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Optimized flocculation of microalgae for fuel oil and antioxidant production

Guzine I. El Diwani, ShadiaA. El Rafei*, Salwa I. Hawash, Sanaa A. El Enin

National Research Centre, Engineering Division, Chemical Engineering Department, Cairo, Egypt

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ABSTRACT

The key technologies for producing microalgae biofuels include identification of preferable culture conditions for high oil productivity, development of effective and economical microalgae cultivation system, as separation and harvesting of microalgae biomass and oil. Microcyst,sp. biomass harvesting by flocculation was studied using inorganic coagulants ($FeCl_3.6H_2O$, $Al_2(SO_4)_3\ 18H_2O$, liquid Bittern rich in Ca^{2+} and Mg^{2+} ions). The results show that $FeCl_3.6H_2O$ proved the best algae removed efficiency when used alone as coagulent. The only drawback is yellow color in the clarified water besides the pollution with elements such as Fe ions. Algae removal efficiency increased remarkably when the culture water was pretreated with Na-ferrate and followed by [$Al_2(SO_4)_3$.$18H_2O$] and pH adjusted to 7.1. The addition of Na-ferrate reduced the optimum dose to one half. As well as the use of sonication significantly enhanced the reduction of algae cells in culture water and reduced the optimum dose down to one third. Coagulation efficiency depends strongly on the coagulant dose and sonication conditions. The optional sonication time (50- 60 sec.), the most effective sonication intensity was 2.5 w/cm^3 and the high removal ratio was 99% by the sonication-coagulation with alum reduced dose. The dried micro algae flocks were collected and subjected for lipids extraction using less toxic solvents. Identification of lipids was studied by Gas Chromatography and the antioxidant activity was determined (IC_{50}= 39.3%) by 2,2diphenylpicryl-hydrazyl (DPPH) free radical using DR-2000 spectrophotometer.

Key words: Micro algae, Chemical coagulation, sonication, Lipids identification, antioxidants.

INTRODUCTION

Today about 80% of global energy demand is produced from fossil fuels. However, extensive utilization of fossil fuels has led to global climate change environmental pollution and health problems [1]. Many countries are thus turning their attention to the development of new, clean, and sustainable energy sources. Among the various potential sources of renewable energy, biofuels are of most interest and are expected to play a role in the global energy infrastructure in the future. Biodiesel, one of the most commonly used biofuels, is recognized as an ideal recyclable energy carrier, and thus also as a possible primary energy source [2]. Microalgae that can grow rapidly and convert solar energy to chemical energy via CO_2 fixation are now being

considered a promising oil source for making biodiesel [3].The ability of algae to survive over a wide range of environmental conditions is, to a large extent, reflected in the treatment diversity and sometimes unusual pattern of cellular lipids as well as the ability to modify lipid metabolism efficiently in response to changes in environmental conditions [4-6].

Microalgae differentiate themselves from other single-cell microorganisms in their natural ability to accumulate large amounts of lipids. Microalgae lipids are also known to contain fatty acids especially valuable as dietary supplements, including omega-3 and omega-6compounds, EPA (Eicosal Pentaenoic Acid) and DAA (Doosan Hexaenoic Acid) are commercially valuable and currently marketed in several different formulation, as dietary supplements for adults, health supplement, in infant nutritional products, and additives to animal feed.

The lipids may include, but are not limited to, neutral lipids, polar lipids, wax esters, sterols and hydrocarbons, as well as Pregl derivatives such as the chlorophylls. Under optimal conditions of growth, algae synthesize fatty acids principally for esterification into glycerol- based membrane lipids, which constitute about 5-20 % of their dry cell weight (DCW). Fatty acids include medium-chain (C_{10} – C_{14}), long-chain (C_{16} – C_{18}) and very long-chain ($\geq C_{20}$) species and fatty acids derivatives. The major membrane lipids are the glucosylglycerides (e.g. monogalactosyldiacylglycerol in dialactosyldiacylglycerol and sulfoquinovosyldiacyl-glycerol), which are enriched in the chloroplast, together with significant amount of phosphoglycerides (e.g. phospha-tidylethanolamine, PE, and phosphatidylglycerol, PG), which mainly reside in the plasma membrane and many endoplasmic membrane system [7], [8],[9],[5]. The major constituents of the membrane glycolipids are various kinds of fatty acids that are polyunsaturated and derived through aerobic desaturation and chain elongation from the " precursor" fatty acids palmitic (16:0) and oleic (18:1 omiga- 9) acids [10].

Under suitable culture conditions, some microalgae species are able to accumulate up to 50-70% of oil / lipid per dry weight[2]. Under unfavorable environmental or stress conditions for growth, however, many algae alter their lipid biosynthetic patways towards the formation and accumulation of neutral lipids (20 – 50% DCW), mainly in the form of triacyl glycerol (TAG). Unlike the glycerolipids found in membranes, TAGs do not perform a structural role but instead serve primarily as a storage form of carbon and energy. However, there is some evidence suggesting that, in algae, the TAG biosynthesis pathway may play a more active role in the stress response, in addition to functioning as carbon and energy storage under environmental stress conditions. The fatty acid profile of microalgae oil is suitable for the synthesis of biodiesel [11]. Hydrocarbons are another type of neutral lipids that can be found in algae at generally < 5% DCW [12]. Only the colinal green algae, *Botryococcus braunii*, has been shown to produce, under adverse environmental conditions, large quantities (up to 80% DCW) of very-long-chain (C_{23}-C_{40}) hydrocarbons, similar to those found in petroleum, and thus has been explored over the decades as a feed stock for biofuels and biomaterial. As many algal species have been found to grow rapidly and produce substantial amounts of TAG or oil, and are thus referred to as oleaginous algae, it has long been postulated that algae could be employed as a cell factories to produce oils and other lipids for biofuels and other biomaterials [13-17]. The potential advantages of algae as feedstock for biofuels and biomaterials include their ability to:

(i) synthesize and accumulate large quantities of neutral lipids\oil (20-50% DCW),
(ii) grow at high rates (e.g. 1-3 doubling per day),
(iii)survive in saline\brackish water \ coastal sea water for which there are few competing demands,

(iv)tolerate marginal lands (e.g. desert, arid – and Semi-arid lands) that are not suitable for conventional agriculture,
(v) utilize growth nutrients such as nitrogen and phosphorus from a variety of wastewater sources (e.g. agricultural run – off, concentrated animal feed operations, and industrial and municipal wastewaters), providing the additional benefit of wastewater bio-remediation ,
(vi)sequester carbon dioxide from flue gases emitted from fossil fuel-fired power plants and other sources, there-by reducing emissions of a major green house gas,
(vii) produce value-added co-products or by-products (e.g. biopolymers, proteins, polysaccharides, pigments, animal feed, fertilizer and H_2),
(viii) can grow in suitable culture vessels (photo-bioreactors) through the year with annual biomass productivity, on area basis, exceeding that of terrestrial plants by approximately ten fold.

The major attraction of using microalgae oil for biodiesel is the treatment oil production capacity by microalgae, as they could produce up to 58-700 L oil per hectare, which is one or two magnitude higher than that of any other energy crop [5]. Based upon the photosynthetic efficiency and growth potential of algae, theoretical calculations indicate that annual oil production of > 30 000 L or about 200 barrels of algal oil per hectare of land may be achievable in mass culture of oleaginous algae, which is 100-fold greater than that of soybeans, a major feedstock currently being used for biodiesel in the USA. Nevertheless, since microalgae production is regarded a feasible approach to mitigate global warning, it is clear that producing oil from microalgae biomass would provide significant benefits, in addition to the fuel. Microalgae have thus been widely recognized as the feedstock for third –generation of biofuels[5]. The biodegradable, renewable and non-toxic properties of biodiesel make it a very promising alternative fuel. Microalgae have advantages over traditional biodiesel feed-stock, these include high growth rate (able to double their biomass within a period of 24 h) [18], high lipid content and the ability to grow on arid regions of land whilst making use of water that is not suitable for conventional agriculture. Dewatering of microalgal cultures requires high costs and energy due to its dilute nature and this highly impact on the economics of biomass engineering. Flocculation is commonly used dewatering technique that has the advantage of using less energy under optimum conditions. Flocculation is a low energy process, but can be expensive if the flocculent is costly and the dosage is high. However, most microalgal systems rely on cheap flocculants such as ferric chloride, aluminum sulphate, chitosan and various polymeric flocculants [19].

Microalgae can be separated from aqueous solution by settling after treatment with flocculants, coagulants and polymers or a combination of these inorganic additives. At the pilot or laboratory scale, this can be performed using a graduated cylinder and measuring the settling speed and final clarification of the aqueous medium. Inorganic coagulants were conducted using Jar tests to evaluate coagulation on algae removal. Microalgae cells are negatively charged, as a result of adsorption of ions originating from organic matter and dissociation of ionization of surface functional groups[20]. By disrupting the stability of the system, successful microalgae harvesting can be obtained. Addition of a coagulant, like iron-based or aluminum-based coagulants, well neutralize reduce the surface charge [19]. Microalgae can also be flocculated by inorganic flocculants at sufficiently low pH [20],[21]. However, despite its advantage coagulation using inorganic coagulants suffers from the following drawbacks:

1-A large concentration of inorganic flocculent is needed to cause solid-liquid separation of the microalgae, thereby producing a large quantity of sludge.
2-The process is highly sensitive to pH level.

3-Although some coagulants may work for some microalgae species, they do not work for others.
4-The end product is contaminated by the added aluminum or iron salts.

The most effective flocculants for the recovery of microalgae are cationic flocculants [22]. Anionic and nonionic polyelectrolytes have been shown to fail to flocculate microalgae, which are explained by the repulsion existing between charges or the insufficient distance to bridge particles. Polymer molecular weight, charge density of molecules, dosage, concentration of microalgae biomass, ionic strength and pH of the broth, and the extent of mixing in the fluid have all has been found to affect flocculation efficiency[19]. Bilanovic et al., 1988 [22] noted that flocculation by cationic polymers can be inhibited by the high salinity of a marine environment. High molecular weight polyelectrolytes are generally better bridging agents.

It was reported that pre-treatment with oxidants may enhance the coagulation process and specifically enhance the removal of algae and other particulate matters in subsequent treatment steps [23], [24],[25]. Sodium- ferrate (Na Fe O_4) is a strong oxidizing agent, which has a strong redox potential through the entire pH range, ranging from 2.2V in acid to -0.7 V in base , the effect of pre-treatment with ferrate VI (ions) on algae coagulation by aluminum sulphate was studied by [26].

Ultrasonic irradiation has been known to cause various chemical, physical, and biological effects [27], [28], [28]. When applied to water, power ultrasound causes acoustic cavitation, in which millions of small bubbles (nuclei) collapse rapidly to reach temperatures as high as 5000 k and pressures as high as 100 MPa (so-called "hot spots"), generates highly active hydroxyl free radicals, and causes high-speed micro-jets [27].

Such extreme conditions endure chemical reactions and inflict mechanical damage on water borne substances, and have been widely employed to accelerate reactions or to eliminate impurities in the laboratory and in industry. Furthermore, micro-jets caused by acoustic cavitation enhance mixing and benefit the coagulation process [30].

Several studies have shown that the quantity and quality of lipids within the cell can vary as a result of changes in growth conditions(temperature and light intensity) or nutrient media in characteristics (concentration of nitrogen, phosphate and iron) A.M. [31-32]. An ideal lipid extraction process for microalgal biodiesel productionneeds to be not only lipid specific in order to minimize the co-extraction of non-lipid contaminants but also selective towards desirable lipid fractions (neutral lipids mono-, di-, and trienoic fatty acid chains) [33-34]. Even though the classic Folch Chloroform-based lipid extraction method [35] is effective for the majority of microalgal lipid analyses, an alternative organic solvent method that is more user-friendly is needed for scale-up. Hexane, ethanol, iso-propanol butanol and various combinations have been investigated as less-toxic substitutes for lipid extraction [33-34, 36-39].

The aim of this work is to study the effectiveness of different flocculants commonly used in the wastewater treatment for the recovery of microalgae. The microalga used in this study is *Microcystis sp*. The oil, lipid and antioxidants were identified[40-42].

The flocculants employed in this work were Fe Cl_3 .$6H_2O$, $Al_2(SO_4)_3$. 18 H_2O and Liquid Bittern (rich in Ca^{2+} and Mg^{2+} ions) were added. Furthermore, Na-ferrate VI and ultrasound irradiation have been used for enhancing the microalgae harvesting. The different compounds have been evaluated at six different concentrations of flocculants values ranging from 10 ppm to 80 ppm

and different concentrations have been tested at pH 7.1 with alum, pH 8 with $FeCl_3$ and pH 10.5 with liquid bittern (LB).

In this study we used solvent mixture for lipid extraction and estimated oil quantity by gravimetric method. Gas Chromatography was used for lipid identification.

MATERIALS AND METHODS

Lyophilized *Microcyst,sp.* biomass of microalgae was used as oil-rich substrate. Cells were grown in photo bioreactor, harvested by precipitation and chemical coagulation enhanced with pre-oxidation and /or pre-sonication.

2.1Raw materials:

2.1.1 Raw water of algae culture after decantation which is in deep green color indicating 10.682 mg/L algae concentration, was selected in this study Table (1) shows water quality.

Table (1) The raw water quality was listed as follows

Parameter	Values
Turbidity, FTUS	104-154
pH	8.3 – 9.5
Temperature °C	18-25
Conductivity MS/cm	2.44
TSS mg/L	10.682
TDS g/L	1.22

2.1.2 Fe CL_3. $6H_2O$ and $Al_2(SO_4)_3$ were added . 18 H_2O were pure grade. HCl and Na OH were pure grade and used to adjust pH of solutions. Distilled water was used for stock solutions preparation. Also methanol and chloroform were pure grade.

2.1.3 Liquid bittern (LB) is obtained as a by- product of seawater after the solar evaporation and NaCl production effluent from saline water conversion plants. Table (2) includes some physicochemical characteristics of (LB).

Table (2) Some physicochemical characteristics of (LB):

Parameter	Values
pH	7.3
Density,(g/ml)	1.297
Conductivity, MS/cm at 25 °C	513
Turbidity, FTUS	131
TDS, mg/L	390
CL^{-1}, mg/L	269,650
Ca^{+2} mg/L,	120
Mg^{+2} mg/L	75,207
Total alkalinity, mg/L(as Ca CO_3)	191

With LB, flocculation is carried out at high alkaline pH because at pH values greater than 10.5 the recovery of the microalgae can be achieved efficiently. At this pH Mg^{2+} and Ca^{2+} ions can be precipitated and as these cations surround the negatively charged microalgal cells, the precipitate will also sweep the algae from the suspension[43]. Reducing the salinity improves flocculation for all cationic polymers (Bilanovic and shelf, 1988).

2.1.4 2,2diphenylpicryl-hydrazyl (DPPH) free radical was used for measuring the scavenging activity of oil extract from *Microcysist, sp*.

2.2 Apparatus used:
2.2.1 Jar test: Standard jar tests were conducted in a mixer equipped with six-paddle jar test apparatus. The effect of various dosages of Ferric chloride and Aluminum sulphate on cell coagulation with and without peroxidation or ultrasound irradiation was tested. The pH of each cultured sample was adjusted 7- 8- and10.14 with 1 N HCL and 0.1 N NaOH.

2.2.2 Ultrasound waves of KHz were provided by a horn system. The literature shows that sonication accelerates the separation of colloids from water which might also contribute to the decrease in algae cells in culture water [44].

2.2.3 Homogenizer model Wise Tis HG – ISD with feedback controller(Wise Tis) for over load protection.

2.2.4 Rotary evaporator model Heidoloph Laborota 4000 efficient with HB digital.

2.2.5 Gas Chromatographic analysis was performed on Perkin Elmer Auto System XL , equipped with a flame ionization detector (FID). Helium was used as carrier gas at a flow rate of 1ml/min.

2.2.6 The disappearance of 2,2diphenylpicryl-hydrazyl (DPPH) free radical.DPPH was measured at 517 nm using (HACH- DR-2000 spectrophotometer) [45], [46].

2.3. Experimental procedure:
2.3.1 A standard jar test was conducted for the coagulation experiments. Each time, algae solution was stirred at high speed for 1 min and then at low speed for 3 min afterwards, samples were allowed to settle quiescently for 30 min. The upper sample was siphoned 1 cm below the water surface for 25 ml and used to determine the residual algae cell concentration. The algae cell concentration was presented by the optical density of the cells suspension at 684 nm (OD 684) using spectrophotometer. The cell calibration curve was established using a known quantity of algae cells as determined by weight. The correlation between light absorbance and the cell concentration was then taken.

2.3.2 Measurement of the ultrasonic field in the reactor was carried out by dipping a horn into solution. The sonic probe was dipped 3 cm below the water surface and sonication was initiated along with rapid mixing. The sonication power was 30 W, under such a power level, sonication itself had visually no impact on algae removal.

2.3.3 Pre-oxidation with Sodium ferrate (Na FeO_4) was prepared in strongly basic media and isolated from saturated NaOH solution. A carefully calculated amount of $NaFeO_4$ solution was injected into beaker a certain time (20 min) before the addition of alum solution. The freshly prepared alum solution using analytical reagent was predetermined (10 mg/L aluminum sulphate). During ferrate and alum addition, samples were stirred at high speed 1 min and then at low speed 3 min. Afterwards samples were allowed to settle quiescently for 30 min There-after, the upper 25 ml of the water samples were siphoned 1 cm below the water surface and taken for determination of residual algae cell concentration.

2.3.4 Oil extraction from coagulated algal salt and cells: Cells were dried at 85 °C in oven till constant weight. The oil was extracted by mixing chloroform-methanol (1:1 v/v) with the dry

cells using homogonizer for 10 minutes at 300 RPM in proportion of 1g in 20 ml of solvent mixture using slightly modified version of [47] and [35]. The homogenate mixture was subjected to stirring with a magnetic stir at room temperature for 4-8 hours. The mixture was then washed with 0.2 volume (4ml for 20 ml) of distilled water. After vortex the cells were shaken in the solvent system and allowed to stand for 18 h. The lipid fraction was separated from the separating funnel and the solvent was evaporated using rotary evaporator. The weight of the crude oil obtained was measured.

2.3.5 The fatty Acid (FA) profile of the biomass *Microcysist,sp.*, determined by gas chromatography (GC) is shown in Table (3) and Fig.(1). The fatty acids were dried under a stream of nitrogen prior to esterification and reduction was pronounced with the shorter chains as shown in the Fig (6). FAs were chromatogram as methyl esters on a fused silica capillary column DB -5 (60mX 0.32 mm i.d). Analysis was performed on Perkin Elmer Auto System XL gas chromatography equipped with a flam ionization detector (FID). Helium was used as carrier gas at a flow rate of 1ml/min. The injection port temperature was 230 ºC and the detector was 250 ºC. The oven temperature was maintained initially at 150 ºC and programmed for 150 to 240 ºC at rate 3 ºC / min, held at 240 ºC for 30 min. Standard used gave rise to well-individualized peak that did not interfere with the FA pattern.

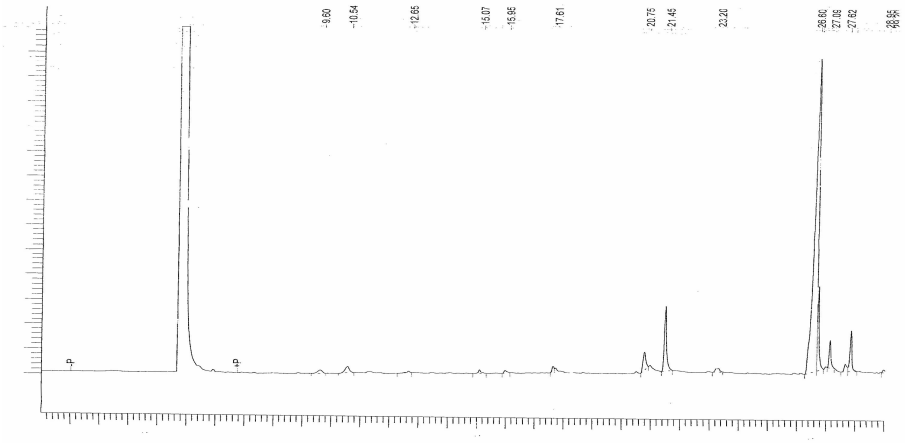


Fig (1) Chromatogram of Gas Chromatography profile of fatty acid methyl esters obtained from extracted of algae oil.

Table (3) Fatty acid Composition of Biomass from *Microcyst sp*. and crude oil extracted from this Microalga.

Fatty acid	Crude oil area%	Name of FA
C_{16}	9.77	Palmitic (n-7)
$C_{18.2}$	78.87	Linoleic (n-6)
$C_{18.1}$	7.99	Oleic (n-9)
$C_{18.0}$	3.38	Stearic

2.3.6 The *2,2diphenylpicryl-hydrazyl (DPPH) free radical.*
DPPH assay was carried as described [48] and [49]. Various concentrations of the sample were mixed with 2 ml (DPPH) solution (0.394µm/L) and filled up with ethanol to final volume 10 ml. After 30 min incubation period at room temperature in dark, the absorbance of the resulting solution and blank (with same chemicals, except for sample) were recorded against tetrbutylated hydroxyl toluene (BTH) as positive control. The disappearance of DPPH was measured at 517 nm using (HACH- DR-2000 spectrophotometer). The percentage of RSC was calculated in the following way:

RSC (%) = 100 X (A_{blank} - A_{Sample}) / A_{blank}

Where A_{blank} is the absorbance of the control reaction (controlling all reagents except the test compound) and A_{Sample} is the absorbance of the test compound.

The parameter EC_{50} (efficient concentration value) or IC_{50} value which is defined as the concentration of substrate that causes 50% loss of the DPPH activity (color) has been calculated by plotting the idealized plots of absorbance A, and the percentage reduction Q, versus the amount of reductant added.

RESULTS AND DISCUSSION

3.1. Effect of $FeCl_3 .6 H_2O$ on algae coagulation:

Fig (2) shows the effects of increasing $FeCl_3 .6H_2O$ dose on algae harvesting. The removal efficiency is expressed as the ratio at the algae concentration before addition of chemicals to algae concentration measured of the end of the coagulation-sedimentation test. It can be seen that $FeCl_3.6H_2O$ coagulation significantly removed the algae cell at dosage of 37.5 mg/L by 80.16%.

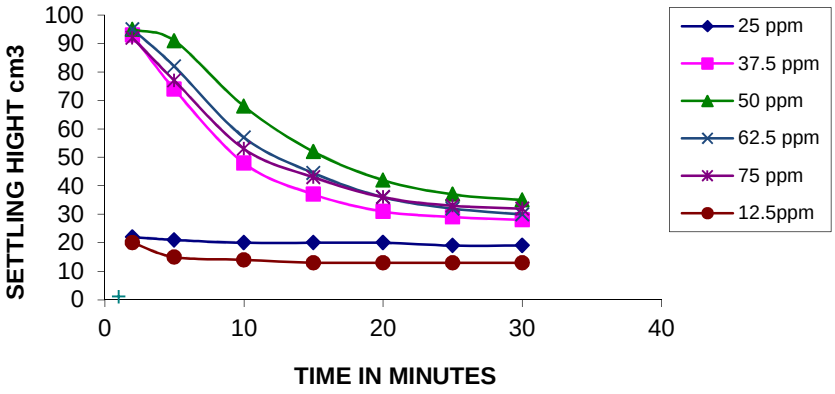


Fig (2)EFFECT OF DIFFERENT FERRIC CHLORIDE DOSES FOR ALGAE SETTLING

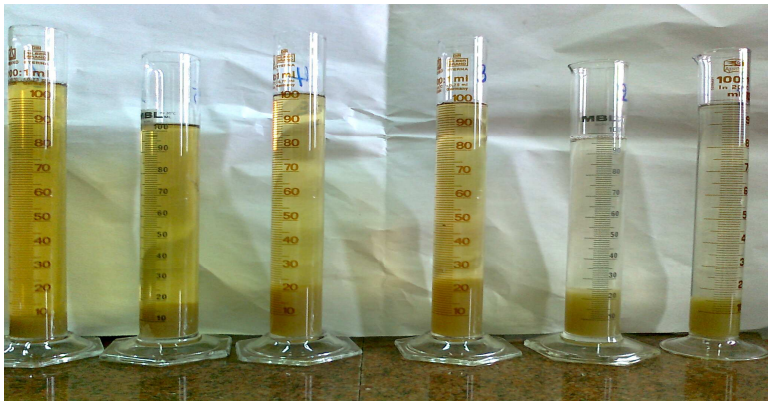
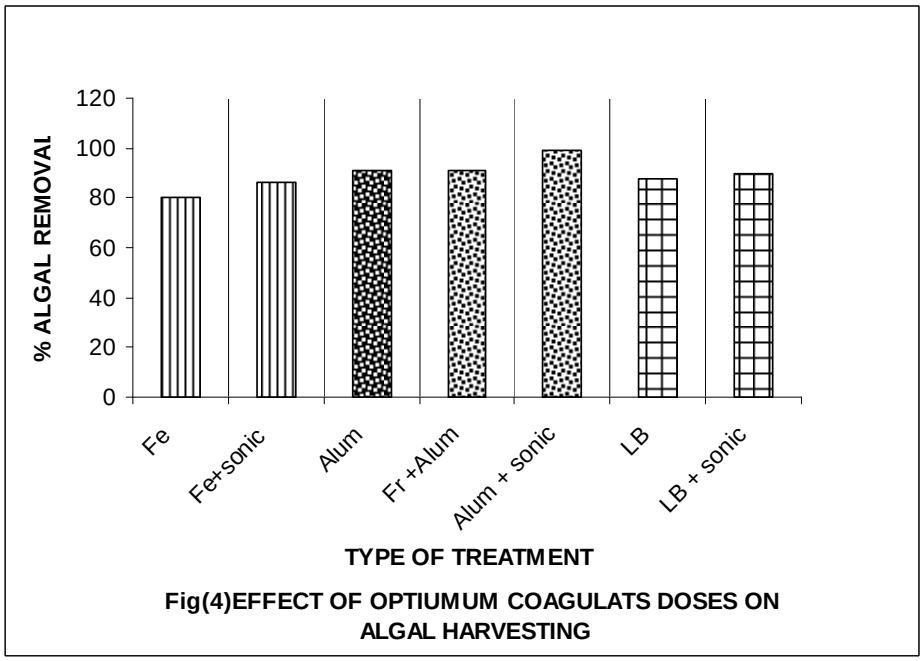


Image (1) illustrating the $FeCl_3.6H_2O$ yellow color

Algae removal was observed at higher $FeCl_3.6H_2O$ dosage (e.g. 75 mg/L see Fig 1) achieved 17% only, while at lower $FeCl_3.6H_2O$ dosage (e.g. 12.75 mg/L, see Fig1) achieved 54.6% algae removal. Also the draw-back of using $FeCl_3.6H_2O$ is the yellow color retained the clarified water besides the pollution with Fe ions see image (1).

3.2. Effect of combination of ultrasound waves and $FeCl_3.6H_2O$ on coagulation. Fig (3) shows the effects of increasing ultrasound sonication time with $FeCl_3.6H_2O$ optimum dose (2.5 mg/L pre-tested treated with sonication 50 sec. at 2.5w/cm^2 and 25 mv.)



Fig(4)EFFECT OF OPTIUMUM COAGULATS DOSES ON ALGAL HARVESTING

Key for Fig (3) of the used doses of coagulants at optimum conditions:-
Dose/Fe=37.5 ppm
Dose/Fe= 2.5 ppm+30 sec Us
Dose/Alum=30ppm
Dose/Alum=10 ppm + Ferrate 3ppm
Dose/Alum=10ppm + 60 sec US
Dose/Liquid Bittern=4 ml i.e (55 ppm Mg++)
Dose/Liquid Bitter= 4 ml & 60 sec US i.e. (55 ppm+60 sec US)

Thus the difference of algae removal between the case of coagulation alone, and that with ultrasound pre-treatment showed a sharp increase of algae removal efficiency when the $FeCl_3.6H_2O$ dose was decreased to 2.5-3 mg/L and algae % removal achieved 86.43 % instead of 80.16% only. Also more clearance appeared in the color of Fe ions in supernatant after settling see image (2). Marcin et al. 2010 investigated the influence of ultrasonic field modification on the efficiency of different flocculants [44].

3.3. Effect of Alum $Al_2(SO_4)_3.18H_2O$ on coagulation dosage of 60 mg/L.
Fig. (4) Shows that alum coagulation partially removed the algae cells by 9.5%. The increase in algae removal was achieved at only optimum alum dose (30 mg/L) where coagulation showed 91.15%. While at low alum dose the algae removal achieved 2.26% only. Thus pre-oxidation with ferrate or sonication was studied for more removal efficiency and higher harvesting of algae cells.

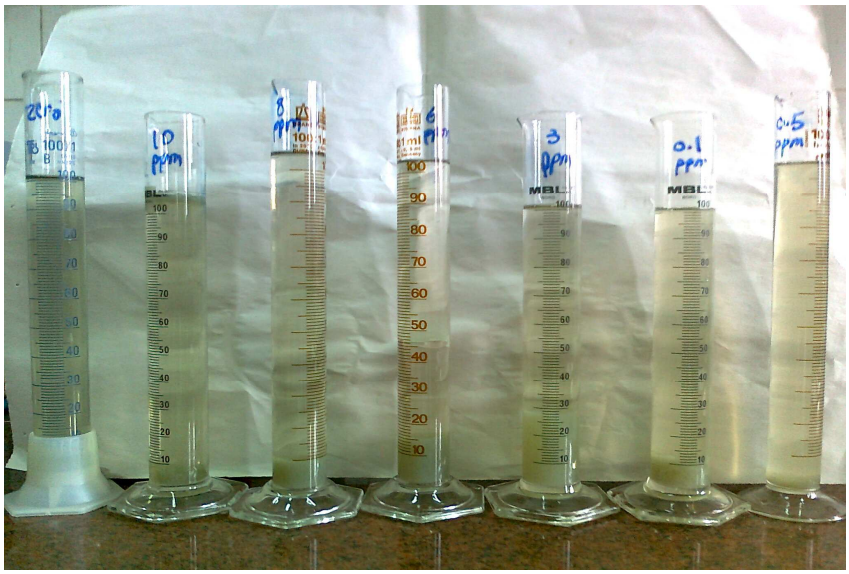


Image (2) illustrating the reduction of $FeCl_3.6H_2O$ dose and the clearly of the supernatant from the yellow color.

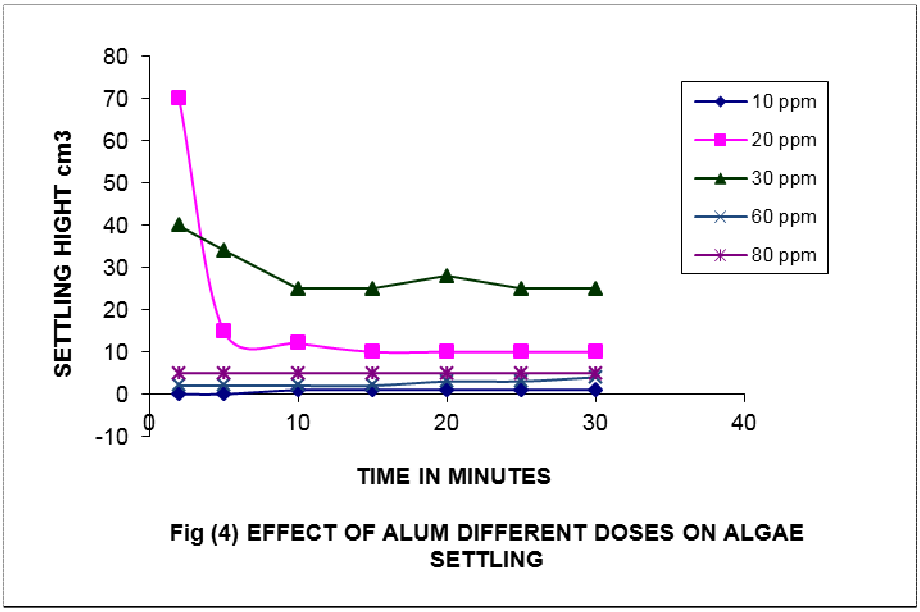


Fig (4) EFFECT OF ALUM DIFFERENT DOSES ON ALGAE SETTLING

3.4. Effect of $Al_2(SO_4)_3.18H_2O$ combined with sodium ferrate pre-oxidation on coagulation.

The difference of algae removal between the case of coagulation alone and that with ferrate pre-oxidation was very obvious for cultured water. It was a sharp increase of algae removal efficiency when the alum dosage was between 10-15 mg/L combined with 3 mg/L ferrate pre-oxidation and pH adjustment 7.1 removal efficiency showed 90.87% with one third dose of coagulant , see Fig.(5) and Image (4).

3.5. Effect of $Al_2(SO_4)_3.18H_2O$ combined with pre-ultrasound waves.

The optimum coagulant dose for Alum alone was 30mg/L showed 91.15% algae removal without sonication. By comparison, when Alum dose was reduced to 10mg/L and culture cells were pretreated with sonication 60 sec., the algae removal reached 99.100% at pH7.1, see image (5).

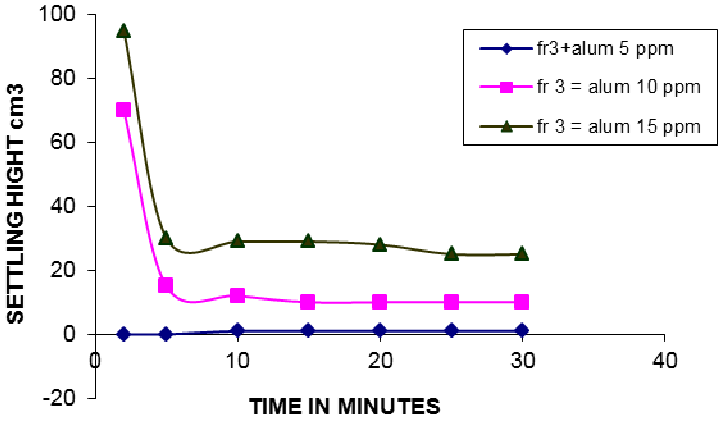


Fig (5)EFFECT OF FERRATE COMBINATION WITH ALUM DIFFERENT DOSE ON ALGAE SETTLING

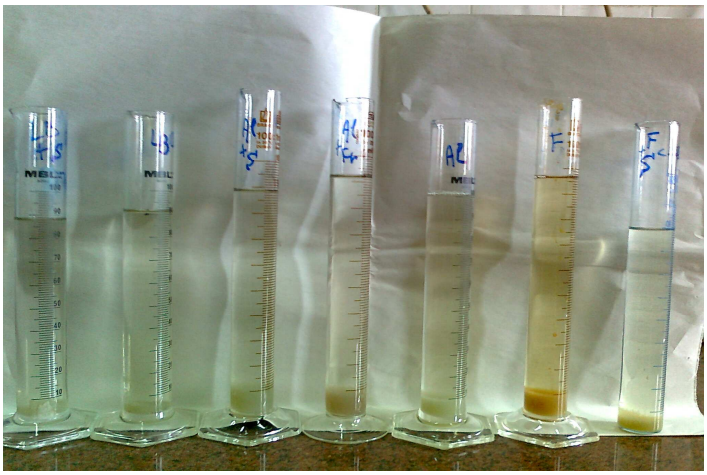


Image (4) Picture of the effect of chemical coagulants optimum doses used and the aid of other techniques (Na- Ferrate oxidation and Ultra sound waves) on settling of micro algae *Microcyst,sp.*

Image (5) illustrating the degree of clear in supernatant when using pre-ultrasound treatment and Alum coagulation.

3.6. Effect of liquid bittern on coagulation:
Fig (3) and image (4) shows optimum LB at pH 10.14 dose was 0.55 mg/L which reduced the algae cells in culture water down to 87.38% removal. Sonication 60 sec. pre– coagulation improved slightly the algae removal up to 89.42%.

4.Profile of Fatty Acid Methyl Esters obtained from Microcystis sp.
The esterified Fatty Acids (FA) is shown in Table (3) and Fig (1). The FAs were dried under a stream of nitrogen prior to esterification. There was a disappearance in the $C_6 – C_{14}$ as shown in Table (3) this result agree with Bach and Babayar (1982); Guy Lepage and Claude (1984). The FA_s $C_{16} – C_{18}$ appeared on the chromatogram as Palmatic and Linoleic, Olic,Stearic (overlapped). The $C_{18.2}$ Linoleic is the main presented FA and exists as 78.88 % as shown in Table (3) and chromatogram Fig (1).

5. Determination of RSC% present in Microcyst sp. Oil using 2,2diphenylpicryl-hydrazyl (DPPH) free radical.
The antioxidant activity of phenolic compounds is mainly due to redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers, heavy metal chelators and hydroxyl radical quenchers [50] the ethanolic extract tested showed poly phenol content of 2.4%. Our results showed that crude oil extract of *Microcyst sp* has IC_{50}= 39.3%. This parameter has the drawback that the higher the antioxidant activity, the lower is the value of IC_{50}. From our results it appears that *Microcyst sp* has good antioxidant activity.

CONCLUSION

Collection and concentration of microalgae biomass from cultivation systems contribute heavily to the operation cost of the overall process. Therefore, more efficient and economic harvesting technology should be developed to enhance the commercial viability of microalgae biofuels industry.

Laboratory studies using cultured algae solution demonstrated that pretreatment with sodium ferrate obviously enhanced the algae removal by coagulation – sedimentation process with $Al_2(SO_4)_3$. 18 H_2O were added. Algae removal efficiency increased remarkably even at a short period of pre-oxidation time.

To achieve a certain extent of algae removal, pretreatment with ferrate can reduce one third the dosage of alum required to cause an efficient coagulation Image (4).

Laboratory studies using cultured water demonstrated that short sonication period improved the coagulation of algae. The sonication enhancement was greater when the coagulant dose was reduced below the optimum dose. Improvements in the removal of algae cells and turbidity were significantly achieved. Liquid Bittern rich with Ca^{2+} and Mg^{2+} ions is a sheep coagulant to algae harvesting unless increasing the salinity in water more than 20%.

The Lyophilized *Microcysist,sp* as shown in the chromatogram Fig (1) is full of unsaturation represented by ($C_{18.2}$ Linoleic (n-6)) showing 87.78 % of the total lipids content of micro algae, but IC_{50} = 39.3% means that *Microcyst sp* has high antioxidant activity.

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