https://doi.org/10.46344/JBINO.2022.v11i01.05

INFLUENCE OF FLOCCULANTS AND HEAVY METALS IN HARVESTING OF ALGAL BIOMASS

E. A. Alwaleed, H. R.M. Galal, M. Abo-Eldahab, H. Saber

Botany and Microbiology Department, Faculty of Science, South Valley University, Qena 83523, Egypt
Corresponding author: E.A. Alwaleed, Botany and Microbiology Department, Faculty of Science, South Valley University, Qena 83523, Egypt;

e–mail: eme_biologist@sci.svu.edu.eg

ABSTRACT

Algal cell have long been deliberated as a useful auspicious biomass feedstock intended for numerous industrial solicitations as biofuels, animal/aquaculture feeds, Nutraceutical, and pharmaceuticals. Numerous biotechnological defies allied with cultivation of algae, containing the minor size and negatively charged on the wall of microbial cells. in addition to the dilution of its cultures, prerequisite to skirted, that proliferations the cost and labor. Hence, proficient biomass restoration or extracting of *Scenedesmus obliques* characterizes a precarious blockage intended for large-scale algal biorefinery process. the flocculation-based procedures ought to assimilate much courtesy owing to their auspicious proficiency and scalability. Efficiency of various flocculants in harvesting *S. obliques* biomass revealed that the highest flocculating efficiencies were observed at 150 ppm of Alum (97.49 %) and 75 ppm of $FeCl_3$ (97.18 %). Also, this research recover the inhibition effect of different concentration of heavy elements; Mn^{+2}, Co^{+2} and Zn^{+2} on algal biomass.

Keywords : Flocculants, Heavy Metals, Microalgae

Introduction

Algal cell have materialized as a supply that can perform both bioremediation of wastewater and biomass generation for biodiesel production [1] . algal biomass has concerned considerably devotion in the theoretical and industrial pitches owing to its numerous industrial solicitations as can then be used in the manufacture of animal feed and the synthesis of various high-valued compounds, such as dietary supplements, cosmetics, and drug products in the manufacture of bio-refinery [2] Also, their ability to extract heavy metals from diverse sources has been checked [3]. Since they function as vitamin precursors and important cofactors in metal enzymes, heavy elements are vital micronutrients for all biota[4] .

Despite this, high heavy metal concentrations can inhibit algal growth and chlorophyll synthesis, also causing variations in photosynthetic activity and cause the construction of reactive oxygen species (ROS) in algal cells causing lipid peroxidation [5], that causes the interruption of membrane functions and destructive possessions on the cells [6] .

The presence of oxidized proteins and lipids in algal wall suggests that many micro-algal organisms are stressed[7], to use ***Scenedesmus obliques*** or any algal species when catalyst for biodiesel production, effective procedures for segregating and extracting algal biomass from culture media were used. As a result, the acceptable level of moisture, salt concentrations, cell damage, and strain features, such as density and size, must all be considered. Furthermore, The fact that algal biomass will be further processed must be considered when selecting an appropriate harvesting procedure; thus, these procedures must not be toxic or contaminate algal biomass. Also it is desirable if the method of extraction used allows for the reuse of the culture medium.[8].

To increase the rate of sedimentation, a chemical separation process for algal cell biomass from culture media is used, which includes the use of multiple chemicals to cause cell flocculation and coagulation, including inorganic salts involving polyvalent metal ions and cationic polymers. Observing flocculants, microalgal cells form clumps that quickly settle to the ground and can be isolated by another effectively achieve because algal biomass has increased in particle size or because algal biomass is extracted from the culture medium by decanting [9].

The accumulation of algal cells by flocculation makes the handling of vast volumes of crops simpler than traditional approaches such as centrifugation and gravity filtration [10]. Considerations such as cost, efficacy, and general consequence on algal biomass should be taken into account when using flocculants for algal cell harvesting[11]. Inorganic salts such as aluminum sulfate $Al_2(SO_4)_3$, ferrous chloride $FeCl_3$, and calcium chloride $CaCl_2$ illustration the combinations having polyvalent metal ions. In other methods, such as wastewater treatment, these inorganic salts have been used to eliminate toxins and others such as phosphorus, as clarifying agents and flocculating agents[12].

The flocculation of algal cells is indeed vulnerable to variations in the pH of the culture media. In wastewater treatment, chemicals such as sodium hydroxide are frequently used to raise the pH level to the point where Mg(OH)2 is formed and serves as the primary flocculants [13]. Much research on the use and mechanism of specific flocculants/techniques is available, but there is no comprehensive comparison of various flocculation-based methods, including traditional inorganic chemicals. As a result, the prosecution's role is to demonstrate a highly efficient and cost-effective harvesting process in order to achieve a commercial scale algae-based procedure, as well as to demonstrate the bioremediation efficiency of S oblique on heavy element removal.

1- Materials and methods

2.1 Experimental layout

The algal cell *Scenedesmus obliques* (Multicellular, Chlorophyta) was separated from the river Nile water at Qena, Egypt. Algal cells were isolated, conveyed to fresh solid medium and endangered to recurrent sub culturing on fresh solid BG 11 [14] . A sterilized clear polyethylene tank containing 8 liters of BG 11 was injected with 80 ml of *S. obliques* culture and incubated at 25±8 °C. Oil-free compressed air from the upper hold was used to aerate the 3-mm polyethylene tubes. The cultures were illuminated by white cool fluorescent lamps (TOSHIBA FL 40 T9D/38) for 13 days with a 16:8 light:dark photoperiod with a light intensity of 5,000 lux

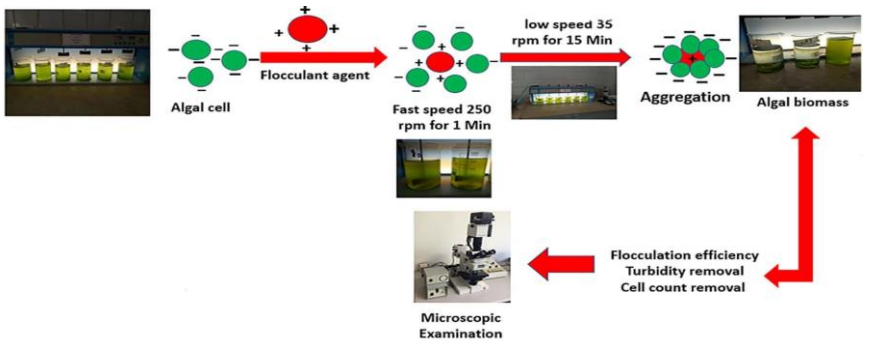
2.2 The effectiveness of various flocculants in biomass harvesting of *S. obliques*.

Four inorganic compounds (aluminum sulphate, ferrous chloride, calcium chloride, and sodium hydroxide) were used in jar tests at different concentrations to determine their flocculating efficiency on S. obliques cultures for harvesting algal biomass. These compounds were chosen based on previous research into their use in wastewater treatment or the harvesting of other algal species [15]. At a concentration of 10,000 ppm, stock solutions for the four inorganic compounds were prepared. Algal culture aliquots (800 mL) were placed in 1000 mL beakers. The test flocculants were

then added in the appropriate concentrations (50, 75, 100, 150, 200 and 250 ppm) from aluminum sulfate, ferrous chloride, calcium chloride, though NaOH were added to reach the pH values of 9, 10, 11 and 12. Each flocculant concentration was tested in triplicate. To stimulate the coagulation process, increasing flocculant concentrations were added to each beaker at the same time, and the beakers were vigorously stirred (250 rpm) for 1 minute. To aid in the flocculation process, the beakers were gently stirred (35 rpm) for 15 minutes. Finally, formed flocs could be allowed to settle for 30 minutes (without stirring) (sedimentation)[16] . At the end of the process, supernatant liquid samples were taken from each beaker, and turbidity and pH were measured using an HI 93703 Hanna Instruments Turbid metre and an Adwa (AD110) pH-meter, respectively. The total number of algae was also counted using a Hemcytometer.

2.2.1 Calculation of flocculation efficacy

The efficacy of flocculation was determined by assessing the initial optical density (OD) with a spectrophotometer at 680 nm **(17),** turbidity, and cell count of the cultures afore flocculation and the enduring optical density, turbidity, and cell count of the supernatant liquid after 30 min. The subsequent formula was used to determine the flocculating efficiency of the various compounds tested. Where Initial refer to the values of OD, turbidity, and cell count before flocculation process. Residuals refer to the values of OD, turbidity, and cell count after flocculation process.



Flocculating Efficiency = (Initial OD – Residual OD)/Initial OD x 100

2.3 **Examination of algal cells and flocculants under the microscope**

To see if the flocculants used had any effect on the integrity of the cell walls and cell structure of *S. obliques* cultures, samples of settled material from each treatment were pipetted out and examined under a light microscope at **40x** magnification.

The effect of different concentration of manganese chloride (0.2, 0.4 and 0.6 mM), cobalt nitrate (0.04, 0.07 and 0.1 mM) and Zinc sulfate (0.1, 0.2,0.3 and 0.4 mM) on growth and some primary metabolites were evaluated.

2.4 Optical density and biomass estimation

The growth of *S. obliques* was scrutinized by assessing the optical density spectrophotometrically at 680 nm (OD_{680}) [18] Biomass productivity was calculated by determination of algal cellular dry weight (CDW, g L^{-1}) using the following formula conferring to **Abomohra *et al.* (6).**

Biomass productivity (CDW g L^{-1} day^{-1}) = (CDW_L – CDW_0)/(T_L-T_0)

Wherever CDW_0 and CDW_L representing the CDW (g L^{-1}) at the starting cultivation day (T_0) and days of late exponential phase (T_L), respectively.

2.5 Determination of dry weight

A weighted glass Fibre filter was used to sieve an aliquot volume of the algal suspension. After being precipitated on the filter, the cells were washed twice with distilled water and dried in a 70°C oven. The dry weight of the algae was calculated as mg.ml^{-1} algal suspension..

2.6 Pigment content estimation

Pigment fractions were determined spectrobolometrically (UV 2300 spectrophotometer). The content of pigment fractions (µg/ml algal suspension) were premeditated using the equations [19].

2.7 Determination of carbohydrates and proteins

For estimation of carbohydrates, Anthrone sulfuric acid method [20–22] was used. Protein content was indomitable [23].

2.8 **Statistical analysis**:

The average and standard deviation (SD) of three replicates are presented. Using the SPSS programme, the collected data were statistically analysed to assess the degree of significance using one-way analysis of variance (ANOVA), LSD, and Duncan test at probability level (P) 0.05. (version IBM 25).

Results

Scenedesmus obliques cultures was treated with different concentration of heavy metal; $MnCL_2$ (0.2, 0.4 and 0.6 mM), Co $(NO_3)_2.6H_2O$ (0.04, 0.07 and 0.1mM) and $ZnSO_4.7H_2O$ (0.1, 0.2, 0.3 and 0.4 mM) to evaluate their effects on growth and photosynthetic pigments as well as carbohydrate, protein, and lipid contents, in addition to the fatty acids profile at late exponential phase. The results of the growth curve in **Fig. 1** show that, the maximum value of optical

density (0.782) was obtained in the culture treated with 0.2 mM $ZnSO_4$ in comparison to the control value (0. 324) after 13 days of cultivation, while the lowest value of optical density was 0.201 and observed in the culture treated with 0.1 mM Co $(NO_3)_2.6H_2O$ after 13 day of the experimental period. On the other hand, biomass productivity results show highly significant value (1.46 g. L^{-1}) in the algal culture treated with 0.2 mM $ZnSO_4$ at late exponential phase which was 119.87 % higher than the control value. While the lowest value of biomass productivity (0.41 g. L^{-1}) was recorded in the culture treated with 0.1 mM Co $(NO_3)_2.6H_2O$, after 13 days of the cultivation period **(Fig. 2).** Furthermore, the changes in dry weight of *S. obliques* as a result of exposure to different concentration of heavy metal at late of exponential phase are shown in **Fig. 3**. The results reveal that, dry weight of the control culture was reached to 1.18 mg/ml after 13 days of incubation. The highest enhancement effect of heavy metal on the dry weight was obtained at 0.2 mM $ZnSO_4$ and the dry weight at this concentration was increased to 1.93 mg/ml after 11 days of incubation, while the lowest value of dry weight 0.87 mg/ml was recorded at 0.1mM Co $(NO_3)_2.6H_2O$ at late exponential phase.

3.1 Photosynthetic pigments

The pigment content of *S. obliques* expressed as µg/ml algal suspension were markedly affected by increased concentration of heavy metals **(Fig. 4).** Chlorophyll a content of *S. obliques* was 2.44 µg/ml in the control culture and increased to 5.63 µg/ml by 0.2 mM $ZnSO_4$ treatment. Chlorophyll b content reached to 0.92 µg/ml in the control culture and increased to 2.59 µg/ml by 0.2 mM $ZnSO_4$ application. The contents of carotenoids ranged between 0.71 µg/ml in control culture and 1.32 µg/ml at 0.2 mM $ZnSO_4$ concentration at the end of the experiment. In general, total pigment of *S. obliques* was significantly increased by increasing heavy metal concentration in the algal cultures.

3.2 Flocculation efficiency for algal biomass

One of the primary goals of this investigation is to devise an effectual technique for recovering *S. obliques* biomass using flocculation–sedimentation operations. Exceedingly effectual flocculants necessity be used for this purpose, and the flocculants chosen must be inexpensive and available on an industrial scale. Furthermore, the flocculants must be safe and not alter the superiority of the detached biomass or the remaining water. Finally, the flocculants of choice should be as versatile as possible in terms of strain variety. Discontinuous sedimentation experiments on *S. obliques* were carried out to determine the yield of various flocculants. In each of them, a different concentration of flocculants was supplementary to the algal cell culture and allowable to settle naturally for 30 minutes. The results in **Fig. (5)** elucidated that, the optimum dose required for turbidity and cell count removal during the experiments was 200 ppm of Alum which reduced turbidity of water samples from 85.50 to 2.52 NTU (97.31%), and the cell count removal reached to 97.92 % as compared with corresponding control. Conversely, the best flocculant in removing turbidity from all flocculant concentration was 50 ppm of $FeCl_3$ at 97.27 % which cause high turbidity removal percentage at low concentration. Furthermore, different pH values and $CaCl_2$ concentrations show lower significant variance in removal percentage of turbidity and algal cell count compared with Alum and $FeCl_3$ after a 30-minute settling period, the flocculating efficiencies of the test flocculants at various concentrations are shown. The highest flocculating efficiency for test flocculants was observed at 75 ppm $FeCl_3$ (97.18 %), 150 ppm of Alum (97.49 %), and pH 12 (95.76%). When the flocculating efficiency of each flocculant at various concentrations is compared, it could be concluded that, the flocculation efficiency was increased significantly by increasing Alum., $FeCl_3$ concentrations and different pH values in contrast flocculating efficiencies of $FeCl_3$ decrease by increase the concentration. In addition, different $CaCl_2$ concentrations show lower flocculation efficiencies than Alum and $FeSO_4$ where the flocculant efficiencies for different $CaCl_2$ concentration (50 ,75 ,100, 150, 200 and 250 ppm) were 76.43, 82.09, 81.15, 75.80, 78.00 and 87.43 %, respectively. Among all flocculant concentration $FeCl_3$ at concentration 75 ppm was the best flocculant for harvesting *S. obliques* because it cause the highest flocculant efficiency at low concentration.

3.3 Turbidity and cell count removal

The results in **Fig. (5)** elucidated that, the optimum dose required for turbidity and cell count removal during the experiments was 200 ppm of Alum which reduced turbidity of water samples from 85.50 to 2.52 NTU (97.31%), and the cell count removal reached to 97.92 % as compared with corresponding control. Conversely, the best flocculant in removing turbidity from all flocculant concentration was 50 ppm of $FeCl_3$ at 97.27 % which cause high turbidity removal percentage at low concentration. Furthermore, different pH values and $CaCl_2$ concentrations show lower significant variance in removal percentage of turbidity and algal cell count compared with Alum and $FeCl_3$.

3.4 Microscopic examination of algal cells

Microscopic exanimation of algal cells treated with various flocculants show that, under all treatments, there were no signs of cell structural damage or plasmolysis, as well as no discernible changes in size and shape (Fig. 6). For cells treated with, the formation of flocks, indicating algal cell aggregation, was clearly observed. $FeCl_3$ and $Al_2(SO_4)_3$. While those treated with $CaCl_2$ and NaOH, although indicating reductions in optical densities, but revealed lower flocs formation as compared with $FeCl_3$ and $Al_2(SO_4)_3$.

2- Discussion

S. obliques cultures was treated with different concentration of the heavy metal; $MnCL_2$ (0.2, 0.4 & 0.6) mM $Co(NO_3)_2.6H_2O$ (0.04 mM, 0.07mM and 1 mM) and $ZnSO_4.7H_2O$ (0.1 mM, 0.2 mM ,0.3 mM and 0.4 mM) was add separately to evaluate their effects on growth and photosynthetic pigments, as well as carbohydrate, protein, and lipid contents, in addition to fatty acids profile. Majority concentration of the heavy elements (Mn^{+2}, Co^{+2} and Zn^{+2}) show positive effects on growth and photosynthetic pigments of *S. obliques*. Maximum stimulation in growth of *S. obliques* was achieved at 0.2 mM Zn^{+2} while dry weight increased by 63.56 %, biomass increased by 119.72 % as compared with control. Total pigment increased to 9.54 µg/ml compared to the control value (4.07 µg/ml) at late exponential phase. In contrast, a non-recoverable decline in growth of *S. obliques* was observed at the low concentration 0.1 µm Co^{+2}.Our result in agree with **Li *et al.*** [24] who reported that Zinc is a necessary element for the normal operation of enzyme systems in algae. Zinc is essential for sundry physiological procedures in algal cells, and it is a constituent of photosynthesis and related metabolism enzymes. Extraordinary Zn^{2+} absorptions that exceed those required for optimal growth can cause the nucleic acid to degrade, suppressing both NADPH formation in the chloroplast and the growth of the chloroplast. [25]**.** Manganese is essential because it is a component of a number of metalloenzymes, proteins, and vitamins that are important in algal metabolism. may explain growth enhancement in high Mn^{2+} supplemented media [26] Also **Battah *et al.*** [27] reported that Manganese is a mineral that plants require., as it participates in a variety of metabolic processes, most notably photosynthesis, and as an enzyme antioxidant-cofactor. Nonetheless, too much of this micronutrient is harmful to plants. Mn phytotoxicity manifests itself as a decrease in biomass and photosynthesis, as well as biochemical disorders such as oxidative stress. Similarly, **Price and Morel** [28] demonstrated that the Co^{2+} effect may promote growth in some metalloenzymes due to Co^{2+} substitution with Zn^{2+} **Whitton** [29] reported on the growth response and tolerance of various algae species to Zn^{2+}, demonstrating that lower concentrations of the metals stimulated algal growth while higher concentrations completely inhibited it. Other investigators conveyed that long -term effect of zinc on algal culture were at concentration ranging from 0.05 to 2.5 mg ml^{-1} [30]**.** Heavy metal stress led to produce a compensatory effect in the homeostatic microalgae regulators that contributes to the activation of toxicity-overcoming mechanisms of metabolic and antioxidant development. For instance, an increment for *S. Capricornus* and *E. gracilis* exposed to contaminated (Zn^{+2}, Cd, or Pb) has been documented to be a hormesis manifestation[31,32] **.**[33] **El-Sheekh *et al.*,** demonstrated that Low Co concentrations were applied to *Monoraphidium minutum* cultures, which resulted in Important changes in different pigment fractions (chlorophylls a and b, and carotenoids) were observed in both species, with maximum values reached at the end of the incubation period. Progressive increases in Co^{2+} concentration for *M. minutum,* on the other hand, resulted in a reduction in pigment content.

The fresh algae have the capability to stimulate Ch. (a) over Ch.(b) as a result of Co^{++} treatment especially at lower concentrations. The same result obtained by **El-Sheekh *et al.,*** [33] who discovered that *M. minutum* treated with high Co^{2+} concentrations had a higher chlorophyll a/b ratio due to the greater sensitivity of chlorophyll b compared to chlorophyll an as the Co^{2+} concentration increased. Even so, some heavy elements may have aided algal growth by acting as a nutrient, thereby providing an additional nutrient source to the medium's previously existing sources. Zn^{+2} is a cofactor in enzymes that is required for DNA synthesis, photosynthetic electron transport, and mitochondrial and chloroplast functions. [31]**.** **Abomohra *et al.*** [34] Both species showed significant improvements in different pigment fractions (chlorophylls a and b, and carotenoids), with maximum values reached at the end of the incubation period. **Afkar *et al.*** [35] zinc is an vital micronutrient for algae metabolism, but it can also be toxic when used at higher concentrations than the optimal level. Heavy element accumulation can inhibit growth through cell division, protein inactivation, chloroplast and mitochondrial decomposition, chloroplastic envelope breakup, membrane integrity degradation, and other mechanisms [36]**.**

Heavy metal deposition by microalgae has been studied in the presence of two stages: a 'fast' phase

characterized by metabolism-independent binding to the cell wall (biosorption), followed by a 'slow' phase characterized by the simultaneous effects of growth and surface adsorption, active uptake, or intra-cellular uptake via passive diffusion. [37] .There is often little discrimination between these possible mechanisms in the literature [38] .These results agree with [39] was recorded accumulation of cobalt by *S. obliques* indicated both fast and slow phases. The initial phase of accumulation, that was independent of light and metabolic inhibitors, was interpreted as biosorption, whereas the second slower phase of uptake, which was dependent on light and inhibited by respiration, was interpreted as an active uptake mechanism, rather than diffusion or increased binding due to growth. Even though cobalt is needed for vitamin B12 production and the bio-methylation of heavy elements by algae, it must be bound to the surface in trace amounts[40]. Because the average concentration of cobalt in the marine environment is about 0.3 mM, *S. obliques* would benefit from a high affinity active mechanism for cobalt uptake [37] .Also, researcher [41] investigated the effect of Co (II) *Monoraphidium minutum*, an algal cell, and *Nitzschia perminuta*, a diatom, were cultured under different concentrations of Co. (II). Low Co (II) concentrations increased growth and pigment content slightly, while high Co (II) concentrations inhibited growth and pigment content..

Regarding to photosynthetic pigments, the obtained results during this study revealed that, cell contents of these pigments of *S. obliques* was significantly affected by the treatment with different heavy metals. Most heavy metals concentration led to significant increase in photosynthetic pigment except 0.1µm Co^{+2} in *S. obliques*. These results in agreement with **Rai & Sharma** [42] whose recorded significant increase in chlorophyll (a)and β-carotene contents of algae cultured under effect of heavy metal. **Saçan & Balcıoğlu (2006)** [43] noticed that chlorophyll contents of algae were significantly stimulated in low concentrations of heavy metal treatments. **Arunakumara & Zhang** [44] attributed the chlorophyll mutilation on the thylakoid membranes to the influence of heavy elements. The inhibition effects of the investigated alga's carotenoid pigment contents were generally lower than those of chlorophyll. **Saçan & Balcıoğlu** [43] noticed that chlorophyll contents of algae were significantly stimulated in low concentrations of heavy metal treatments. However, high waste concentrations reduced chlorophyll contents of these two algal species.

The accumulation of carbohydrates within the algal cells is regarded as the main organic compounds derived from photosynthetic activity. Carbohydrate contents of the investigated alga were found to be affected due to the influence of different heavy metals concentration. The contents of total carbohydrates of *S. obliques* increase significantly in algal cultures treated with different heavy metals. Our results agreed with **Sharma & Agrawal** [45] whose recorded significant increases in carbohydrate contents of algal cultures under effect of heavy metals. The huge accumulation of soluble carbohydrate fraction could using to detoxify heavy metals stress, which seemed to be a suitable mechanism [44] . **Costa & Spitz** [46] showed that the carbohydrates levels increased at lower concentrations of Mn^{+2}. Moreover, the stimulatory effect of suitable concentrations of copper on the soluble carbohydrates may be due to the stimulation of photosystems I and II. [47]found a relation amid copper and ferredoxin on the reducing site of PS I, where Cu^{+2} stimulated the level of overall electron transfer from water to NADP.

The response of *S. obliques* to different heavy metal in the form of total protein was change according to type of heavy metal and dose. The role of heavy elements on *S. obliques* reveal variation in the protein content according to the dose of the studied element. Increased reactive oxygen species caused by heavy metals can cause protein oxidation and degradation [48]. Under heavy metal stress, mutually decreases and increases in total protein content have been observed in algae [49]. Furthermore, the decrease in protein content in heavy metal-treated algae may be endorsed to protein degradation due to oxidative damage. The increases in total protein content caused by heavy metals, on the other hand, were most likely due to an increase in specific stress-related proteins, such as enzymes involved in antioxidant metabolism and photo heating biosynthesis [48]. It may be argued that one way for algae to remove their toxic effects is to accumulate protein fractions at low heavy metal concentrations, or that increasing respiration causes carbohydrate utilization to be prioritized over protein accumulation [39]. Protein accumulation may be suppressed due to a lack of carbon skeleton caused by a low photosynthetic rate [35]. In addition, the reduction in proteins might be due to the hydrolysis of proteins into amino acids allows the possibility of amino acids being used osmotically [50] or decreased availability of amino acids which it share in proteins synthesis and /or denaturation of enzymes intricate in the synthesis of amino acids and proteins [51].

4.1 Flocculation

For harvesting *S. obliques*, this study found a clear relationship between concentration and flocculating efficiency for NaOH, $Al_2(SO_4)_3$, $CaCl_2$, and $FeCl_3$. These chemicals are simple to use, inexpensive, and pose no significant environmental hazards to the recovered biomass resources for more manufacturing. Many benefits have included ease of operation and the prospect of reusing the culture medium and recycling the nutrients. Harvesting microorganisms biomass from growth medium is a critical step in the development of microalgae biodiesel, accounting for about 20–30% of the total cost [9].

Coagulation-flocculation is a technique for aggregating microbial cells and increasing the appropriate particle size, resulting in increased biomass production. To adequately settle microalgae cells, the sedimentation speed must be higher than 10^{-4} m/s, so it is important to increase it by collating cells[52] . The flocculant was inserted to the microalga culture and resettled naturally for 30 minutes to determine only those flocculants that are truly successful, producing flocs with sedimentation velocity greater than 210^{-4} m/s [53]. Flocs were developed and settled in some cases, and then an interphase was detected. Instead that, some flocs emerge and settle, but there is no discernible interphase. The height variance of the interphase was calculated over time. Different doses of each flocculant were evaluated to determine the minimum doses of flocculant able to properly recover the biomass [53] As a result, the flocculant quality, cell count removal, and turbidity removal were all assessed. As metal ion flocculants are added to a microalga suspension, the negative surface charge that prevents the cells from clumping together is reduced or neutralized[54]. Metal cations (e.g., Al^{3+}, Fe^{3+}) can also act as a bridge between cells, causing them to clump together and settle out of suspension. [55] [56].

This study revealed that with increase the concentration of alum,$CaCL_3$ and pH value the flocculation efficiency , turbidity removal and cell count removal of *S. obliques* cells from culture media increase until reach to max. level at 200 ppm alum (250 ppm) $CaCl_3$ and pH 12 while with increase the concentration of $FeCl_3$ flocculation efficiency , turbidity removal and cell count removal significantly decrease utile reach to the lowest level at 250 ppm $FeCl_3$. In line with this, it has previously been stated that alum. sulphate outperformed other inorganic salts in terms of optimal dosage, pH, and the consistency of the resulting water and algal slurry [53]. **Lee *et al.*** [10] demonstrated that Aluminum sulphate was also found to be more effective at extracting algae cells than pH adjustment with NaOH. The direct relationship observed between concentration and flocculating efficiency for $Al_2(SO_4)_3$, $CaCl_2$, and $FeCl_3$ as well as pH adjustment using NaOH is also shown in this research. **Vandamme *et al.*** [52] testified flocculation reactions can be subtle to a number of factors, one of which is flocculant concentration. The flocculant efficiency, turbidity removal and cell count removal [57] .

Aziz *et al.* [58] indicated that $FeCl_3$ as a instructive agent for landfill leachate at a regulated pH (6) resulted in a significant decrease in $FeCl_3$ efficacy as concentrations varies from 0 to 500 mgL^{-1}. Data also revealed that *S. obliques* separation induced by pH was highly effective in this experiment where the highest value of flocculant efficiency recorded was 95.76 % and the highest turbidity removal was 95.34 % at pH 12. [59] Metal ions in the growth medium, such as Mg^{2+} and Ca^{2+}, are hydrolyzed to form positive precipitates, which coagulate negative microalgae cells by sweeping flocculation and charge neutralization, according to the study. [60] *Scenedesmus* auto-flocculation induced by photosynthesis [10] mediated auto-flocculation of *Arthrospira* at an optimal pH level of 9 with a flocculation efficiency of nearly 90%, demonstrating that this method is certainly useful to harvest microalgae in large-scale culture without any drawbacks.

[59] Generated a flocculation method for harvesting a specific microalgae with self-flocculating microalgae by lowering pH to below isoelectric point; this mechanism is more efficient than flocculation only by lowering pH. [61] When particles were simply destabilized by charge neutralization, the flocculation efficiency was significantly improved. It has been demonstrated that maintaining cell wall integrity during the harvesting process improves the shelf life of harvested cells & the preservation of cell metabolites[62].

This analysis revealed that all flocculants used with different ratios showed evidence of cell wall destruction, suggesting that these flocculants should be used to harvest algal cells[63]. In terms of pH, this survey suggests that independently to concentration, all flocculants had a major impact on changing the pH of the culture media after flocculation.

Excepting NaOH, that had the contradictory influence, all flocculants lowered the pH of the subsequent supernatant. Lower pH values in the culture media resulted in higher biomass concentrations [52]. Other parameters such as nutrient concentration (i.e., ammonia, nitrite, nitrate, and phosphorous) should be included in future studies about the probability of recycling wastewater

after flocculation, even though pH of the culture media plays an important role in algae culture. Despite the fact that all therapies had substantial pH increases, these levels were just within the algal culture's tolerable range of pH 6.47 to 8.53. NaOH was used to raise the pH of the culture medium from 9 to 12, resulting in coagulation after 30 minutes. When NaOH and $CaCl_2$ were added to the coagulated cells, they endured suspended in the medium, but when Alum and $FeCl_3$ were added, they formed a large floc.

Reference

[1] D.C. Kligerman, E.J. Bouwer, Prospects for biodiesel production from algae-based wastewater treatment in Brazil: A review, Renew. Sustain. Energy Rev. 52 (2015) 1834–1846. https://doi.org/10.1016/j.rser.2015.08.030.

[2] D. Vandamme, I. Foubert, K. Muylaert, Flocculation as a low-cost method for harvesting microalgae for bulk biomass production, Trends Biotechnol. 31 (2013) 233–239. https://doi.org/10.1016/j.tibtech.2012.12.005.

[3] B. Kiran, N. Rani, A. Kaushik, FTIR spectroscopy and scanning electron microscopic analysis of pretreated biosorbent to observe the effect on Cr (VI) remediation, Int. J. Phytoremediation. 18 (2016) 1067–1074. https://doi.org/10.1080/15226514.2016.1183577.

[4] I.M. MUNDA, V. HUDNIK, The Effects of Zn, Mn, and Co Accumulation on Growth and Chemical Composition of Fucus vesiculosus L under Different Temperature and Salinity Conditions, Mar. Ecol. 9 (1988) 213–225. https://doi.org/10.1111/j.1439-0485.1988.tb00329.x.

[5] S. Siripornadulsil, S. Traina, D.P.S. Verma, R.T. Sayre, Molecular mechanisms of proline-mediated tolerance to toxic heavy metals in transgenic microalgae, Plant Cell. 14 (2002) 2837–2847.

[6] A.M. Abo-Shady, A.H. El-Naggar, M.M. El-Sheekh, A.E.-F. Abomohra, Impact of UV-B radiation on antioxidant enzymes and protein electrophoretic pattern of the green algaChlorococcum sp., Ann. Microbiol. 58 (2008) 195–201.

[7] B.N. Tripathi, J.P. Gaur, Physiological behavior of Scenedesmus sp. during exposure to elevated levels of Cu and Zn and after withdrawal of metal stress, Protoplasma. 229 (2006) 1–9.

[8] M.K. Danquah, L. Ang, N. Uduman, N. Moheimani, G.M. Forde, Dewatering of microalgal culture for biodiesel production: exploring polymer flocculation and tangential flow filtration, J. Chem. Technol. Biotechnol. Int. Res. Process. Environ. Clean Technol. 84 (2009) 1078–1083.

[9] Z. Wu, Y. Zhu, W. Huang, C. Zhang, T. Li, Y. Zhang, A. Li, Evaluation of flocculation induced by pH increase for harvesting microalgae and reuse of flocculated medium, Bioresour. Technol. 110 (2012) 496–502.

[10] S.J. Lee, S.B. Kim, J.E. Kim, G.S. Kwon, B.D. Yoon, H.M. Oh, Effects of harvesting method and growth stage on the flocculation of the green alga Botryococcus braunii, Lett. Appl. Microbiol. 27 (1998) 14–18.

[11] E. Molina Grima, E.H. Belarbi, F.G. Acién Fernández, A. Robles Medina, Y. Chisti, Recovery of microalgal biomass and metabolites: Process options and economics, Biotechnol. Adv. 20 (2003) 491–515. https://doi.org/10.1016/S0734-9750(02)00050-2.

[12] G. Prochazkova, I. Safarik, T. Branyik, Harvesting microalgae with microwave synthesized magnetic microparticles, Bioresour. Technol. 130 (2013) 472–477.

[13] I.A. Matter, V.K. Hoang Bui, M. Jung, J.Y. Seo, Y.E. Kim, Y.C. Lee, Y.K. Oh, Flocculation harvesting techniques for microalgae: A review, Appl. Sci. 9 (2019). https://doi.org/10.3390/app9153069.

[14] W.W. Carmichael, J.-W. He, J. Eschedor, Z.-R. He, Y.-M. Juan, Partial structural determination of hepatotoxic peptides from Microcystis aeruginosa (cyanobacterium) collected in ponds of central China, Toxicon. 26 (1988) 1213–1217.

[15] N.S. Caetano, A.A. Martins, M. Gorgich, D.M. Gutiérrez, T.J. Ribeiro, T.M. Mata, Flocculation of Arthrospira maxima for improved harvesting, Energy Reports. 6 (2020) 423–428.

[16] R. Gutiérrez, F. Passos, I. Ferrer, E. Uggetti, J. García, Harvesting microalgae from wastewater treatment systems with natural flocculants: effect on biomass settling and biogas production, Algal Res. 9 (2015) 204–211.

[17] A.K. Lee, D.M. Lewis, P.J. Ashman, Microbial flocculation, a potentially low-cost harvesting technique for marine microalgae for the production of biodiesel, J. Appl. Phycol. 21 (2009) 559–567.

[18] C.-H. Hsieh, W.-T. Wu, Cultivation of microalgae for oil production with a cultivation strategy of urea limitation, Bioresour. Technol. 100 (2009) 3921–3926.

[19] H. Metzner, H. Rau, H. Senger, Untersuchungen zur synchronisierbarkeit einzelner pigmentmangel-mutanten von Chlorella, Planta. 65 (1965) 186–194.

[20] S.S.A. Badour, Analytisch-chemische Untersuchung des Kaliummangels bei Chlorella im Vergleich mit anderen Mangelzuständen, (1960).

[21] F.W. Fales, The assimilation and degradation of carbohydrates by yeast cells, J. Biol. Chem. 193 (1951) 113–124.

[22] A. Schlesinger, D. Eisenstadt, A. Bar-Gil, H. Carmely, S. Einbinder, J. Gressel, Inexpensive non-toxic flocculation of microalgae contradicts theories; overcoming a major hurdle to bulk algal production, Biotechnol. Adv. 30 (2012) 1023–1030.

[23] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with the Folin phenol reagent., J. Biol. Chem. 193 (1951) 265–275.

[24] M. Li, C. Hu, Q. Zhu, L. Chen, Z. Kong, Z. Liu, Copper and zinc induction of lipid peroxidation and effects on antioxidant enzyme activities in the microalga Pavlova viridis (Prymnesiophyceae), Chemosphere. 62 (2006) 565–572.

[25] Z.Y.L. Dongchao, Effect of Four Kinds of Trace Metal Element on Growth, Chlorophyll-a and Size of Pavlova viridis [J], J. Zhanjiang Ocean Univ. 1 (2003).

[26] M. Battah, Y. El-Ayoty, A.E.F. Abomohra, S.A. El-Ghany, A. Esmael, Effect of Mn2+, Co2+ and H2O2 on biomass and lipids of the green microalga Chlorella vulgaris as a potential candidate for biodiesel production, Ann. Microbiol. 65 (2015) 155–162. https://doi.org/10.1007/s13213-014-0846-7.

[27] R. Millaleo, M. Reyes-Díaz, A.G. Ivanov, M.L. Mora, M. Alberdi, Manganese as essential and toxic element for plants: transport, accumulation and resistance mechanisms, J. Soil Sci. Plant Nutr. 10 (2010) 470–481.

[28] N.M. Price, F.M.M. Morel, Cadmium and cobalt substitution for zinc in a marine diatom, Nature. 344 (1990) 658–660.

[29] B.A. Whitton, Toxicity of heavy metals to freshwater algae: a review, Phykos. (1971).

[30] M. Paulsson, V. Månsson, H. Blanck, Effects of zinc on the phosphorus availability to periphyton communities from the river Göta Älv, Aquat. Toxicol. 56 (2002) 103–113.

[31] M. Einicker-Lamas, G.A. Mezian, T.B. Fernandes, F.L.S. Silva, F. Guerra, K. Miranda, M. Attias, M.M. Oliveira, Euglena gracilis as a model for the study of Cu2+ and Zn2+ toxicity and accumulation in eukaryotic cells, Environ. Pollut. 120 (2002) 779–786.

[32] D. Spoljaric, A. Cipak, J. Horvatic, L. Andrisic, G. Waeg, N. Zarkovic, M. Jaganjac, Endogenous 4-hydroxy-2-nonenal in microalga Chlorella kessleri acts as a bioactive indicator of pollution with common herbicides and growth regulating factor of hormesis, Aquat. Toxicol. 105 (2011) 552–558.

[33] M.M. El-Sheekh, A.H. El-Naggar, M.E.H. Osman, E. El-Mazaly, Effect of cobalt on growth, pigments and the photosynthetic electron transport in Monoraphidium minutum and Nitzchia perminuta, Brazilian J. Plant Physiol. 15 (2003) 159–166.

[34] M. Battah, Y. El-Ayoty, A.E.-F. Abomohra, S. Abd El-Ghany, A. Esmael, Effect of Mn 2+, Co 2+ and H 2 O 2 on biomass and lipids of the green microalga Chlorella vulgaris as a potential candidate for biodiesel production, Ann. Microbiol. 65 (2015) 155–162.

[35] E. Afkar, H. Ababna, A.A. Fathi, Toxicological response of the green alga Chlorella vulgaris, to some heavy metals, Am. J. Environ. Sci. 6 (2010) 230.

[36] N. La Rocca, C. Andreoli, G. áM Giacometti, N. Rascio, I. Moro, Responses of the Antarctic microalga Koliella antarctica (Trebouxiophyceae, Chlorophyta) to cadmium contamination, Photosynthetica. 47 (2009) 471–479.

[37] G.W. Garnham, G.A. Codd, G.M. Gadd, Kinetics of uptake and intracellular location of cobalt, manganese and zinc in the estuarine green alga Chlorella salina, Appl. Microbiol. Biotechnol. 37 (1992) 270–276.

[38] D. Khummongkol, G.S. Canterford, C. Fryer, Accumulation of heavy metals in unicellular algae, Biotechnol. Bioeng. 24 (1982) 2643–2660.

[39] M.E.H. Osman, A.H. El-Naggar, M.M. El-Sheekh, E.E. El-Mazally, Differential effects of Co2+ and Ni2+ on protein metabolism in Scenedesmus obliquus and Nitzschia perminuta, Environ. Toxicol. Pharmacol. 16 (2004) 169–178.

[40] F.E. Brinckman, G.J. Olson, Global biomethylation of the elements: Its role in the biosphere translated to new organometallic chemistry and biotechnology, Biol. Alkylat. Heavy Elem. R. Soc. Chem. London. (1988) 168–196.

[41] M.A. Saito, J.W. Moffett, S.W. Chisholm, J.B. Waterbury, Cobalt limitation and uptake in Prochlorococcus, Limnol. Oceanogr. 47 (2002) 1629–1636.

[42] A.K. Rai, N.K. Sharma, Phosphate metabolism in the cyanobacterium Anabaena doliolum under salt stress, Curr. Microbiol. 52 (2006) 6–12.

[43] M.T. Saçan, I.A. Balcıoğlu, A case study on algal response to raw and treated effluents from an aluminum plating plant and a pharmaceutical plant, Ecotoxicol. Environ. Saf. 64 (2006) 234–243.

[44] K. Arunakumara, X. Zhang, Heavy metal bioaccumulation and toxicity with special reference to microalgae, J. Ocean Univ. China. 7 (2008) 60–64.

[45] R.K. Sharma, M. Agrawal, Biological effects of heavy metals: an overview, J. Environ. Biol. 26 (2005) 301–313.

[46] G. Costa, E. Spitz, Influence of cadmium on soluble carbohydrates, free amino acids, protein content of in vitro cultured Lupinus albus, Plant Sci. 128 (1997) 131–140.

[47] A.S. Fahmy, T.M. Mohamed, S.A. Mohamed, M.M. Saker, Effect of salt stress on antioxidant activities in cell suspension cultures of cantaloupe (Cucumis melo), Egypt. J. Physiol. Sci. 22 (1998) 315–326.

[48] Z.B. Doganlar, S. Cakmak, T. Yanik, Metal uptake and physiological changes in Lemna gibba exposed to manganese and nickel, Int. J. Biol. 4 (2012) 148.

[49] P. Vajpayee, R.D. Tripathi, U.N. Rai, M.B. Ali, S.N. Singh, Chromium (VI) accumulation reduces chlorophyll biosynthesis, nitrate reductase activity and protein content in Nymphaea alba L., Chemosphere. 41 (2000) 1075–1082.

[50] M.A. Shaddad, A.M. Ismail, M.M. Azooz, A. Abdel-Latef, Effect of salt stress on growth and some related metabolites of three wheat cultivars, Assuit Univ. J. Bot. 34 (2005) 477–491.

[51] W.A. Kasim, Effect of copper and cadmium on some growth criteria and physiological aspects of Sorghum bicolor. Egypt, J. Biotechnol. 9 (2001) 298–310.

[52] D. Vandamme, I. Foubert, I. Fraeye, B. Meesschaert, K. Muylaert, Flocculation of Chlorella vulgaris induced by high pH: role of magnesium and calcium and practical implications, Bioresour. Technol. 105 (2012) 114–119.

[53] M.R. Granados, F.G. Acién, C. Gomez, J.M. Fernández-Sevilla, E.M. Grima, Evaluation of flocculants for the recovery of freshwater microalgae, Bioresour. Technol. 118 (2012) 102–110.

[54] A. Richmond, Handbook of microalgal culture: biotechnology and applied phycology, Wiley Online Library, 2004.

[55] A. Papazi, P. Makridis, P. Divanach, Harvesting Chlorella minutissima using cell coagulants, J. Appl. Phycol. 22 (2010) 349–355.

[56] P. Kondzior, A. Butarewicz, Effect of heavy metals (Cu and Zn) on the content of photosynthetic pigments in the cells of algae Chlorella vulgaris, J. Ecol. Eng. 19 (2018).

[57] S.A. Shehata, G.H. Ali, S.Z. Wahba, Distribution pattern of Nile water algae with reference to its treatability in drinking water, J. Appl. Sci. Res. 4 (2008) 722–730.

[58] H.A. Aziz, S. Alias, F. Assari, M.N. Adlan, The use of alum, ferric chloride and ferrous sulphate as coagulants in removing suspended solids, colour and COD from semi-aerobic landfill leachate at controlled pH, Waste Manag. Res. 25 (2007) 556–565.

[59] J. Liu, Y. Tao, J. Wu, Y. Zhu, B. Gao, Y. Tang, A. Li, C. Zhang, Y. Zhang, Effective flocculation of target microalgae with self-flocculating microalgae induced by pH decrease, Bioresour. Technol. 167 (2014) 367–375.

[60] A. Sukenik, G. Shelef, Algal autoflocculation—verification and proposed mechanism, Biotechnol. Bioeng. 26 (1984) 142–147.

[61] J. Duan, J. Gregory, Coagulation by hydrolysing metal salts, Adv. Colloid Interface Sci. 100 (2003) 475–502.

[62] R.M. Knuckey, M.R. Brown, R. Robert, D.M.F. Frampton, Production of microalgal concentrates by flocculation and their assessment as aquaculture feeds, Aquac. Eng. 35 (2006) 300–313.

[63] V.M.E.N. Ferriols, R.O. Aguilar, Efficiency of various flocculants in harvesting the green microalgae Tetraselmis tetrahele (Chlorodendrophyceae: Chlorodendraceae), Aquac. Aquarium, Conserv. Legis. 5 (2012) 265–273.