was the lowest removal compared to other days of treatment for microalgae beads with 1×10^6 cell/ml. After 15 days, it was discovered that the *Botryococcus* sp. microalgae were performing less effectively at removing TP, as evidenced by a decline in performance.

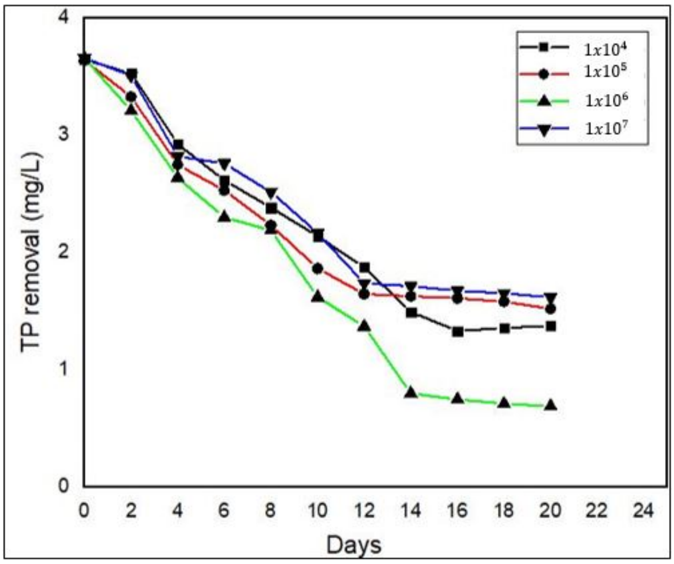

Fig. 3. Removal of TP in kitchen wastewater

Ammonia is extremely toxic and also an oxygen consuming compound, which can deplete the dissolved oxygen in water [19]. It is well recognized that microalgae are capable to remove ammonia nitrogen from wastewater. According to Mujtaba et al. [20], it's stated that immobilized microalgae *chlorella vulgaris* achieved the highest removal of ammonia nitrogen about 60%. Therefore, the potential of immobilized *Botryococcus* sp. in treating kitchen wastewater is presented in Figs. 4 and 5. A considerable reduction of ammonia nitrogen was recorded for each initial cell concentration for all KW and these results demonstrated that relatively ammonia nitrogen can be eliminated from KW by using the immobilized method. The microalgae *Botryococcus* sp. beads with 1×10^6 cell/ml were effective in removing ammonia nitrogen from 4.07% to 60.53% of kitchen wastewater after 2 days of cultures.

Ammonia is the preferred nitrogen compound for microalgae absorption. Nutrients first adsorb on the surface of the bead in immobilized states, then pass through the matrix and are continuously sorbed into cells [21]. This environment may expose cells to ammonia at higher concentrations and at a close distance. As a result, it is known that the nature of alginate allows for ionic interaction with ammonium ions, facilitating ammonia nitrogen elimination in immobilized cell systems.

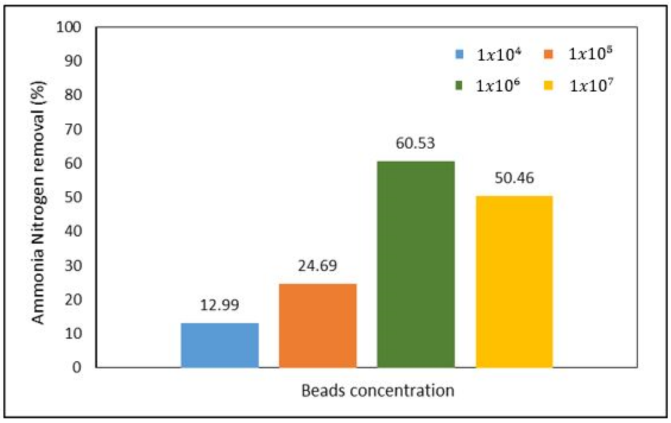

Fig. 4. Percentage ammonia nitrogen removal (%)

The recent findings in this study clearly show the significance of microalgae, *Botryococcus* sp., for phycoremediation and prospective biomass productivity. The maximum growth rate and biomass productivity have been determined in this study. The biomass productivity assessment and pollutants removal efficiencies that can be reached utilizing immobilized microalgae *Botryococcus* sp. have established this. The best concentration selected in enhancing this finding is 1×10^6 cell/ml.

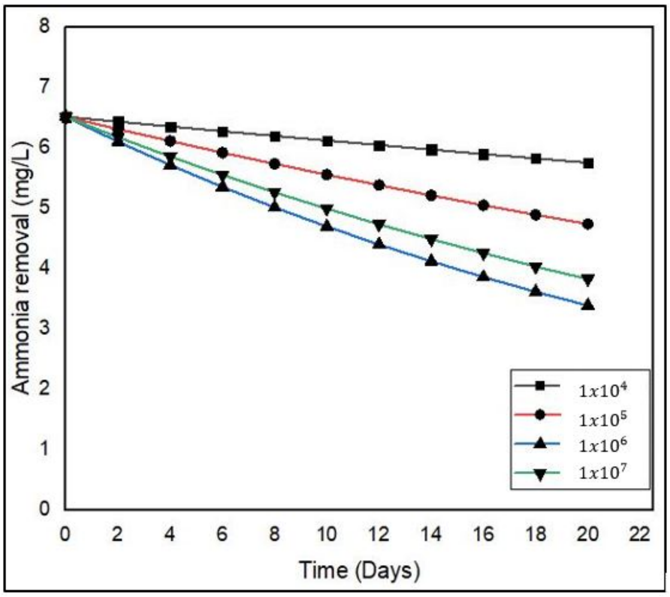

Fig. 5. Ammonia nitrogen removal in kitchen wastewater

3.4. Scanning Electron Microscopy – SEM

The surface morphology of the microalgae bead was determined using SEM before and after nutrient removal as shown in Figs. 6 and 7. SEM analysis was used to provide additional

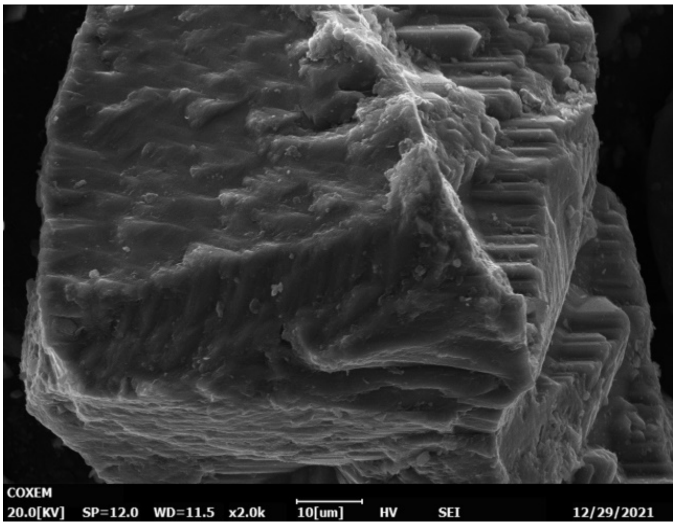

Fig. 6. Morphology of alginate beads before nutrients removal

information to better understand the underlying process mechanisms. Fig. 6 shows the morphology of microalgae beads before they were used to treat KW, demonstrating that the beads were stable enough to remove nutrients from the water. After biosorption, the surface areas of immobilized *Botryococcus* sp. are irregular and heterogeneous, and contain different pores on the algal biomass surfaces. The morphology of the content changed after the adsorption process, with cracks on the surface and a rough texture compared to before adsorption as shown in Fig. 6. Aside from that, the surface shines after the nutrients are removed from the kitchen wastewater. Changes in the morphology of beads could be due to the pores being used to exchange materials like Co$_2$, oxygen, nutrients, and metabolites between the inside and outside of the beads [22].

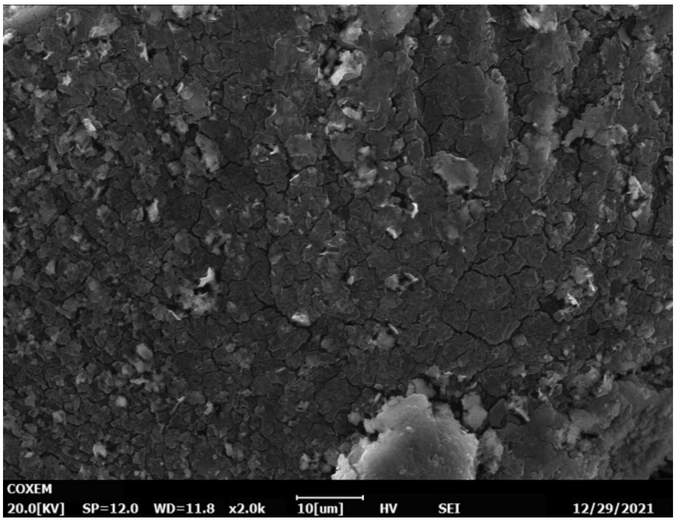

Fig. 7. Morphology of alginate beads after nutrients removal

4. Conclusion

This study reveals that *Botryococcus* sp. is able to grow in the immobilized state by using calcium alginate. The growth curves for different initial concentrations of *Botryococcus* sp. beads during 21 days are shown in Fig. 1. This study also proven that the growth of microalgae beads *Botryococcus* sp. as effected by the different concentrations 1×10^4, 1×10^5, 1×10^6 and 1×10^7 cells/ml. The study verified that the highest specific growth rate at concentration 1×10^6 cells/ml of microalgae *Botryococcus* sp. was 0.2896 µmax/d compared to other concentrations. In addition, the TP and ammonia concentration was also successfully reduced by more than 50% total in raw kitchen wastewater which was 78.14% and 60.53% respectively.

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