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

Zenebe Yirgu\textsuperscript{a,b,*}, Seyoum Leta\textsuperscript{a}, Ahmed Hussen\textsuperscript{a}, Mohammed Mazharuddin Khan\textsuperscript{a}, Temesgen Aragaw\textsuperscript{a}

\textsuperscript{a} Center for Environmental Science, Addis Ababa University, Addis Ababa, Ethiopia
\textsuperscript{b} Department of Environmental Science, Wolaita Sodo University, Wolaita Sodo, Ethiopia

ARTICLE INFO

Keywords:
Carbohydrate extraction
Brewery effluent
Algal biomass
Microwave
Central composite design
Response surface methodology

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\textsuperscript{2} (0.9899) and adjusted R\textsuperscript{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\textsuperscript{-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\textsubscript{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...
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 (Fontoura 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 *Scene-desmus obliquus* on anaerobically digested brewery 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\(_4\)-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 sugar-cane bagasse (Binod et al., 2012), rice straw (Akhhtar 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 microalgal: biodiesel production from *Papaya* oil (Nayak and Vyas, 2019), lipid extraction from *Scenedesmus quadricauda* (Onumahbhu 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 and Kawachi (2005). The concentrations 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\(_4\)-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 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\(_2\)O and 3 N HCl, H\(_2\)SO\(_4\), 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 for microwave (Onumaegbu et al., 2019), carbohydrate extraction from *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.

### 2.4. 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: $2^k + 2k + n_c$, where $n$ is the number of independent variables ($n = 4$), $2^k$ 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).

$$Y = b_0 + \sum_{i=1}^{k} b_i X_i + \sum_{i=1}^{k} b_{ij} X_i^2 + \sum_{i<j}^{k} b_{ij} X_i X_j + \ldots + e$$  \hspace{1cm} (1)

where, $Y$ is response variable. $b_0$, $b_i$, $b_{ij}$ and $b_{ij}$ 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.

$$\text{DW (g L}^{-1}) = 0.95\text{OD}_{680} - 0.037 \ R^2 = 0.9916$$  \hspace{1cm} (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).

$$P_b = \frac{X_t - X_0}{t_t - t_0}$$  \hspace{1cm} (3)

where $X_t$ and $X_0$ are the biomass yield at time, $t_t$ and at initial time, $t_0$, 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).

$$R = \frac{C_i - C_f}{C_i} \times 100\%$$  \hspace{1cm} (4)

where $C_i$ and $C_f$ 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$_2$CO$_3$) (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 alkanes in microwave.

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

| Independent variables | Unit | Symbol | Levels |
|-----------------------|------|--------|--------|
| 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     |

Heliyon 7 (2021) e07115
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) under continuous cultivation; however, they reported higher biomass productivity (217 mg L g⁻¹) than the present study. A comparable biomass productivity (217 mg L g⁻¹) was obtained by Ansari et al. (2017) in brewery effluent using *Scenedesmus obliquus* (0.93 g L⁻¹) under anaerobiocally 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 NH₄-N and phosphorus as PO₄-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 NH₄-N and 37.79 ± 2.65 mg L⁻¹ of PO₄-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 microalgal growth. Therefore, the ammonium nitrogen and phosphate concentrations obtained in this study were sufficient for microalgal cultivation. Figure 2 shows the changes of NH₄-N and PO₄-P concentration and removal efficiency during the cultivation period of *Scenedesmus* sp. on brewery effluent. The concentrations of both NH₄-N and PO₄-P decreased with an increase of cultivation time. NH₄-N concentration reduced from 41.52 ± 4.73 to 0.06 ± 0.02 mg L⁻¹ while PO₄-P concentration dropped from 37.79 ± 2.65 to 12.58 ± 1.03 mg L⁻¹. The removal efficiencies of NH₄-N and PO₄-P obtained at the end of cultivation were more than 99% and 66.7%, respectively. The result in NH₄-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 PO₄-P removal efficiency (around 40%) than the present study. However, Tripathi et al. (2019) and Ansari et al. (2017) achieved a removal efficiency of 100% and 80.5% PO₄-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 will 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, H₂SO₄, 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 ± 0.33 mg g⁻¹ and 62.02 ± 0.33 mg g⁻¹ using an acid HCl and H₂O, respectively. 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, Shokkar et al. (2017) found that HCl was the most effective acid for the hydrolysis of mixed algae culture compared to H₂SO₄, H₂PO₄, and NaOH. In contrast, Miranda et al. (2012) compared HCl, H₂SO₄, and NaOH for the pretreatment of *Scenedesmus obliquus* to produce sugar and the result indicated that the use of H₂SO₄ provided a higher sugar content compared to HCl and NaOH. Therefore, the

![Figure 1. Biomass yield and productivity of Scenedesmus sp. in brewery effluent.](image-url)

---

**Figure 1.** Biomass yield and productivity of *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.

\[
Y = -806 + 68.83A + 2.34B + 10.643C + 0.000685D - 0.01363A^2 - 0.0695B^2 - 0.01248CD + 0.00849CD
\]

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 synergistic 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 (A², B², and C²) 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 (R²-values) (Pandey et al., 2020). The values of R², adjusted R², and predicted R² obtained were 0.9899, 0.9811, and 0.9363, respectively. The larger value of R² (correlation coefficient) showed a high reliability of the model in the predicting of carbohydrate production (Sarrai et al., 2016); the adjusted R² measured the amount of variation about a mean explained by the model (Behera et al., 2018). The R²-value indicated 98.99% of the variability in carbohydrate extraction explained by the quadratic model in this study. The high value of adjusted R² 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 R² and adjusted R² is too small, showing that they are in reasonable agreement with each other (Vasaki et al., 2021). The values of R² and adjusted R² are close to 1, indicating a high degree of correlation between the observed and predicted carbohydrate production from microalgal biomass.
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 predicted 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 carbohydrate yield.

---

**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   | 2.55 | 1000 | 130 | 17.5  | 253.29 | 251.06    |
| 23       | 2   | 1   | 0    | 0   | 0   | 2.55 | 1000 | 130 | 5     | 235.83 | 234.61    |
| 4        | 3   | 1   | 1    | -1  | -1  | 5    | 1200 | 80  | 5     | 117.00 | 105.82    |
| 22       | 4   | 1   | 0    | 0   | 1   | 2.55 | 1000 | 180 | 17.5  | 243.92 | 238.65    |
| 31       | 5   | 1   | 0    | 0   | 0   | 2.55 | 1000 | 130 | 17.5  | 253.29 | 251.06    |
| 2        | 6   | 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  | 5    | 800  | 80  | 30    | 102.31 | 108.10    |
| 12       | 9   | 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  | 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   | 0    | 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    |

**Figure 4.** Diagnostic plot (a) predicted values versus actual values of carbohydrate yield and (b) normal plot of residuals.
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 significance 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 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 for carbohydrate extraction from the biomass of indigenous Scenedesmus sp. is shown in Figures 5, 6, 7, 8, 9 and 10.

Table 4. 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   |
| A²              | 1                 | 10592         | 10591.5     | 121.65  | 0.000   |
| B²              | 1                 | 1951          | 1950.9      | 22.41   | 0.000   |
| C²              | 1                 | 3013          | 3012.8      | 34.60   | 0.000   |
| D²              | 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        |             |         |         |

R² = 0.9899, R² (adjusted) = 0.9811, R² (predicted) = 0.9363, CV = 3.6%.

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   | 1.0 |
| A     | 14.10       | 2.20    | 6.41    | 0.000   | 1.0 |
| B     | 17.97       | 2.20    | 8.17    | 0.000   | 1.0 |
| C     | 21.66       | 2.20    | 9.85    | 0.000   | 1.0 |
| D     | 5.59        | 2.20    | 2.54    | 0.022   | 1.0 |
| A²    | -63.88      | 5.79    | -11.03  | 0.000   | 2.9 |
| B²    | -27.42      | 5.79    | -4.73   | 0.000   | 2.9 |
| C²    | -34.07      | 5.79    | -5.88   | 0.000   | 2.9 |
| D²    | -10.86      | 5.79    | -1.88   | 0.079   | 2.9 |
| AB    | -6.12       | 2.33    | -2.62   | 0.019   | 1.0 |
| AC    | 2.20        | 2.33    | 0.94    | 0.359   | 1.0 |
| AD    | 2.36        | 2.33    | 1.01    | 0.326   | 1.0 |
| BC    | 10.35       | 2.33    | 4.44    | 0.000   | 1.0 |
| BD    | -1.99       | 2.33    | -0.81   | 0.428   | 1.0 |
| CD    | 5.30        | 2.33    | 2.27    | 0.037   | 1.0 |
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 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...
obliquus on municipal wastewater supplemented with food wastewater and flue gas of 5%, 10%, and 15% CO2 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% NH4-N and 66.7% PO4-P. The effect of microwave pretreatment using acids (HCl and H2SO4) 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 optimum 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

Author contribution statement

Zenebe Yirgu: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.
Seyoum Leta & Ahmed Hussen: Conceived and designed the experiments; Analyzed and interpreted the data.
Mohammed Mazharuddin Khan & Temesgen Aragaw: Performed the experiments.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

The authors would like to thank Addis Ababa University and Wolaita Sodo University for providing necessary equipment and laboratory facilities. The Authors would also like to thank BGI Ethiopia for accessing UASB effluent from their wastewater treatment plant.

References

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

Chai, S.H., Nam, H., Choi, H., 2017. Response surface methodology for optimization of microwave assisted pretreatment for reducing sugar recovery and nutrient removal by Scenedesmus obliquus biomass. Process Saf. Environ. Protect. 1 (111), 355–362.

Gouveia, L., Sousa, C., Ambrosano, L., Ribeiro, B., Botrel, E.P., Castro, P., Ferreira, A.F., 2017. Effect of the N/P ratio on microalga isolated wastewater. Energy Procedia 136, 28–38.

Shokrkar, H., Ebrahimi, S., Zamani, M., 2017. Bioethanol production from acidic and alkaline wastewater treatment: effect of dilution rate on nutrient removal rates, biomass, biochemical composition, and cell morphology. J. Biofuels. 10 (3), 1583–1595.

McGinn, P.J., Dickinson, K.E., Bhatti, S., Frigon, J.-C., Guiot, S.R., O’Leary, S.J.B., 2015. Optimization of biomass cultivation with industrial wastewater treatment and bioenergy production: opportunities and limitations. Photosynth. Res.

Miranda, J.R., Passarini, P.C., Gouveia, L., 2012. Pre-treatment optimization of Scenedesmus obliquus biomass for bioethanol production. Bioresour. Technol. 104, 2028–2036.

Yu, X., Li, X., Zou, Y., Zhang, Y., Angelidaki, I., 2011. Biomass pretreatment of Trichosarcina aestuarii biomass for fed-batch enzymolysis. J. Biofuels. 10 (17), 9307–9746.

Pandey, A., Gupta, A., Suna, S., Kumar, S., Srivastava, S., 2020. Multi-objective optimization of media components for improved algae biomass, fatty acid and starch biosynthesis. Bioresour. Technol. 342, 120–121.

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, 34–40.

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, 34–40.

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, 34–40.

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, 34–40.

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, 34–40.

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.
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, 784–792.

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

Vasik, E., Karri, R.R., Ravindran, G., Panmasivan, 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 digested 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., Kidir, Z., Thommes, A.R., Schmidt, J.E., Feng, 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., Becharar, F., Awad, D., Qusra, F., Mehlman, 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. Bioreour. 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.