Contents lists available at ScienceDirect

Heliyon

Research article

Optimization of microwave-assisted carbohydrate extraction from indigenous *Scenedesmus* sp. grown in brewery effluent using response surface methodology

Zenebe Yirgu [a,b,*], Seyoum Leta [a], Ahmed Hussen [a], Mohammed Mazharuddin Khan [a], Temesgen Aragaw [a]

[a] *Center for Environmental Science, Addis Ababa University, Addis Ababa, Ethiopia*
[b] *Department of Environmental Science, Wolaita Sodo University, Wolaita Sodo, Ethiopia*

ARTICLE INFO

ABSTRACT

The use of wastewater as a nutrient source for microalgae cultivation is considered as a cost-effective approach for algal biomass and biofuel production. The microalgal biomass contains carbohydrates that can be processed into bioethanol through different extraction methods. The objective of this study is to optimize the microwave-assisted extraction (MAE) of carbohydrates from the indigenous *Scenedesmus* sp. grown on brewery effluent. Optimization of independent variables, such as acid concentration (0.1–5 N), microwave power (800–1200 W), temperature (80–180 °C) and extraction time (5–30 min) performed by response surface methodology. It was found that all independent variables had a significant and positive effect on microwave-assisted carbohydrate extraction. The quadratic model developed on the basis of carbohydrate yield had F value of 112.05 with P < 0.05, indicating that the model was significant to predict the carbohydrate yield. The model had a high value of R^2 (0.9899) and adjusted R^2 (0.9811), indicating that the fitted model displayed a good agreement between the predicted and actual carbohydrate yield. An optimum carbohydrate yield obtained was 260.54 mg g^{-1} under the optimum conditions of acid concentration (2.8 N), microwave power (1075 W), temperature (151 °C) and extraction time (22 min). The validation test showed that the model has adequately described the microwave-assisted extraction (MAE) of carbohydrates from microalgal biomass. This study demonstrated that the indigenous *Scenedesmus* sp. grown on brewery effluent provides a promising result in carbohydrate production for bioethanol feedstock.

1. Introduction

Microalgae have been considered as an alternative biofuel feedstock that has the potential to offer a solution for the ever-increasing energy demand worldwide (Younes et al., 2020). Algal biomass is recognized as a promising bioenergy source for bioethanol production compared to traditional energy crops (Qu et al., 2020). This is because microalgae have characteristics, such as fast growth, no need of arable land, high CO_2 capturing efficiency, have short growth cycle, and use different water sources (fresh, saline, and wastewater) for their growth (Harun and Danquah, 2011; Zhao et al., 2013; Wu et al., 2012; Yang et al., 2015). Furthermore, microalgae are more efficient in converting solar energy into macro-metabolic products such as lipids, carbohydrates and proteins (Gupta et al., 2017).

Microalgae store a substantial amount of carbohydrate contents, in terms of starch and cellulose polysaccharides with the absence of lignin, which makes them suitable for bioethanol production (Wang et al., 2014; Sivaramakrishnan and Incharoensakdi, 2018). The carbohydrate contents in microalgae reached up to 40% under normal cultivation conditions, which mean without nutrient limitations (de Farias Silva and Bertucco, 2016). The microalgae such as *Chlorella*, *Chlamydomonas*, *Scenedesmus*, and *Spirulina* are known with high amount of carbohydrates (mainly starch) (Zhao et al., 2013; Ho et al., 2013). These microalgae have also been widely studied for nutrient removal and found to be effective in removing nitrogen and phosphorus from various wastewaters (Salama et al., 2017).

The use of wastewater as a medium for microalgae growth is an alternative for biomass production with lower environmental impacts (Fernández-Linares et al., 2017). Wastewater contains essential nutrients

* Corresponding author.
  E-mail addresses: zenebe.yirgu@aau.edu.et, zeneyw944@gmail.com (Z. Yirgu).

such as nitrogen and phosphorus, and other trace elements, which make it useful as a potential available medium for microalgae cultivation (Tan et al., 2018). Various wastewater types such as municipal (Cho et al., 2011; Caporgno et al., 2015), agricultural (Abou-Shanab et al., 2013; Ji et al., 2014), industrial (Fontouraa et al., 2017), and anaerobically digested effluent (Cai et al., 2013) have been successfully used for microalgae cultivation with the advantages, including simultaneous nutrient removal, producing oxygenated effluent, reducing sludge production, capturing carbon, abating secondary pollution and generating useful biomass (Gouveia et al., 2016; Ferreira et al., 2017).

The microalgae species such as *Chlorella* and *Scenedesmus* have been cultivated on anaerobically digested effluent in various studies for nutrient removal and biomass production. For instance, Wang et al. (2016) cultivated six microalgae, including *Chlorella vulgaris* and *Scenedesmus obliquus* on anaerobically digested piggery effluent at different dilution levels (25%, 50%, 75% and 100%) to evaluate their nutrient removal potential. Darpito et al. (2014) cultivated *Chlorella protothecoides* on anaerobically digested brewery effluent and found a maximum removal of 96% of TN and 90% of TP with biomass production of 1.88 g L^{-1}. Ferreira et al. (2017) also conducted a study on anaerobically digested brewery effluent for cultivating *Scenedesmus obliquus* with the supply of CO_2 and reported a maximum removal efficiency of 92.9% of NH_4^+-N, 40.8% of PO_4^{3-}-P, and 62% of COD with 20%–26% of carbohydrate and 37%–40% of protein production. These studies showed that anaerobically digested brewery effluent is suitable to cultivate microalgae for nutrient removal and produce useful biomass for carbohydrate production.

The carbohydrate production from microalgal biomass using an appropriate pretreatment method is considered as a preliminary step for bioethanol production. Pretreatment is used to breakdown complex carbohydrates into fermentable sugar and enhance the efficiency of bioconversion for bioethanol production (Harun et al., 2011; Sankaran et al., 2020). Different pretreatment methods have been employed to pretreat the microalgal biomass so far. For instance, autoclave, microwave, oven heating, homogenization, sonication and bead-beating were used for pretreating microalgal biomass (Hernández et al., 2015; Harun et al., 2011; Miranda et al., 2012). Microwave-assisted extraction (MAE) of carbohydrates using either acids or alkalis for bioethanol production has been successfully proven for lignocellulosic biomass, such as sugarcane bagasse (Binod et al., 2012), rice straw (Akhtar et al., 2017), rape straw (Lu et al., 2011), and wheat straw (Xu et al., 2011). Therefore, the utilization of microwave-assisted acid or alkali hydrolysis with optimization studies for microalgal biomass has to be performed to enhance carbohydrate extraction.

Optimization of the extraction process should be required to maximize the carbohydrate production achieved from microalgal biomass with less chemical utilization and time. This often employs using single-factor optimization in which the combinations of all variables were tested and thus it requires long time with the involvement of large number of experiments (Kassim and Bhattacharya, 2016). Nevertheless, response surface methodology (RSM) is employed as an option to optimize the extraction of carbohydrate. The key purpose of the RSM is to find and recognize the interaction between the optimizing parameters and developing a statistical model (Behera et al., 2018). RSM in optimization makes to reduce the experimental numbers and then it saves time, space, and raw materials (Ye and Jiang, 2011).

RSM has been widely used for MAE optimization of different products from various biomass including microalgae: biodiesel production from Papaya oil (Nayak and Vyas, 2019), lipid extraction from *Scenedesmus quadricauda* (Onumaegbu et al., 2019), carbohydrate extraction from corn starch (Yoshida et al., 2010), and transesterification of microalgal biomass (Patil et al., 2011). Moreover, many studies have used RSM optimization for carbohydrate/reducing sugar/extraction from microalgal biomass using autoclaved pretreatment (Dong et al., 2016; Kassim and Bhattacharya, 2016). However, MAE of carbohydrate/reducing sugar from microalgal biomass was performed without optimization (Nur

et al., 2016; Kassim et al., 2019). This indicated that optimization of MAE of carbohydrates using RSM should be employed from microalgal biomass to evaluate the extraction efficiency and subsequently used for bioethanol production.

Therefore, the objective of this study is to optimize MAE of carbohydrate as preliminary step to produce bioethanol from indigenous *Scenedesmus* sp. The optimization was carried out using face-centered composite design under RSM to study the effects of acid concentration, microwave power, temperature, and extraction time on carbohydrate extraction from the biomass of indigenous *Scenedesmus* sp. obtained after aerobically digested brewery effluent treatment. Moreover, biomass production and nutrient removal potential of *Scenedesmus* sp. on an anaerobically digested brewery effluent were also evaluated.

2. Materials and methods

2.1. Microalgae isolation and identification

Water samples for microalgae isolation and identification were taken from Lake Ziway, Ethiopia. The microalgae isolation process and inoculum preparation were made using a sterilized BBM (Basal Bold Medium) (Nichols and Bold, 1965). The microalgae isolation was performed using procedures described by Anderson Andersen and Kawachi (2005). The combinations of agar plating with pipetting and serial dilution were used to isolate the indigenous microalga, which was identified as *Scenedesmus* sp. Identification was achieved under a light microscope based on morphology feature of the *Scenedesmus* sp. described in Bellinger and Sigee (2010) and Shubert and Gärtner (2015).

2.2. Microalgae cultivation in brewery effluent

The anaerobically digested brewery effluent (hereafter, brewery effluent) was obtained at the outlet of up-flow anaerobic sludge blanket (UASB) reactor from St. George Brewery Industry, Addis Ababa, Ethiopia. The effluent sample was characterized for pH, NH_4^+-N and PO_4^{3-}-P using standard methods. The *Scenedesmus* sp. was cultivated in batch mode in brewery effluent. A 2 L conical flask was used as a photobioreactor (Oliveira et al., 2017). A 10% algal suspension inoculum (Ansari et al., 2017) was added in each flask with 1.6 L working volume. The flasks were then illuminated with a maximum light intensity of 5500 lux (Li et al., 2014b) at room temperature (18–24 °C). The light-dark cycle was kept at 12:12 h period using time switcher. Aeration was supplied using an aerator to provide atmospheric CO_2 and prevent he sedimentation of microalgal cells. The cultivation lasted for 18 days. After this day, the biomass of *Scenedesmus* sp. was collected using a centrifuge with washing using distilled water. Then, biomass was dried in an oven at 60 °C and kept at 4 °C until the determination of carbohydrate content.

2.3. Screening of pretreatment method

Microwave-assisted acid or alkali extraction of carbohydrate was conducted with 5% (w/v) dried microalgal biomass suspended on H_2O and 3 N HCl, H_2SO_4, NaOH, and KOH in 100 mL Teflon tube. The Teflon tube was sealed with Teflon cap and then subjected to pretreatment in Microwave (Milestone SK-10 and SK-12, Italy), having power of 1000 W and temperature of 120 °C for 15 min as modified form Boonmanumsin et al. (2012). After pretreatment, the best acid or alkali was selected for the optimization of carbohydrate extraction using response surface methodology (RSM).

2.4. Experimental design

2.4.1. Optimization of carbohydrate extraction

RSM with a CCD (central composite design) was used to optimize the microwave-assisted carbohydrate extraction from microalgal biomass using MINITAB software version 18. This software was used for the

design of the experiment. CCD with an alpha (α) value equal to 1 was employed in this study, which is known as face-centered central composite design (FC-CCD) and it has three levels (-1, 0, 1) (Chellamboli and Perumalsamy, 2014). The four independent variables such as acid concentration (N), microwave power (W), temperature (°C), and extraction times (min) at three levels (-1, 0, 1) were investigated for the optimization of carbohydrate extraction in this study. The acid concentration, microwave, temperature, and extraction time symbolized by letter A, B, C and D, respectively. Table 1 shows the range and the levels of each independent variable used in this study. The experimental design consists of 31 runs, which were obtained from the formula: $2^n + 2n + n_c$, where n is the number of independent variables (n = 4), 2^n is the number of factorial points, 2n is the number of axial points and n_c is the replicate number of central points (Maran et al., 2013). Therefore, FC-CCD in this study consisted of 6 factorial points, 8 axial points and 7 central points with one block.

2.4.2. Statistical analysis

In RSM, the experimental results were analyzed using a MINITAB software version 18. The mathematical model was developed and established to get a functional relationship between independent variables and the response. The mathematical model is provided using a second-order polynomial (Eq. (1)), which was used to describe the effect of variables regarding linear, quadratic, and interaction terms (Bajpai et al., 2012).

$$Y = b_o + \sum_{i=1}^{k} b_i X_i + \sum_{i=1}^{k} b_{ii} X_i^2 + \sum_{i<j}^{k} b_{ij} X_i X_j + \ldots + e \quad (1)$$

where, Y is response variable. b_0, b_i, b_{ij} and b_{ii} are the intercept, linear coefficient, the interaction effect and the quadratic coefficients, respectively and e represents the random error.

The statistical significance of the developed quadratic model and the model terms were evaluated by analysis of variance (ANOVA). The quality of the quadratic model was determined by the coefficients of determination (R^2). The significance of the model terms was determined according to the P-value and a 95% of confidence level. The contour plots with its corresponding surface plots were constructed to estimate the relationship between the independent variables and response.

2.4.3. Model validation

The suitability of the model for predicting the optimum carbohydrate yield was confirmed using the optimal conditions. Triplicate experiments were performed under the optimal conditions and the average value of the experiments was compared with the predicted value of the developed model to check the accuracy and relevance of the optimized conditions.

2.5. Analytical methods and calculations

2.5.1. Biomass yield

Optical density was used to evaluate the daily microalgae growth in the culture. Microalgae growth was evaluated daily by measuring algal density in the culture. The algal suspension sample was collected from the culture and measured its optical density using a JENWAY spectrophotometer (model: 6705, UK) at 680 nm (OD_{680}) (Lee et al., 2013). The

dry weight (DW, g L^{-1}) of biomass yield was also determined gravimetrically as suspended solid according to the standard method (APHA, 1999). The regression Eq. (2) was established by plotting a graph between optical density and dry weight of the microalgal biomass.

$$DW\ (g\ L^{-1}) = 0.95 OD_{680} - 0.037\ R^2 = 0.9916 \quad (2)$$

The biomass productivity, P_B (mg L^{-1} d^{-1}), was determined through the difference in biomass concentration (g L^{-1}) with cultivation time according to Eq. (3) (Zhu et al., 2013).

$$P_B = \frac{X_t - X_0}{t_t - t_0} \quad (3)$$

where X_t and X_o are the biomass yield at time, t_t, and at initial time, t_o, respectively.

2.5.2. Nitrogen and phosphorus removal

The concentration of NH_4^+-N and PO_4^{3-}-P was determined by collecting samples every second day from the microalgae culture using a JENWAY spectrophotometer (model 6705, UK). The concentration of NH_4^+-N and PO_4^{3-}-P was determined after filtration through a 0.45 syring filter. The filtrates were appropriately diluted and used for NH_4^+-N and PO_4^{3-}-P determination by phenate and ascorbic methods (APHA, 1999), respectively. HACK pH meter (HACK®, HQ440d, Loveland, USA) was used for pH measurement of the wastewater and the microalgal culture. Eq. (4) was used to calculate the NH_4^+-N and PO_4^{3-}-P removal efficiencies (Renuka et al., 2013).

$$R_f = \frac{C_0 - C}{C_0} x\ 100\% \quad (4)$$

where C_o and C are the initial and final concentrations of nutrients, respectively.

2.5.3. Determination of carbohydrate content

The total carbohydrate content analysis in microalgae biomass was employed using the Dubois et al. (1956) method after neutralizing the supernatant using sodium carbonate (Na_2CO_3) (Kassim and Bhattacharya, 2016). The volume of the supernatant was adjusted to 50 mL using distilled water. After that, to 2 mL of the supernatant solution in a test tube, 1 mL of 5% phenol solution and 5 mL of 98% sulfuric acid were added and vortexed for 1 min, and kept it in a 30 °C water bath for 30 min. The absorbance was measured at 490 nm using the JENWAY spectrophotometer (model: 6705, UK), using a distilled water as blank. The calibration curve was prepared using glucose as a standard with regression equation of Y = 11.741 + 0.0027 (R^2 = 0.9993) within the test ranges for analysis.

2.6. Data analysis

The data of nutrient removal, biomass yield and productivity are presented as the mean ± standard deviation in a Figure. Statistical analysis was performed at p < 0.05 using one-way analysis of variance (ANOVA) followed by post hoc Tukey's test on R-software to evaluate the significant difference in carbohydrate production using acids and alkalis in microwave.

Table 1. Independent variables and their levels in the response surface design.

Independent variables	Unit	Symbol	Levels		
			Low (-1)	Center (0)	High (1)
HCl concentration	N	A	0.1	2.55	5
Microwave power	W	B	800	1000	1200
Temperature	°C	C	80	120	180
Extraction Time	min	D	5	17.5	30

3. Results and discussion

3.1. Biomass yield and productivity

The biomass yield and productivity of indigenous *Scenedesmus* sp. in brewery effluent over the cultivation period is depicted in Figure 1. The biomass yield of the *Scenedesmus* sp. gradually increased with the increase of cultivation time until it reached a maximum value of 1.05 g L^{-1} on day 18. Unlike biomass yield, biomass productivity increased for the first four days of cultivation but decreased on day 5. After day 5, the biomass productivity has started to increase until it reached the maximum biomass productivity of 64.33 ± 2.26 mg L^{-1} d^{-1} on day 13. Finally, it was dropped until the end of cultivation. The biomass produced in the present study was similar to that obtained by Ferreira et al. (2017) in brewery effluent using *Scenedesmus obliquus* (0.93 g L^{-1}) under continuous cultivation; however, they reported higher biomass productivity (217 mg L g^{-1}). Darpito et al. (2014) cultivated *Chlorella protothecoides* on an anaerobically digested brewery effluent and reported a higher biomass yield (1.88 g L^{-1}) and biomass productivity (240 mg L^{-1} d^{-1}) than the present study. A comparable biomass productivity (58.7 mg L^{-1} d^{-1}) with the present study was obtained by Ansari et al. (2017) in institutional wastewater using *Scenedesmus* sp. Nevertheless, they reported a lower biomass yield (0.445 g L^{-1}) compared to the present result. The variation in microalgal biomass yield and productivity in wastewater was due to the availability of nutrients, light, pH, temperature, and the initial inoculum density (Abou-Shanab et al., 2013).

3.2. Nutrient consumption by indigenous microalgae in brewery effluent

The nitrogen as NH_4^+-N and phosphorus as PO_4^{3-}-P removal by indigenous *Scenedesmus* sp. was evaluated in this study. The brewery wastewater used in this study had a pH of 7.45 and an initial concentration of 41.52 ± 4.73 mg L^{-1} of NH_4^+-N and 37.79 ± 2.65 mg L^{-1} of PO_4^{3-}-P. These nutrient concentrations were comparable to those reported by McGinn et al. (2011), who suggested that 40 mg L^{-1} ammonium nitrogen and 1–10 mg L^{-1} phosphates were enough to support microalgae growth. Therefore, the ammonium nitrogen and phosphate concentrations obtained in this study were sufficient for microalgae cultivation. Figure 2 shows the changes of NH_4^+-N and PO_4^{3-}-P concentration and removal

efficiency during the cultivation period of *Scenedesmus* sp. on brewery effluent. The concentrations of both NH_4^+-N and PO_4^{3-}-P decreased with an increase of cultivation time. NH_4^+-N concentration reduced from 41.52 ± 4.73 to 0.06 ± 0.02 mg L^{-1} while PO_4^{3-}-P concentration dropped from 37.79 ± 2.65 to 12.58 ± 1.03 mg L^{-1}. The removal efficiencies of NH_4^+-N and PO_4^{3-}-P obtained at the end of cultivation were more than 99% and 66.7%, respectively. The result in NH_4^+-N removal efficiency obtained in this study was slightly higher than that achieved by Ferreira et al. (2017) (91%), but in accordance with that reported by Marchão et al. (2018) (99%) using *Scenedesmus* obliquus in brewery effluent. However, these two studies were reported a lower PO_4^{3-}-P removal efficiency (around 40%) than the present study. However, Tripathi et al. (2019) and Ansari et al. (2017) were achieved a removal efficiency of 100% and 80.5% PO_4^{3-}-P from municipal wastewater and institutional wastewater using *Scenedesmus* sp., respectively. The initial phosphorus concentration and its chemical forms in wastewater and environmental conditions such as temperature light intensity, and pH affect the variation of phosphorus removal efficiencies in different studies (Choi and Lee, 2014).

3.3. Selection of pretreatment method

Carbohydrate extraction was carried out from microalgal biomass in a microwave using HCl, H_2SO_4, NaOH, and KOH as pretreatment. Figure 3 displays the effects of acid and alkali hydrolytic agents for carbohydrate extraction. The maximum and the minimum carbohydrate contents obtained in the microwave-assisted extraction were 207.70 ± 8.58 mg g^{-1} and 62.02 ± 0.33 mg g^{-1} using an acid HCl and H_2O, respectively. This maximum result was significantly different ($P < 0.05$) from the other results obtained by H_2SO_4, NaOH, KOH, and H_2O. This shows that the degree of carbohydrate extraction in the microwave is affected by different types of hydrolytic agents. The extraction of carbohydrate/ sugar/from microalgal biomass using acids and bases is not well understood and thus this makes it hard to reproduce the results in different studies. For example, Shokrkar et al. (2017) found that HCl was the most effective acid for the hydrolysis of mixed algae culture compared to H_2SO_4, H_3PO_4, and NaOH. In contrast, Miranda et al. (2012) compared HCl, H_2SO_4, and NaOH for the pretreatment of *Scenedesmus obliquus* to produce sugar and the result indicated that the use of H_2SO_4 provided a higher sugar content compared to HCl and NaOH. Therefore, the
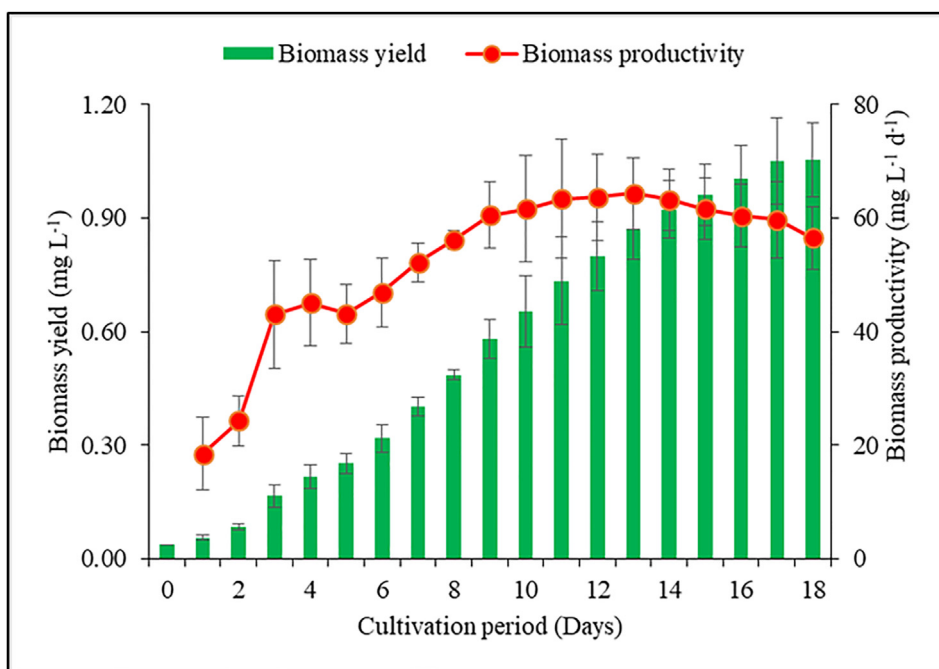

Figure 1. Biomass yield and productivity of *Scenedesmus* sp. in brewery effluent.
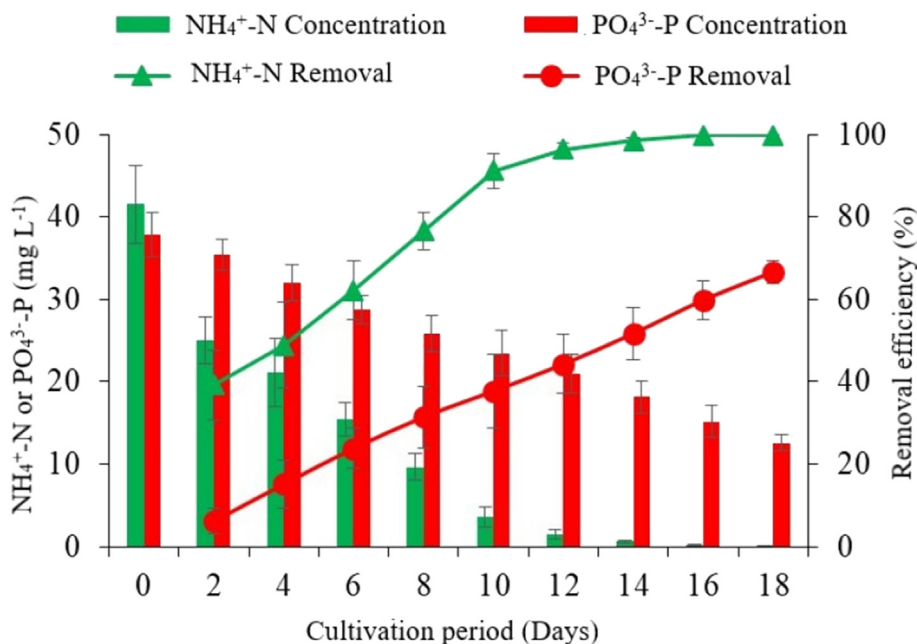
Figure 2. Change of concentration and removal efficiencies of NH_4^+-N and PO_4^{3-}-P by *Scenedesmus* sp. in brewery effluent.

selection of a hydrolytic agent is mostly dependent on the types of microalgae species. Based on the result obtained in this study, the acid HCl was chosen for the optimization process for microwave-assisted carbohydrate extraction using RSM.

3.4. Optimization of carbohydrate extraction

3.4.1. Regression model development

A total of 31 experiments were performed to optimize the four parameters (acid concentration, microwave power, temperature, and extraction time) using RSM. The complete experimental design with actual and predicted values of carbohydrate content is provided in Table 2. The results show that the maximum carbohydrate yield obtained was 257.3 mg g^{-1} using an acid concentration of 2.55 N, microwave power of 1000 W, a temperature of 130 °C, and extraction time of 17.5 min, while the minimum carbohydrate yield obtained was 64.51 mg g^{-1} using an acid concentration of 0.1 N, microwave power of 800 W, a temperature of 80 °C, and extraction time of 5 min. A quadratic model (Eq. (5)) was generated through a multiple nonlinear regression analysis of the experimental data to predict the carbohydrate yield obtained from microalgal biomass.

$$Y = -806 + 68.83\,A + 1.371\,B + 2.747\,C + 2.34\,D - 10.643\,A^2 - 0.000685\,B^2 - 0.01363\,C^2 - 0.0695\,D^2 - 0.01248\,AB + 0.0180\,AC + 0.0771\,AD + 0.001035\,BC - 0.000759\,BD + 0.00849\,CD \tag{5}$$

where A, B, C, and D are acid concentration, microwave power, temperature, and extraction time, respectively, and Y is the predicted carbohydrate content. In a regression equation, a positive parameter indicates a synergetic effect in which the response increases with an increase in the input of independent variables. On the other hand, a negative sign denotes an antagonistic effect where response increases with the decrease of input variables (Li et al., 2014a).

3.4.2. Statistical analysis

A statistical test for a regression model and individual model terms was performed to evaluate the significance of the model. Table 3 displays the analysis of variance (ANOVA) for the data generated by Eq. (5) for carbohydrate extraction. A high Fisher's F and a smaller P-value (Prob. > F) show the significance of the developed model and the model's terms

(Hamouda et al., 2015). The model F-value of 112.05 and P-value of 0.000 in this study indicated that the model was significant. All linear terms, three quadratic terms (A^2, B^2, and C^2) and three interactive terms (AB, BC, and CD) had significant effects on carbohydrate extraction. The F value and the P-value for lack of fit were 2.48 and 0.139, which showed that the lack of fit was not significant relative to the pure error and the model fit is good (Vasaki et al., 2021).

The goodness of fit of the quadratic model is evaluated using coefficients of determination (R^2-values) (Pandey et al., 2020). The values of R^2, adjusted R^2, and predicted R^2 obtained were 0.9899, 0.9811, and 0.9363, respectively. The larger value of R^2 (correlation coefficient) showed a high reliability of the model in the predicting of carbohydrate production (Sarrai et al., 2016); the adjusted R^2 measured the amount of variation about a mean explained by the model (Behera et al., 2018). The R^2-value indicated 98.99% of the variability in carbohydrate extraction explained by the quadratic model in this study. The high value of adjusted R^2 showed a reasonable agreement between the observed and predicted values of the carbohydrate yields and suggested that the proposed quadratic model equation offers satisfactory and accurate results.

Furthermore, the difference between the predicted R^2 and adjusted R^2 is too small, showing that they are in reasonable agreement with each other (Vasaki et al., 2021). The values of R^2 and adjusted R^2 are close to 1, indicating a high degree of correlation between the observed and
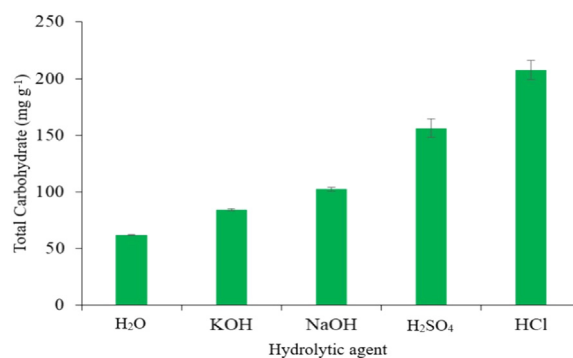

Figure 3. Effect of microwave pretreatment using acids and alkalis on total carbohydrate production from microalgal biomass.

Table 2. Experimental design, actual and predicted values of carbohydrate yield.

StdOrder	Run	Blk	Variable levels				Actual value of variable				Carbohydrate (mg g^{-1})	
			A	B	C	D	A	B	C	D	Actual	Predicted
29	1	1	0	0	0	0	2.55	1000	130	17.5	253.29	251.06
23	2	1	0	0	0	-1	2.55	1000	130	5	235.83	234.61
4	3	1	1	1	-1	-1	5	1200	80	5	117.00	105.82
22	4	1	0	0	1	0	2.55	1000	180	17.5	243.92	238.65
31	5	1	0	0	0	0	2.55	1000	130	17.5	253.29	251.06
2	6	1	1	-1	-1	-1	5	800	80	5	101.78	99.01
24	7	1	0	0	0	1	2.55	1000	130	30	245.41	245.79
10	8	1	1	-1	-1	1	5	800	80	30	102.31	108.10
12	9	1	1	1	-1	1	5	1200	80	30	103.27	107.32
27	10	1	0	0	0	0	2.55	1000	130	17.5	252.23	251.06
1	11	1	-1	-1	-1	-1	0.1	800	80	5	64.51	67.72
3	12	1	-1	1	-1	-1	0.1	1200	80	5	90.07	98.99
14	13	1	1	-1	1	1	5	800	180	30	153.95	145.73
6	14	1	1	-1	1	-1	5	800	180	5	116.36	115.43
18	15	1	1	0	0	0	5	1000	130	17.5	197.92	201.27
20	16	1	0	1	0	0	2.55	1200	130	17.5	250.95	241.62
5	17	1	-1	-1	1	-1	0.1	800	180	5	78.67	75.32
19	18	1	0	-1	0	0	2.55	800	130	17.5	197.18	205.67
30	19	1	0	0	0	0	2.55	1000	130	17.5	257.33	251.06
17	20	1	-1	0	0	0	0.1	1000	130	17.5	177.27	173.08
8	21	1	1	1	1	-1	5	1200	180	5	150.01	163.62
11	22	1	-1	1	-1	1	0.1	1200	80	30	90.60	91.04
15	23	1	-1	1	1	1	0.1	1200	180	30	157.78	161.25
13	24	1	-1	-1	1	1	0.1	800	180	30	85.49	96.18
26	25	1	0	0	0	0	2.55	1000	130	17.5	236.25	251.06
21	26	1	0	0	-1	0	2.55	1000	80	17.5	190.89	195.33
16	27	1	1	1	1	1	5	1200	180	30	190.04	186.34
7	28	1	-1	1	1	-1	0.1	1200	180	5	154.27	147.98
9	29	1	-1	-1	-1	1	0.1	800	80	30	80.27	67.36
28	30	1	0	0	0	0	2.55	1000	130	17.5	252.22	251.06
25	31	1	0	0	0	0	2.55	1000	130	17.5	250.31	251.06

predicted values of carbohydrate yields. At the same time, the low values of the coefficient of variance (3.6%) of the model indicated a high degree of precision and good reliability of the experimental data (Ren et al., 2017). Therefore, the developed model was adequate for predicting carbohydrate yield in the range of experimental variables.

The adequacy of the developed model was also evaluated through diagnostic plots such as predicted versus actual and normal probability plots (Alexander et al., 2020). Figure 4a shows the plot of the predicted value versus the actual value of carbohydrate yield from microalgal biomass. It was observed that values lie reasonably close to a straight line, indicating that the predicated values obtained from the developed

model adequately agreed to the experimental values. The normal probability plot of the residuals for carbohydrate extraction is displayed in Figure 4b, which demonstrated that the errors were normally distributed across straight line. This indicates that the residuals for carbohydrate yield fitted a normal distribution (Zhu et al., 2010).

3.4.3. Effect of variables on carbohydrate yield

Contour and surface plots were used to visualize the individual independent variable and their interaction effects on carbohydrate extraction. These plots were drawn in the basis of the quadratic model equation to investigate the effect of the process variables on
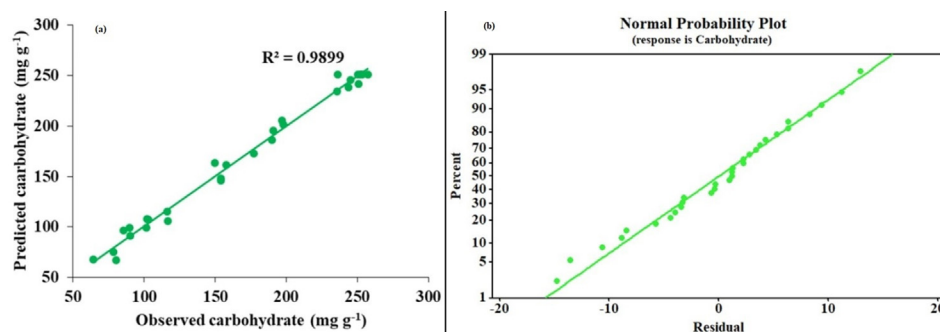

Figure 4. Diagnostic plot (a) predicted values versus actual values of carbohydrate yield and (b) normal plot of residuals.

Table 3. ANOVA of the developed model for carbohydrate yield.

Source	Degree of freedom	Sum of Square	Mean square	F-value	P-value
Model	14	136582	9755.9	112.05	0.000
Linear	4	18393	4598.3	52.81	0.000
A	1	3577	3576.5	41.08	0.000
B	1	5813	5812.8	66.76	0.000
C	1	8441	8441.3	96.95	0.000
D	1	562	562.4	6.46	0.022
Square	4	115203	28800.7	330.80	0.000
A^2	1	10592	10591.5	121.65	0.000
B^2	1	1951	1950.9	22.41	0.000
C^2	1	3013	3012.8	34.60	0.000
D^2	1	306	306.1	3.52	0.079
2-Way Interaction	6	2986	497.7	5.72	0.002
AB	1	598	598.4	6.87	0.019
AC	1	78	77.6	0.89	0.359
AD	1	89	89.3	1.03	0.326
BC	1	1713	1713.3	19.68	0.000
BD	1	58	57.6	0.66	0.428
CD	1	450	450.1	5.17	0.037
Error	16	1393	87.1		
Lack-of-Fit	10	1122	112.2	2.48	0.139
Pure Error	6	271	45.2		
Total	30	137975			

$R^2 = 0.9899$, R^2 (adjusted) $= 0.9811$, R^2 (predicted) $= 0.9363$, CV $= 3.6\%$.

carbohydrate extraction (Ibrahim et al., 2019). The plots show the interaction of two variables with keeping the other two variables at zero levels (at fixed value). They were employed to achieve the optimum condition of each independent variable for maximum carbohydrate production. The coefficients of each independent variable and their interaction with Student t-test and p-value are given in Table 4, which are utilized to identify the significant of quadratic and interaction terms of the model. The contour plot and corresponding surface plot of the interactions of the acid concentration, microwave power, temperature, and extraction time for carbohydrate extraction from the biomass of indigenous *Scenedesmus* sp. is shown in Figures 5, 6, 7, 8, 9 and 10.

Figures 5, 6, and 7 show the contour and the corresponding surface plots of the interaction of acid concentration with microwave power, temperature, and extraction time on carbohydrate production, respectively. All these plots show similar trends with the acid concentration. Figure 5 shows the interaction effect of acid concentration and microwave power on carbohydrate production at fixed values of temperature

and extraction times. The results indicated that the carbohydrate yield first increased as the acid concentration changed from 0.1 to 2.82 N or microwave power changed from 800 to 1062.7 W, then decreased with the increase of both variables. Therefore, the optimal acid concentration and microwave power for carbohydrate extraction were 2.82 N and 1062.7 W, respectively. The interaction effect of acid concentration and microwave power was significant but antagonistic on carbohydrate extraction. Both acid concentration and microwave power had significant linear on carbohydrate extraction. Furthermore, they had a significant quadratic but antagonistic effect on carbohydrate production.

Figure 6 shows the interaction effect of acid concentration and temperature on carbohydrate production at fixed values of microwave power and extraction time. The carbohydrate yield increased with increasing acid concentration from 0.1 to 2.8 N and temperature from 80 °C to 145 °C. However, the carbohydrate yield reduced when the acid concentration and temperature were increased further from 2.8 to 5 N and 145 °C–180 °C. Therefore, the acid concentration of 2.8 N and temperature of

Table 4. Regression coefficients and the corresponding T and P values of the predicted model.

| Term	Coefficient	SE Coef	T-Value	P-Value	VIF
Constant	251.06	2.77	90.70	0.000	
A	14.10	2.20	6.41	0.000	1.00
B	17.97	2.20	8.17	0.000	1.00
C	21.66	2.20	9.85	0.000	1.00
D	5.59	2.20	2.54	0.022	1.00
A^2	-63.88	5.79	-11.03	0.000	2.91
B^2	-27.42	5.79	-4.73	0.000	2.91
C^2	-34.07	5.79	-5.88	0.000	2.91
D^2	-10.86	5.79	-1.88	0.079	2.91
AB	-6.12	2.33	-2.62	0.019	1.00
AC	2.20	2.33	0.94	0.359	1.00
AD	2.36	2.33	1.01	0.326	1.00
BC	10.35	2.33	4.44	0.000	1.00
BD	-1.90	2.33	-0.81	0.428	1.00
CD	5.30	2.33	2.27	0.037	1.00
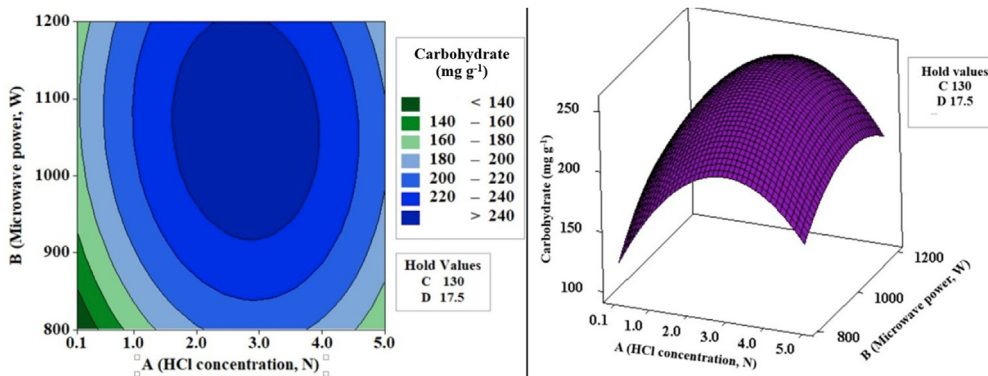
Figure 5. Response contour plot (left) and the corresponding surface plot (right) showing effect of acid concentration and microwave power on carbohydrate yield.
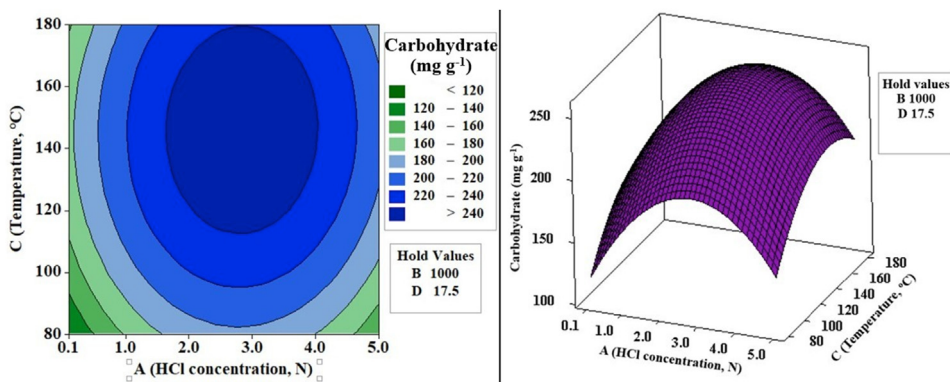

Figure 6. Response contour plot (left) and the corresponding surface plot (right) showing effect of acid concentration and temperature on carbohydrate yield.

145 °C were the optimal values for carbohydrate extractions. The interaction of acid concentration and the temperature had an insignificant but synergic effect on carbohydrate extraction. However, the temperature had a significant linear effect on carbohydrate production. Moreover, the quadratic effect of temperature was significant on carbohydrate production.

Figure 7 shows the interaction of acid concentration and extraction time on carbohydrate production at hold values of microwave power and temperature. The carbohydrate yield increased with a raise in acid concentration from 0.1 to 2.83 N for a given extraction time. At the acid concentration of 2.83 N, increasing extraction time from 5 to 21.1 min improved the carbohydrate yield from 235.78 to 252.63 mg g^{-1}. Therefore, the acid concentration of 2.83 N and extraction time of 21.1 min were the optimal conditions for carbohydrate extraction. The interaction of acid concentration and extraction time was insignificant,

but synergic effect on carbohydrate extraction. Extraction time had an insignificant quadratic effect on carbohydrate extraction. However, it had a significant linear effect on carbohydrate extraction.

Figure 8 shows the contour and the corresponding surface plots for the interaction between the microwave and temperature on carbohydrate yields when the value of acid concentration and extraction time were kept constant. It could be seen the carbohydrate yield first increased as the microwave power increased from 800 to 1080 W and temperature increased from 80 °C to 149 °C, and then decreased as both variables increased. The optimum microwave power and the temperature obtained for carbohydrate extraction were 1080 W and 149 °C, respectively. The interaction effect between microwave power and the temperature was significant and synergic on carbohydrate production. Both microwave power and temperature had a significant quadratic but antagonistic effect on carbohydrate production.
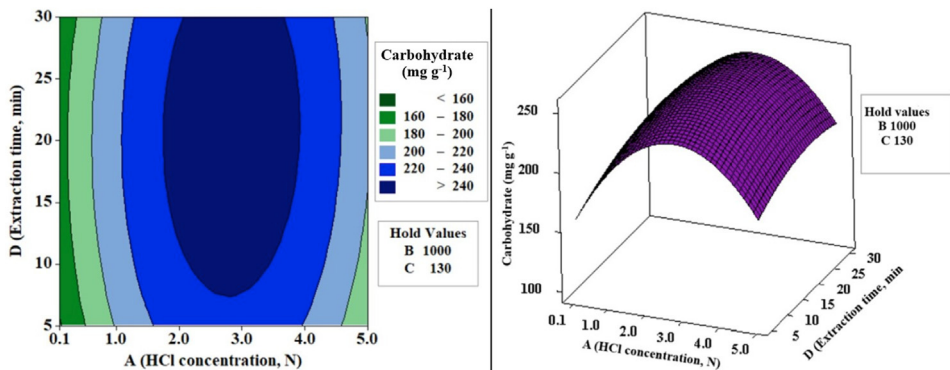

Figure 7. Response contour plot (left) and the corresponding surface plot (right) showing effect of acid concentration and extraction time on carbohydrate yield.
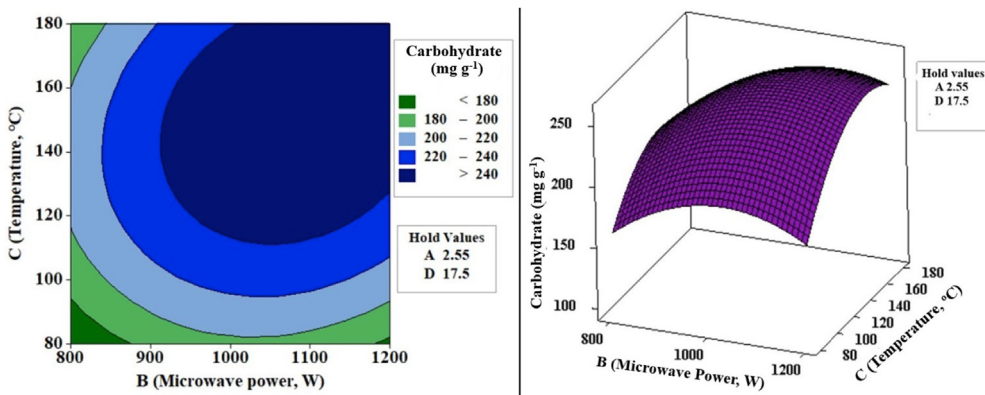
Figure 8. Response contour plot (left) and the corresponding surface plot (right) showing effect of microwave power and temperature on carbohydrate yield.
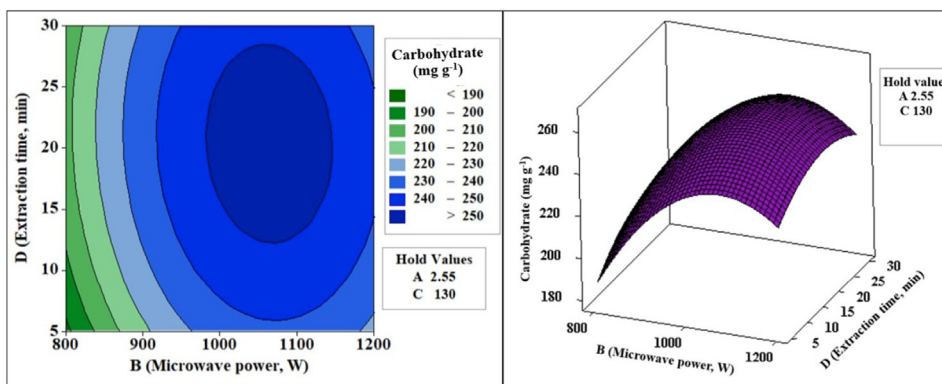

Figure 9. Response contour plot (left) and the corresponding surface plot (right) showing effect of microwave power and extraction time on carbohydrate yield.

Figure 9 shows the relationship between carbohydrate yield and the two variables (microwave power and extraction time) using the fixed values of acid concentration and temperature. The carbohydrate yield increased to its maximum as both microwave power and extraction time initially increased, and then it decreased with an increase in microwave power and extraction time. The optimum values for a maximum carbohydrate yield were 1064.4 W and 20 min. Moreover, the interaction of extraction time and microwave power had antagonistic and insignificant effect on carbohydrate extraction. The linear term of microwave power had positive and significant effect; however, the quadratic terms of microwave power had a negative but significant effect on carbohydrate extraction.

Figure 10 depicts the interactive effect of temperature and extraction time on carbohydrate extraction at fixed values of acid concentration and temperature. The carbohydrate yield first increased as both temperature

and extraction time increased and then reduced as both variables increased. The maximum carbohydrate yield was attained at an extraction time of 21.8 min and temperature of 147.3 °C, which were the optimum values for carbohydrate production. The interaction effect of temperature and extraction time was significant and synergic on carbohydrate production. The extraction time and temperature had insignificant and significant quadratic effects on carbohydrate extraction, respectively. The quadratic of temperature and extraction time had antagonistic effect on carbohydrate extraction. However, extraction time had a significant linear effect on carbohydrate production.

The maximum carbohydrate yield obtained in the present study was higher than those obtained by Sivaramakrishnan and Incharoensakdi (2018), who found around 220 mg g^{-1} of carbohydrate from *Scenedesmus* sp. cultured on BG11 medium. Comparable carbohydrate content with this study reported by Ji et al. (2015), who cultivated *Scenedesmus*
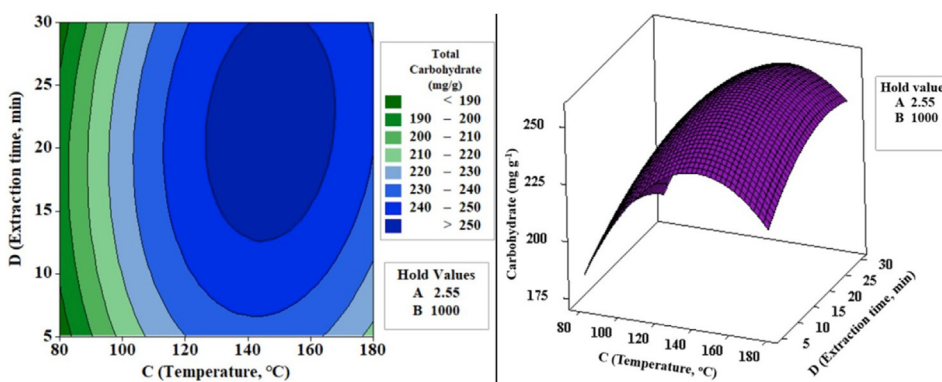

Figure 10. Response contour (left) and the corresponding surface (right) plots showing effect of temperature and extraction time on carbohydrate yield.
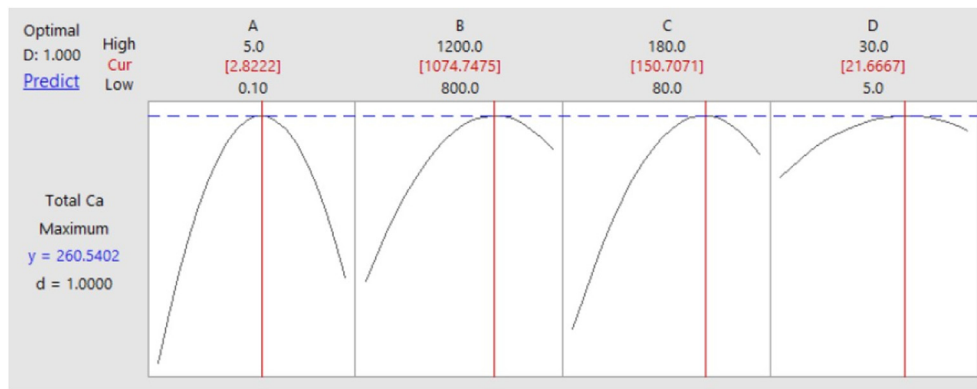
Figure 11. Process optimization curve for carbohydrate extraction form microalgal biomass.

obliquus on municipal wastewater supplemented with food wastewater and flue gas of 5%, 10%, and 15% CO_2 and they found carbohydrate contents between the range of 20.5% and 28.8%. Ansari et al. (2019) also reported a comparable carbohydrate content (27.5%) from *Scenedesmus obliquus* grown on municipal wastewater with the present study. Hence, the use of brewery effluent for indigenous microalgae growth and biomass production shows promising results for carbohydrate production, which can be used as a potential feedstock for bioethanol production.

3.4.4. Validation of optimized carbohydrate extraction conditions

The appropriateness of the model equation to predict the optimal carbohydrate yield was verified with triplicate experiments under optimum conditions. The predicted carbohydrate yield obtained was 260.54 mg g^{-1} under optimum conditions of acid concentration of 2.8 N, microwave power of 1075 W, a temperature of 151 °C, and extraction time of 22 min. The predicted value of the carbohydrate yield and the optimum conditions of each variable is provided in Figure 11. The predicted carbohydrate yield was validated by carrying out experiments in triplicate at optimal conditions. The actual result obtained was 259.88 ± 0.24 mg g^{-1}, which is in good agreement with the predicted value. Thus, the model was appropriate and adequate to describe the microwave-assisted extraction of carbohydrates from the indigenous microalgal biomass in this study.

4. Conclusion

In this study, the optimization of MAE of carbohydrate is carried out from microalgal biomass, which was obtained after brewery effluent treatment. The maximum biomass production achieved was 1.05 g L^{-1} with removal efficiency of around 99% NH_4^+-N and 66.7% PO_4^{3-}-P. The effect of microwave pretreatment using acids (HCl and H_2SO_4) and alkali (NaOH and KOH) was investigated for carbohydrate extraction and HCl was found to be given a maximum carbohydrate yield. The optimization of MAE of carbohydrate was performed using RSM to evaluate the effects of process variables (acid concentration, microwave power, temperature, and extraction time) and to determine the optimum conditions. Results indicated that model predictions are in line with experimental results. The statistical analysis also showed that all single parameters significantly influenced the efficiency of carbohydrate extraction. The optimum conditions were 2.8 N, 1075 W, 151 °C and 22 min for acid concentration, microwave power, temperature and extraction time, respectively, with a predicted value of 260.54 mg g^{-1}. The average actual result obtained under these optimal conditions was 259.88 mg g^{-1}, which was in good agreement with the predicted value. This investigation showed that the MAE of carbohydrate using RSM provides a promising result to use brewery wastewater as a growth medium for microalgae cultivation and further production of bioethanol from indigenous microalgae.

Declarations

References

Abou-Shanab, R.A.I., Ji, M., Kim, H., Paeng, K., Jeon, B., 2013. Microalgal species growing on piggery wastewater as a valuable candidate for nutrient removal and biodiesel production. J. Environ. Manag. 115, 257–264.

Akhtar, N., Goyal, D., Goyal, A., 2017. Characterization of microwave-alkali-acid pretreated rice straw for optimization of ethanol production via simultaneous saccharification and fermentation (SSF). Energy Convers. Manag. 141, 133–144.

Alexander, R.A., Innasimuthu, G.M., Rajaram, S.K., Jeganathan, P.M., Somasundarar, S.C., 2020. Process optimization of microwave-assisted alkali pretreatment for enhanced delignification of *Prosopis juliflora* biomass. Environ. Prog. Sustain. Energy 39, 1–11.

Andersen, R.A., Kawachi, M., 2005. Traditional microalgae isolation techniques. In: Andersen, R.A. (Ed.), Algal Culturing Techniques. Elsevier/Academic Press, London, UK, pp. 83–100.

Ansari, A.A., Khoja, A.H., Nawar, A., Qayyum, M., 2017. Wastewater treatment by local microalgae strains for CO_2 sequestration and biofuel production. Appl. Water Sci. 7, 4151–4158.

Ansari, F.A., Ravindran, B., Gupta, S.K., Nasr, M., Rawat, I., Bux, F., 2019. Techno-economic estimation of wastewater phycoremediation and environmental benefits using *Scenedesmus obliquus* microalgae. J. Environ. Manag. 240, 293–302.

APHA, 1999. Standard Methods for the Examination of Water and Wastewater, twentieth ed. Washington, D.C., USA.

Bajpai, S., Gupta, S.K., Dey, A., Jha, M.K., Bajpai, V., Joshi, S., Gupta, A., 2012. Application of Central Composite Design approach for removal of chromium (VI) from aqueous solution using weakly anionic resin: modeling, optimization, and study of interactive variables. J. Hazard Mater. 227–228, 436–444.

Behera, S.K., Meena, H., Chakraborty, S., Meikap, B.C., 2018. Application of response surface methodology (RSM) for optimization of leaching parameters for ash reduction from low-grade coal. Inte. J. Min. Sci. Technol. 28, 621–629.

Bellinger, E.G., Sigee, D.C., 2010. Freshwater Algae: Identification and Use as Bioindicators Edward. John Wiley & Sons, Ltd, UK.

Binod, P., Satyanagalakshmi, K., Sindhu, R., Janu, K.U., Sukumaran, R.K., Pandey, A., 2012. Short duration microwave assisted pretreatment enhances the enzymatic saccharification and fermentable sugar yield from sugarcane bagasse. Renew. Energy 37, 109–116.

Boonmanumsin, P., Treeboobpha, S., Jeamjumnunja, K., Luengnaruemitchai, A., Chaisuwan, T., Wongkasemjit, S., 2012. Release of monomeric sugars from *Miscanthus sinensis* by microwave-assisted ammonia and phosphoric acid treatments. Bioresour. Technol. 103, 425–431.

Cai, T., Park, S.Y., Racharaks, R., Li, Y., 2013. Cultivation of *Nannochloropsis salina* using anaerobic digestion effluent as a nutrient source for biofuel production. Appl. Energy 108, 486–492.

Caporgno, M.P., Taleb, A., Olkiewicz, M., Font, J., Pruvost, J., Legrand, J., Bengoa, C., 2015. Microalgae cultivation in urban wastewater: nutrient removal and biomass production for biodiesel and methane. ALGAL 10, 232–239.

Chellamboli, C., Perumalsamy, M., 2014. Application of response surface methodology for optimization of growth and lipids in Scenedesmus abundans using batch culture system. RSC Adv. 4, 22129–22140.

Cho, S., Thao, T., Lee, D., Oh, Y., Lee, T., 2011. Reuse of effluent water from a municipal wastewater treatment plant in microalgae cultivation for biofuel production. Bioresour. Technol. 102 (18), 8639–8645.

Choi, H.J., Lee, S.M., 2014. Effect of the N/P ratio on biomass productivity and nutrient removal from municipal wastewater. Bioproc. Biosyst. Eng. 38 (4), 761–766.

Darpito, C., Shin, W.-S., Jeon, S., Lee, H., Nam, K., Kwwon, J.-H., Yang, J.-W., 2014. Cultivation of *Chlorella protothecoides* in anaerobically treated brewery wastewater for cost-effective biodiesel production. Bioproc. Biosyst. Eng. 38 (3), 523–530.

de Farias Silva, C.E., Bertucco, A., 2016. Bioethanol from microalgae and cyanobacteria: a review and technological outlook. Process Biochem. 51, 1833–1842.

Dong, T., Van Wychen, S., Nagle, N., Pienkos, P.T., Laurens, L.M.L., 2016. Impact of biochemical composition on susceptibility of algal biomass to acid-catalyzed pretreatment for sugar and lipid recovery. Algal Res. 18, 69–77.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28 (3), 350–356.

Fernández-linares, L.C., Barajas, C.G., Páramo, E.D., Corona, J.A.B., 2017. Assessment of *Chlorella vulgaris* and indigenous microalgae biomass with treated wastewater as growth culture medium. Bioresour. Technol. 244, 400–406.

Ferreira, A., Ribeiro, B., Marques, P.A.S.S., Ferreira, A.F., Paula, A., Pinheiro, H.M., Reis, A., Gouveia, L., 2017. *Scenedesmus obliquus* mediated brewery wastewater remediation and CO_2 bio fixation for green energy purposes. J. Clean. Prod. 165, 1316–1327.

Fontouraa, J.T., Rolim, G.S., Farenzena, M., Gutterres, M., 2017. Influence of light intensity and tannery wastewater concentration on biomass production and nutrient removal by microalgae *Scenedesmus* sp. Process Saf. Environ. Protect. 1 (111), 355–362.

Gouveia, L., Sousa, C., Ambrosano, L., Ribeiro, B., Botrel, E.P., Castro, P., Ferreira, A.F., Silva, C.M., 2016. Microalgae biomass production using wastewater: treatment and costs Scale-up considerations. Algal Res. 16, 167–176.

Gupta, S.K., Ansari, F.A., Nasr, M., Rawat, I., Nayunigari, M.K., Bux, F., 2017. Cultivation of Chlorella sorokiniana and Scenedesmus obliquus in wastewater: fuzzy intelligence for evaluation of growth parameters and metabolites extraction. J. Clean. Prod. 147, 419–430.

Hamouda, H.I., Nassar, H.N., Madian, H.R., Amr, S.S.A., El-gendy, N.S., 2015. Response surface optimization of bioethanol production from sugarcane molasses by Pichia veronae strain HSC-22. Biotechnol. Res. Int. 1–10.

Harun, R., Danquah, M.K., 2011. Influence of acid pre-treatment on microalgal biomass for bioethanol production. Process Biochem. 46 (1), 304–309.

Harun, R., Jason, W.S.Y., Cherrington, T., Danquah, M.K., 2011. Exploring alkaline pre-treatment of microalgal biomass for bioethanol production. Appl. Energy 88, 3464–3467.

Hernández, D., Riaño, B., Coca, M., García-González, M.C., 2015. Saccharification of carbohydrates in microalgal biomass by physical, chemical and enzymatic pre-treatments as a previous step for bioethanol production. Chem. Eng. J. 262, 939–945.

Ho, S.-H., Huang, S.-W., Chen, C.-Y., Hasunuma, T., Kondo, A., Chang, J.-S., 2013. Bioethanol production using carbohydrate-rich microalgae biomass as feedstock. Bioresour. Technol. 135, 191–198.

Ibrahim, A.P., Omilakin, R.O., Betiku, E., 2019. Optimization of microwave-assisted solvent extraction of non-edible sandbox (Hura crepitans) seed oil: a potential biodiesel feedstock. Renew. Energy 141, 349–358.

Ji, F., Liu, Y., Hao, R., Li, G., Zhou, Y., Dong, R., 2014. Biomass production and nutrients removal by a new microalgae strain *Desmodesmus* sp . in anaerobic digestion wastewater. Bioresour. Technol. 161, 200–207.

Ji, M., Yun, H., Park, Y., Kabra, A.N., Oh, I., Choi, J., 2015. Mixotrophic cultivation of a microalga *Scenedesmus obliquus* in municipal wastewater supplemented with food wastewater and fl ue gas CO_2 for biomass production. J. Environ. Manag. 159, 115–120.

Kassim, M.A., Bhattacharya, S., 2016. Dilute alkaline pretreatment for reducing sugar production from *Tetraselmis suecica* and *Chlorella* sp . biomass. Process Biochem. 51, 1757–1766.

Kassim, M.A., Meng, T.K., Seng, K.Y., Serri, N.A., 2019. Alkaline-assisted microwave pretreatment of *Tetraselmis suecica* biomass for fed-batch enzymatic hydrolysis. J. Eng. Technol. Sci. 51 (2), 272–289.

Lee, Y., Chen, W., Shen, H., Han, D., Li, Y., Jones, H.D.T., Timlin, J.A., Hu, Q., 2013. Basic culturing and analytical measurement techniques. In: Richmond, A., Hu, Q. (Eds.), Handbook of Microalgal Culture: Applied Phycology and Biotechnology. Blackwell Publishing Ltd, pp. 37–68.

Li, Y., Shue, M., Hsu, Y., Lai, W., Chen, J., 2014a. Application of factorial design methodology for optimization of transesterification reaction of microalgae lipids. Energy Proc. 52, 377–382.

Li, Y., Tsai, W., Hsu, Y., Xie, M., Chen, J., 2014b. Comparison of autotrophic and mixotrophic cultivation of green microalgal for biodiesel production. Energy Proc. 52, 371–376.

Lu, X., Xi, B., Zhang, Y., Angelidaki, I., 2011. Microwave pretreatment of rape straw for bioethanol production: focus on energy efficiency. Bioresour. Technol. 102 (17), 7937–7940.

Maran, J.P., Mekala, V., Manikandan, S., 2013. Modeling and optimization of ultrasound-assisted extraction of polysaccharide from *Cucurbita moschata*. Carbohydr. Polym. 92 (2), 2018–2026.

Marchão, L., da Silva, T.L., Gouveia, L., Reis, A., 2018. Microalgae-mediated brewery wastewater treatment: effect of dilution rate on nutrient removal rates, biomass biochemical composition, and cell physiology. J. Appl. Phycol. 30 (3), 1583–1595.

McGinn, P.J., Dickinson, K.E., Bhatti, S., Frigon, J.-C., Guiot, S.R., O'Leary, S.J.B., 2011. Integration of microalgae cultivation with industrial waste remediation for biofuel and bioenergy production: opportunities and limitations. Photosynth. Res.

Miranda, J.R., Passarinho, P.C., Gouveia, L., 2012. Pre-treatment optimization of *Scenedesmus obliquus* microalga for bioethanol production. Bioresour. Technol. 104, 342–348.

Nayak, M.G., Vyas, A.P., 2019. Optimization of microwave-assisted biodiesel production from Papaya oil using response surface methodology. Renew. Energy 138, 18–28.

Nichols, H.W., Bold, H.C., 1965. Trichosarcina polymorpha. J. Phycol. 1, 34–38.

Nur, M.M.A., Kristanto, D., Setyoningrum, T.M., 2016. Utilization of microalgae cultivated in palm oil mill wastewater to produce lipid and carbohydrate by employing microwave- assisted irradiation. Recent Innov. Chem. Eng. 9, 107–116.

Oliveira, O., Gianesella, S., Silva, Vânia, Mata, T., Caetano, N., 2017. Lipid and carbohydrate profile of microalga isolated wastewater. Energy Procedia 136, 468–473.

Onumaegbu, C., Alaswad, A., Rodriguez, C., Olabi, A., 2019. Modelling and optimization of wet microalgae *Scenedesmus quadricauda* lipid extraction using microwave pre-treatment method and response surface methodology. Renew. Energy 132, 1323–1331.

Pandey, A., Gupta, A., Sunny, A., Kumar, S., Srivastava, S., 2020. Multi-objective optimization of media components for improved algae biomass , fatty acid and starch biosynthesis from *Scenedesmus* sp . ASK22 using desirability function approach. Renew. Energy 150, 476–486.

Patil, P.D., Gnaneswar, V., Mannarswamy, A., Cooke, P., Munson-mcgee, S., Nirmalakhandan, N., Lammers, P., Deng, S., 2011. Optimization of microwave-assisted transesterification of dry algal biomass using response surface methodology. Bioresour. Technol. 102, 1399–1405.

Qu, W., Loke, P., Hasunuma, T., Ho, S., 2020. Optimizing real swine wastewater treatment e ffi ciency and carbohydrate productivity of newly microalga *Chlamydomonas* sp . QWY37 used for cell- displayed bioethanol production. Bioresour. Technol. 305, 1–9.

Ren, B., Chen, C., Li, C., Fu, X., You, L., Hai, R., 2017. Optimization of microwave-assisted extraction of *Sargassum thunbergii* polysaccharides and its antioxidant and hypoglycemic activities. Carbohydr. Polym. 173, 192–201.

Renuka, N., Sood, A., Ratha, S.K., 2013. Evaluation of microalgal consortia for treatment of primary treated sewage effluent and biomass production. J. Appl. Phycol. 25, 1529–1537.

Salama, E., Kurade, M.B., Abou-shanab, R.A.I., El-dalatony, M.M., 2017. Recent progress in microalgal biomass production coupled with wastewater treatment for biofuel generation. Renew. Sustain. Energy Rev. 79, 1189–1211.

Sankaran, R., Parra Cruz, R.A., Pakalapati, H., Show, P.L., Ling, T.C., Chen, W.H., Tao, Y., 2020. Recent advances in the pretreatment of microalgal and lignocellulosic biomass: a comprehensive review. Bioresour. Technol. 298, 122476.

Sarrai, A.E., Hanini, S., Merzouk, N.K., Tassalit, D., Szabó, T., Hernádi, K., Nagy, L., 2016. Using central composite experimental design to optimize the degradation of tylosin from aqueous solution by photo-fenton reaction. Materials 9 (428), 1–11.

Shokrkar, H., Ebrahimi, S., Zamani, M., 2017. Bioethanol production from acidic and enzymatic hydrolysates of mixed microalgae culture. Fuel 200, 380–386.

Shubert, E., Gärtner, G., 2015. Nonmotile coccoid and colonial green algae. In: Wehr, J.D., Sheath, R.G., Kociolek, J.P. (Eds.), Freshwater Algae of North America. Elsevier Inc, london, UK, pp. 321–370.

Sivaramakrishnan, R., Incharoensakdi, A., 2018. Utilization of microalgae feedstock for concomitant production of bioethanol and biodiesel. Fuel 217, 458–466.

Tan, X., Zhao, X., Zhang, Y., Zhou, Y., Yang, L., Zhang, W., 2018. Enhanced lipid and biomass production using alcohol wastewater as carbon source for *Chlorella pyrenoidosa* cultivation in anaerobically digested starch wastewater in outdoors. Bioresour. Technol. 247, 784–793.

Tripathi, R., Gupta, A., Thakur, I.S., 2019. An integrated approach for phycoremediation of wastewater and sustainable biodiesel production by green microalgae, *Scenedesmus* sp. Renew. Energy 135, 617–625.

Vasaki, Em., Karri, R.R., Ravindran, G., Paramasivan, B., 2021. Predictive capability evaluation and optimization of sustainable biodiesel production from oleaginous biomass grown on pulp and paper industrial wastewater. Renew. Energy 168, 204–215.

Wang, H., Ji, C., Bi, S., Zhou, P., Chen, L., Liu, T., 2014. Joint production of biodiesel and bioethanol from filamentous oleaginous microalgae Tribonema sp. Bioresour. Technol. 172, 169–173.

Wang, M., Yang, Y., Chen, Z., Chen, Y., Wen, Y., Chen, B., 2016. Removal of nutrients from undiluted anaerobically treated piggery wastewater by improved microalgae. Bioresour. Technol. 222, 130–138.

Wu, X., Ruan, R., Du, Z., Liu, Y., 2012. Current status and prospects of biodiesel production from microalgae. Energies 5, 2667–2682.

Xu, J., Chen, H., Kádár, Z., Thomsen, A.B., Schmidt, J.E., Peng, H., 2011. Optimization of microwave pretreatment on wheat straw for ethanol production. Biomass Bioenergy 35 (9), 3859–3864.

Yang, L., Tan, X., Li, D., Chu, H., Zhou, X., Zhang, Y., Yu, H., 2015. Nutrients removal and lipids production by *Chlorella pyrenoidosa* cultivation using anaerobic digested starch wastewater and alcohol wastewater. Bioresour. Technol. 181, 54–61.

Ye, C., Jiang, C., 2011. Optimization of extraction process of crude polysaccharides from *Plantago asiatica* L . by response surface methodology. Carbohydr. Polym. 84 (1), 495–502.

Yoshida, T., Tsubaki, S., Teramoto, Y., Azuma, J., 2010. Optimization of microwave-assisted extraction of carbohydrates from industrial waste of corn starch production using response surface methodology. Bioresour. Technol. 101, 7820–7826.

Younes, S., Bracharz, F., Awad, D., Qoura, F., Mehlmer, N., Brueck, T., 2020. Microbial lipid production by oleaginous yeasts grown on *Scenedesmus obtusiusculus* microalgae biomass hydrolysate. Bioproc. Biosyst. Eng. 43, 1629–1638.

Zhao, G., Chen, X., Wang, L., Zhou, S., Feng, H., Chen, W.N., Lau, R., 2013. Ultrasound assisted extraction of carbohydrates from microalgae as feedstock for yeast fermentation. Bioresour. Technol. 128, 337–344.

Zhu, L., Wang, Z., Shu, Q., Takala, J., Hiltunen, E., Feng, P., Yuan, Z., 2013. Nutrient removal and biodiesel production by integration of freshwater algae cultivation with piggery wastewater treatment. Water Res. 47 (13), 4294–4302.

Zhu, T., Heo, H.J., Row, K.H., 2010. Optimization of crude polysaccharides extraction from *Hizikia fusiformis* using response surface methodology. Carbohydr. Polym. 82, 106–110.