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A Novel and Rapid Harvesting Method of Microalgae Using Saw Dust Coated Polypyrrole

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P A P E R I N F O

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A B S T R A C T

In this study a simple and rapid harvesting method using electro conductive polymer coated saw dust has been presented as a new coagulant for separating *Chlorella vulgaris* from a diluted suspension. Polypyrrole (PPy) coated saw dust as a novel coagulant was prepared via in-situ polymerization of pyrrole (Py) monomer using $FeCl_3$ oxidant in aqueous medium in which saw dust particles were suspended. The zeta potential of coagulant and *C. vulgaris* and X-ray photoelectron spectroscopic (XPS) analysis of coagulant were characterized. PPy maintain predominantly positive charge over a wide pH range (2-10) with an isoelectric point 10.4 while, *C. vulgaris* maintained negative surface charge from pH 5 and onward with isoelectric point 3.8. The microalgae showed the highest separation efficiency at pH 10. The maximal recovery efficiency reached more than 90% for microalgae at a stirring speed of 120 rpm within 7 min. The maximal adsorption capacity of *C. vulgaris* was 28.8 mg dry biomass/mg-saw dust coated PPy. The concentration factor obtained is higher than 32 which save energy and time associated with microalgal harvesting and allows a reduction in the equipment size necessary for biomass dewatering and improves the feasibility of using these microorganisms in biofuel or wastewater processes.

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INTRODUCTION

Microalgae have received significant global attention for the production of numerous value-added products, such as pigments, steroids, pharmaceuticals and biofuels [1]. Moreover, the research interest in oil-accumulating microalgae renewed since past few years are due to sharp rise in non-renewable fossil fuel prices [2] and air pollution [3]. Microalgae are considered as one of the most promising candidate for biodiesel production among others biodiesel feedstock.

Growing algae as renewable energy source; a substantial reduction in the processing costs is needed in every steps of upstream and downstream process. However, the ongoing debate on the production of the microalgal biomass is due to its weak economic viability [4]. In fact, despite of the remarkable efforts to reduce the costs of algae production and processing up to date, there remains a major price gap between microalgae-derived biofuels and fossil fuels, especially the harvesting costs of microalgae which are very high [5].

The efficient microalgae biomass harvesting is one of the expensive step of algal biofuel production which accounts more than 30% of the total cost in case of algal production in open ponds and the main reasons are their small cells size (range vary from 5 to 50 μm) and their electronegative surface charge which enable them to survive as stable suspension in culture media [6]. To overcome the above mentioned limitations several methods have been developed for harvesting microalgae to reduce the cost of energy production [7]. Centrifugation process involves centripetal acceleration to separate algal cells; and water is separated by draining the excess medium. The use of centrifugation for harvesting the relatively low concentration of total suspended cells in the pond culture is restricted by the high cost of energy required in handling large quantities of media [8]. Moreover, processing large quantities of culture consumes a lot of time and exposure of microalgal

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cells to high gravitational, and shear forces reduces the cells integrity [7], and consequently might affect the production of biodiesel. Gravity sedimentation process allows suspended cells to settle down at the bottom of media under the influence of gravitational force, and form concentrated slurry and left the clear culture media above. It is highly energy efficient method [9], however, it is a very slow process [5] and consumes a lot of time, which is the main hurdle in its popularity. Filtration process allows culture media to pass through filters, which hold back algae. In spite of being an effective method of recovering biomass it has extensive running and pre-concentration costs. Moreover, processing large quantities of culture consumes a lot of time and blocking in filter causes fouling. Flocculation process allows algae cells to join together with flocculant (chemical and biological) via charge dispersion mechanism to form aggregates called floc [5], the bigger and heavier flocs helps in settling microalgae cells [7].

The cationic polymers and polyvalent cations coagulants are being used to neutralize the negative charge of the algae cells to get flocs. Additionally, in most of the algae cell harvest system low acidic pH are required along with the high dosage of costly coagulant to achieve a satisfactory result [10].

However, most of the heavy metal salts especially aluminium salts, caused cell lysis [11] and poses a carcinogenic effect [12]. In electrolytic process, polyvalent cations are released from the anode such as Al^{3+} and Fe^{2+}/Fe^{3+}, and form positively charged metal hydroxides by reacting with water molecules, which neutralized the negative surface of the microalgae cells to aggregate the biomass. The electrical energy input of the electrolytic harvest approach is quite high, especially for fresh water microalgae and consequently the high cost of energy required in handling large quantities of culture. Flotation process the microalgal cells are attached with upward gas bubbles in flotation, and collected on top of the media layer. There are two types of flotation methods depending upon the size of bubbles (a) dissolved air flotation (DAF), (b) dispersed flotation (DF) [7]. However, a common problem associated with dissolved air flotation systems is that oversized bubbles break up the floc [8]. While dispersed flotation process is an expensive process [9].

$$H_2O + MO\text{-}O^- \underset{OH^-}{\overset{H^+}{\rightleftharpoons}} MO\text{-}OH \underset{OH^-}{\overset{H^+}{\rightleftharpoons}} MO\text{-}OH_2^+$$

Magnetic process is relatively new technique; both the microalgal cells and the magnetic particles have negatively charged surfaces in aqueous medium, which may undergo protonation/deprotonation as per the following reaction [13], where MO refer as metal oxide.

The magnetic particles and microalgal cells are incorporated through direct linking or electrostatic interactions. The limitation of this technique is low pH requirement. Moreover, processing large quantities of culture consumes a lot of acid to recover the biomass and again need to neutralize the water before discharge.

To minimize the cost and energy consumption of harvesting microalgae, an integrated approach is needed [14]. So it is necessary to develop cost-effective technologies that would permit efficient harvesting.

In order to recover targeted microalgae biomass from an aqueous culture media, saw dust waste can be improved by using functionalized polymers to enhance surface reactivity. Electroactive conducting polymers (ECP) such as Polypyrrole (PPy) is a subject of intense investigation of many research groups worldwide. PPy only exists in a neutral reduced state and a positive oxidized state, however, PPy can exchange the ions present in electrolytic solution with its counter ions depending upon their characteristics. The ion exchange behaviour of PPy also depends upon the polymerization conditions. During polymerization reactions, PPy receives positive charge on its matrix due to the contribution of positively charged nitrogen atoms present in the polymer matrix and simultaneously to maintain the charge neutrality of the PPy matrix counter ions are combined into the growing polymer chains as dopant [15].

The existence of positively charged nitrogen atoms in PPy provides a good opportunity for its applications in biomass recovery by attachment with negative charge possessing algae cells. With increasing pH of solution ($pH \geq 10$), polypyrrole doped with chloride ions (PPy/Cl) become undoped (forming emeraldine base), and then less negative charges is available to attached with PPy surface [16]. In this study, polypyrrole coated on saw dust PPy/SD particles were encapsulated by polypyrrole (PPy) to achieve a well dispersed particle that can be separated and has a high capacity to recover microalgae biomass. The objective of this current study is to develop an efficient method for recovering the algal cells of *Chlorella vulgaris* by low-cost PPy/SD. The operation parameters in this microalgal harvesting process such as stirring speed, solution pH and dosage were characterized, and the adsorption mechanism of PPy/SD particles was investigated.

MATERIALS AND METHODS

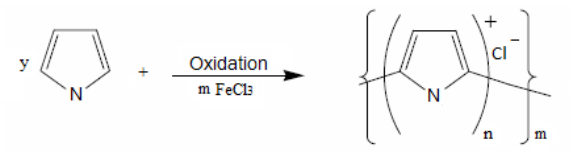
Materials

Pyrrole (Py) and ferric chloride ($FeCl_3$) as oxidant were purchased from Sigma–Aldrich, Germany. Py was distilled under reduced pressure prior to use. The saw

dust was ground and sieved through 30–50 µm mesh before use. All other solvents used, were of analytical grade and were freshly distilled before use.

Preparation of saw dust coated polypyrrole particles

The saw dust coated polypyrrole particle (PPy/SD) was synthesized via in situ chemical oxidative polymerization of Py monomer in the presence of oxidant, $FeCl_3$ as shown in scheme 1, where y = n × m.



Scheme 1. Polymerization reaction of polypyrrole.

The polymerization of Py took place on the surface of suspended saw dust particles. The precipitating PPy moieties encapsulate the suspended saw dust particles as discussed earlier [16]. For polymerization of Py, 0.1 g of saw dust particles was added into 30mL deionized water in a conical flask and ultrasonicated for 10 min for better dispersion of saw dust particles into water. A 3 g of $FeCl_3$, oxidant, was added into the de-ionized water containing saw dust particles and was shaken for 10 min and followed by addition of 0.5 mL of Py. The reaction mixture was kept under constant shaking for 2.5h at ambient temperature. Finally, the acetone was added into the reaction mixture to stop the reaction. The black powder obtained was filtered and washed with distilled water until the filtrate became colourless and finally washed with acetone. Then the PPy/SD particles were dried at 80 ◦C for 5 h under vacuum, until the total mass became constant. The total weight of the saw dust coated polypyrrole particles was 0.50 g, determined gravimetrically. The weight ratio of saw dust particles and conducting polymer PPy were obtained as 1:2.

Microalgae source and cultivation

The fresh water microalgae *C. vulgaris* UTEX2714 was obtained from the University of Texas at Austin, USA. The culture was maintained in 250 mL flasks containing 150 mL BG11 medium of culture at 28 ◦C with an irradiance of 120 µmol/m^2/s and L:D cycle of 14:10 h for 14 days. The photo bioreactor was supplied with 1.5% (v/v) CO_2 enriched air with flow rate of 100 mL/min and continuous stirring at 120 rpm in photo bioreactor, (Model: INFORS HT Minitron). The initial biomass concentration was 0.1 g/L dry cell weight of C. *vulgaris*. The final maximal microalgal biomass concentration was 1.1 g/L dry cell weight.

Coagulation-flocculation–sedimentation experiments

Coagulation-flocculation–sedimentation experiments were carried out by jar test using 250 mL of glass beaker, after 14 days of cultivation of microalgae in BG11 medium, when culture reached to its stationary phase. The beaker were filled with 50 mL of microalgal suspensions of a defined concentration (DW = 1.0 g/L). Specific amounts (6-30 mg/L) of PPy/SD particles were added in microalgae cells culture and mixed by stirring at 120 rpm for 2-3 min and left undisturbed to favour flocs formation and settlement. An interphase had appeared with time. After the formation of interphase the flocs of microalgal cells associated with PPy/SD particle were further separated from the medium by decantation.

Recovery efficiency

Biomass recovery was calculated from the absorbance ratio at the clarified zone after attaining constant interphase height (concentration factor) against the absorbance of homogenous culture at the beginning of the experiment using Eq. (1);

$$\text{Recovery\%} = \frac{\text{ODt0} - \text{ODt}}{\text{ODt0}} \times 100 \quad (1)$$

where ODt_0 is the optical density at time zero and ODt is the optical density of the sample taken at time t. The absorbance of the supernatant (3 mL) was measured at 750 nm and all experiments were performed in triplicate and presented results are mean values with standard deviation.

Concentration factor

After 14 days of cultivation of microalgae in BG11 medium, 50 mL of microalgal suspensions of a defined concentration (DW = 1.0 g/L) was taken into 250 mL of beaker. Specific amounts (5-32.5 mg/L) of PPy/SD particle were added in microalgae cells culture and mixed by stirring at 120 rpm for 2-3 min and shifted it into calibrated glass cylinder 0.20 m height by 0.05 m diameter (h/D = 4) and left for flocs formation and settlement. An interphase had appeared with time and the concentration factor was calculated as the height ratio of the total liquid to the interphase height.

Zeta potential

The Zeta (ζ) potential of PPy/SD particle and microalgae cells was analysed by Zeta Potential Analyser (Beckman Coulter Inc.) at 25 °C in the culture medium. All samples were measured three times and presented results are mean values with their standard deviation.

Recovery and reusability of PPy/SD particle

For recovery of PPy/SD particle, 2g of harvested microalgae cells associated with PPy/SD particle were

dried at 60 ºC for 24 hours for extraction of lipids. Lipids were extracted with hexane in a Soxhlet apparatus operated at 80 ºC for 10 hours [17]. After lipid extraction the cell debris with PPy/SD particle was treated with 25 mL of water at different pH values (from range pH 2 to 12) for 30 min. After recovery, the ability of the PPy/SD particle to coagulate microalgal cells was again tested using similar procedure as described in section 2.4. The pH was controlled by adding 2M NaOH or HCl solutions.

Integrity of *C. vulgaris* cell wall

Cell integrity was determined by the Evans blue [18] and lipid extraction methods. For Evans blue method, 0.1 mg flocculated biomass of algae were treated with 2M NaOH solution to remove the PPy/SD particle and the re-suspended cells collected from the supernatant. The cells were treated with 100 µL of 1% Evans blue solution and incubated for 10 min at room temperature [11] then washed twice in deionized water and observed under light microscopy. While, for lipid extraction method the same procedures were repeated as described before except 2g of recovered biomass of microalgae was treated with 2M NaOH solution causing microalgae cells to detached from PPy/SD particle and goes for suspension again, the re-suspended biomass of algae was collected and dried at 60 ºC for 24 hours. A 1g dry weight biomass of re-suspended microalgae was taken for extraction of lipids and compared with the amount of lipid obtained from 1g dry weight biomass of microalgae harvested by filtration process. Lipids were extracted with hexane in a Soxhlet apparatus operated at 80 ºC for 10 hours.

Study of stirring speed on recovery efficiency

To analyse the effect of stirring speed on recovery efficiency of microalgae 26 mg/L) of PPy/SD particles were added in microalgae cells culture and mixed by stirring from 60 to 140 rpm for 2-3 min and left undisturbed to favour flocs formation and settlement.

RESULTS AND DISCUSSION

The objective of this research was to develop an efficient method for the recovery of microalgae biomass based on electrostatic aggregation–sedimentation operations. The developed method was found useful for various strains and showed quick electrostatic aggregation – sedimentation operations with high concentration factors.

Characterization of the PPy/SD particlebiomass

PPy/SD particle tested in this work was consisting of saw dust with average diameter of 40µm. The polymer coated as a very thin film on the surface of saw dust was allowed to dry at 80 ºC for 5 h under vacuum and sieved (35–50 mesh size). These features indicate that the micro dimensional saw dust particles were embedded in the PPy matrix, forming a core–shell structure. In this study, the main interest was focused on the proportion of positively charged nitrogen atoms in PPy/SD particle. Kang et al., [19] has reported that the PPy synthesized by oxidative polymerization have a proportion of positively charged nitrogen atoms in the range of 25 to 30%. Here, XPS analysis was used to analyse the oxidation states of nitrogen in PPy. PPy only exists in a neutral reduced state and a positive oxidized state can be achieved by applying an oxidant during the chemical polymerization [16]. The N atoms in the PPy structure may exist as; imine (==N–), amine (–NH–), and/or electron deficient nitrogen (N^+). With reference of Fig 1, it was concluded that 29.5% of positively charged nitrogen atoms (N^+) contributed the polypyrrole structure while rest of the nitrogen was amine (–NH–), however no signal for imine was observed.

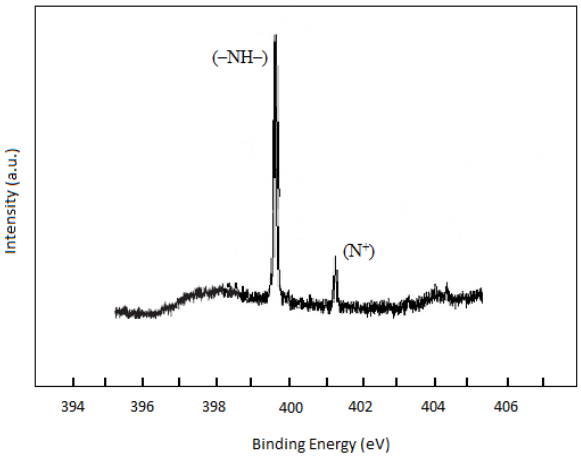


Figure 1. XPS spectrum of PPy/SD particle.

Effect of PPy/SD particle dosage

The effect of PPy/SD particle doses on harvesting efficiency of *C. vulgaris* was investigated. Different concentrations of PPy/SD particles were investigated at initial concentration of 1g/L microalgal suspensions at 27 ºC. From Figure 2 it was observed that, the percentage of microalgal suspension removal increased from 15.9 to 90.8% with an increase in PPy/SD particles from 6.0 to 30.0 mg/L for *C. vulgaris* respectively. The removal of microalgal suspensions were analysed as optical densities which decreased with increase in PPy/SD particles dose, also referred as harvesting efficiency. Even in low dose the total time consumption in algae harvesting were 7 min, which are supposed to be as the rapid harvesting method, comparing to other harvesting process as listed in Table 1.

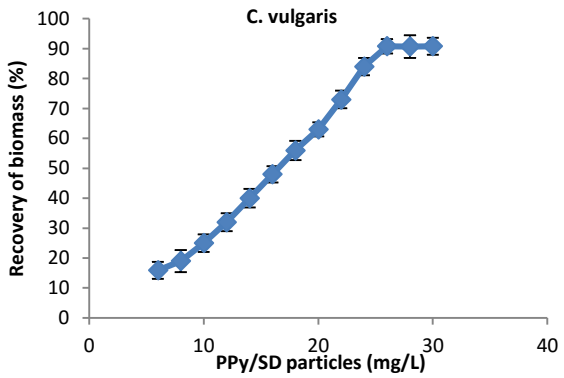


Figure 2. The effect of PPy/SD Particles dose on harvesting efficiency.

The effect of microalgae biomass concentration on recovery

Microalgae can grow with different rates under different conditions of culture and thus possessed different biomass to harvest. The effectiveness of PPy/SD for microalgae recovery with different biomass concentration was therefore evaluated. Several mathematical models have been used in description of the equilibrium relationship between the amount of PPy/SD particles or adsorbate per unit of settled microalgae or adsorbent (*Qe*) and suspended biomass or equilibrium biomass concentration (*Ce*) of algae after achieving the constant concentration factor. Among these models, Langmuir and Freundlich models are the most popular ones due to their relative simplicity and reasonable accuracy [13, 20, 21].

The adsorbent which fit with the Langmuir adsorption isotherms are consider as possessing homogenous surface consists of small adsorption unit which carry equal level of energy. The Langmuir Eq. 2 was followed during the adsorption of microalgae cells onto PPy/SD particles to achieving the equilibrium state

$$Ce/Qe = 1/Q_0b + Ce/Q_0 \quad (2)$$

where *Ce* is the equilibrium concentration of microalgae in supernatant (g/L) and *Qe* the amount of microalgae adsorbed on the PPy/SD particles (mg/mg-particles), Q_0 (mg/mg) is the maximum monolayer adsorption capacity, and *b* the equilibrium Langmuir adsorption constant related to the stability of the combination between microalgal cells and PPy/SD particles surface (L/g). The isotherm data Q_0 and *b* were calculated and reported in Table 2. The high value of correlation coefficient (R^2 = 0.999) indicate a good agreement between the parameters and confirms the monolayer adsorption of microalgae cells onto saw dust coated with polypyrrole surface.

TABLE 2. Adsorption isotherms parameters of PPy/SD articles at 25 °C and pH = 10.0.

Microalga e	Langmuir Model			Freundlich Model		
	Q_0 (mg/mg)	b (L/g)	R^2	K_f (mg/mg)	b_f	R^2
C. vulgaris	28.8	7.02	0.999	12.41	0.331	0.955

The Freundlich isotherm describes the heterogeneous surface energies by multilayer adsorption [21] and is expressed as Eq (3)

$$\ln Qe = \ln K_f + b_f \ln Ce \quad (3)$$

where *Ce* is the equilibrium concentration of microalgae in supernatant (g/L) and *Qe* the amount of microalgae adsorbed on the PPy/SD particles (mg/mg-particles), K_f is Freundlich equilibrium constant, and b_f is an experimental parameter which describe the intensity of adsorption related with heterogeneity of the adsorbent surface. The adsorption is considered as favourable if the value of b_f lies in between 0.1-1 [22].

Freundlich model hints towards the conclusion that the adsorbent deals with the multilayer adsorption process due to the presence of energetically heterogeneous adsorption sites. The related experimental parameters were determined and listed in Table 2. The closer the values of b_f towards 1 better is the favourability of adsorption.

From Table 2, the Langmuir adsorption isotherm model yielded the best fit as indicated by the highest R^2 values compared to the other model for microalgae, showing the adsorption of microalgal cells on the PPy/SD particles were homogeneous and monolayer in nature. The maximum adsorption capacity (Q_0) of the PPy/SD particles for *C. vulgaris* (28.8 mg-dry biomass/mg-PPy/SD). As discussed earlier [20], that higher the values of *b* better is the binding affinity for the absorbent and algae cells. From Table 2 *C. vulgaris* possessed high value of *b* indicated that *C. vulgaris* cells formed the strong bond with PPy/SD particles, and consequently favours sustainable floc.

Concentration factor and zeta-potential

To obtain comparable results all experimental trials were performed at the same initial biomass concentration of 1.0 g/L and over the same time. Concentration factor is a parameter to evaluate the degree of harvest. It is the ratio of the liquid to the interphase height, after aggregation–sedimentation operations thus concentration factors allow a reduction in the equipment size necessary for biomass dewatering. This parameter obviously depends on PPy/D particles

TABLE 1. Comparisons of harvesting methods for microalgae.

Microalgae	Method of recovery	Time required for recovery	Recovery efficiency (%)	References
Chlorella minutissima	(i) Flocculation-sedimentation using $Al_2(SO_4)_3$	2 hours	80	Papazi et al. [11]
	(ii) Flocculation-sedimentation using $Fe_2(SO_4)_3$	4 hours	80	Papazi et al. [11]
Chlorella vulgaris	(i) Gravity sedimentation	1 hour	60	Ras et al., [28]
	(ii) Flocculation	-	95	Ras et al., [28]
	(iii) Bioflocculation	11 min	83	Oh et al., [29])
	(iv) PPy/SD particles	7 min	90.8	Present study
Botryococcus braunii	(i) Electroflocculation	30 min	93.6	Xu et al., [30]
	(ii)Electroflocculation-Dispersed air floatation (DAF)	14 min	98.9	Xu et al., [30]
	(iii) Magnetic separation	2-3 min	98	Xu et al. [20]

dose. The concentration factors were measured at various PPy/SD particles dosages. It was observed that in the absence of PPy/SD particles, no interphase formed in microalgae culture and the concentrations were homogenous throughout the culture. When PPy/SD particles were added, flocs or aggregation of algal biomass started appearing within 4 min and interphase was clearly observed in 6 min after the addition of PPy/SD particles. The interphase height diminished with time which became constant in total 7 min for *C. vulgaris* after addition of PPy/SD particles. From Figure 3 it was clear that the variations in height slope was directly proportional to the dosage of PPy/SD particles for microalgae sample. Data in Figures 2 and 3, showed that for *C. vulgaris* 30 mg/L PPy/SD particles was optimum to achieve the highest biomass recovery as 90.8 % while the concentration factor was reported as 30.5. The concentration factor further enhanced by almost 15 % with increase of PPy/SD particle (32.5 mg/L) although the biomass recovery was same as it was at 30 mg/L, however both biomass recovery and concentration factor decreased when PPy/SD particles dosage was increased more than 40 mg/L for *C. vulgaris* (Data not shown).

The results indicated that overdosing of PPy/SD particles resulted in dispersion and charge re-stabilization. Similar results were obtained by other researcher [23]. The zeta potentials of the microalgal suspensions before flocculation were -18 mV for *C. vulgaris*, while at optimal dosage of PPy/SD particles it was +0.92 mV. However, the zeta potential increased to +14.02 mV for at excess of 7.5 mg/L of PPy/SD particles. These analytical data pointed out those PPy/SD particles were adsorbed on the surface of the microalgae cells resulting surface charge neutralization which caused a reduction of surface potential. Eventually adsorption beyond the point of charge neutralization due to excess of PPy/SD particles caused charge reversal and re-stabilization or re-suspension of microalgae cells.

In order to further understand the impact of pH on PPy/SD particles and microalgae interactions, zeta potential measurements of the PPy/SD particles and

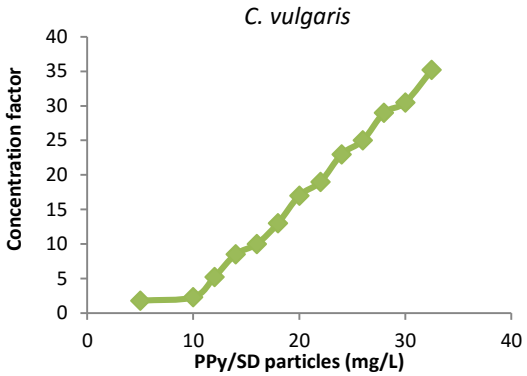


Figure 3. The effect of PPy/SD particles dose on concentration factors.

microalgae were carried out (Figure 4). Zeta potential measurements were performed to study the electrical properties of PPy/SD particles and *C. vulgaris*. The most noticeable effect of pH was that the zeta potentials of the PPy/SD particles, maintain predominantly positive charge over a wide pH range (2-10) with an isoelectric point at pH 10.4 (Figure 4). The *C. vulgaris* shows negative surface charge over a wide scale of pH (3.5-12). It was also previously reported [20, 24, 25] that *C. vulgaris* cells maintain predominantly a negative surface charge over a wide pH range. However *C. vulgaris* showed positive surface charge at low pH or in highly acidic condition with isoelectric point at 3.8. The microalgae showed the highest separation efficiency at pH 10 as represented in Figure 5. These findings confirmed the electrostatic attractions between microalgae and the PPy/SD particles.

It was noticed that the most prominent difference between zeta potentials of the algae cells and PPy/SD particles results in the strongest electrostatic interactions and so contributed to the highest separation efficiencies. Less separation efficiency below pH 10, might had happened due to stabilization of algae cells with the hydrogen ions and protonation of PPy matrix (scheme 2) at low pH [26]. The attractions between PPy/SD particles

and microalgal cells were highest at pH 10, just below the isoelectric point of PPy/SD particles, ensuring a strong electrostatic attraction and subsequently high separation efficiencies.

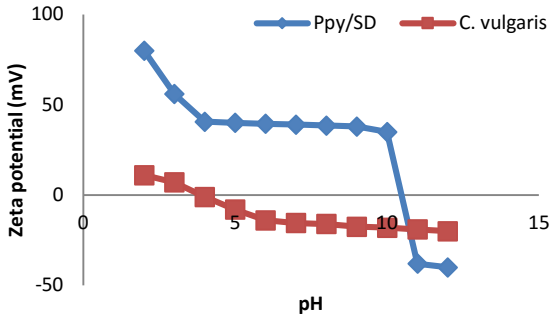


Figure 4. Zeta-potentials of PPy/SD particles as a function of pH.

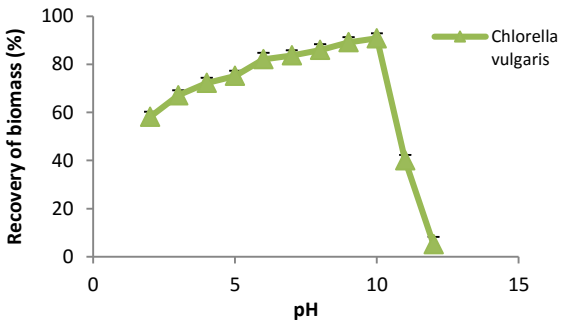
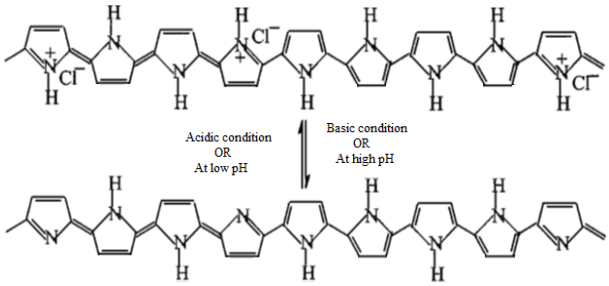


Figure 5. The effect of pH on algae biomass recovery.



Scheme 2. Protonated and deprotonated phase of PPy matrix under the influence of acid and base.

The positive zeta potential of PPy/SD particles over the range of pH 2-10 was due to the presence of positive nitrogen atom in PPy matrix, while with increasing pH of solution (pH ≥ 10), PPy/Cl become partially deprotonated or undoped (forming emeraldine base), and consequently less negative charges are available to attached with PPy surface [26]. While few existing positive nitrogen are neutralize by hydroxide anions by forming H bonds [16], which might be strong enough to be replaced by microalgae cells, resulting less biomass recovery at high pH.

Effect of PPy/SD on cell integrity

Generally the harvesting processes caused algae cell disruption and thus affect downstream processing and lipid production [23]. In order to assess the impact of PPy/SD particles on the microalgal lipid production, the direct effects of PPy/SD particles on the cell wall of *C. vulgaris* (Figure 6) were observed using light microscopes. Figure 6 shows the state of microalgal cells after harvesting with PPy/SD. The integrity of the microalgae cells were tested with Evans blue solution, cells with broken cell walls should appeared blue, as Evans blue solution diffused in the protoplasm region and stained the cells blue. In present study the microalgae showed no any blue stain, advocating PPy/SD as a safe and appropriate for recovery of microalgae biomass. The effect of PPy/SD on lipid production of microalgae was analysed by comparing the lipid obtained from microalgae harvested by filtration process and by PPy/SD particles. Table 3 showed that there were no losses in lipids obtained from the microalgae harvested by PPy/SD particles

TABLE 3. Comparison between amounts of lipid extracted from microalgae harvest by two methods.

Microalgae	Lipid content (% of dry weight)	
	PPy/SD particle	Filtration
C. vulgaris	19.62±1.19	20.02±1.23

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Figure 6. Integrity status of cell-walls of *C. vulgaris* before (A) and after (B) the harvesting.

Effect of stirring speed on recovery efficiency of microalgal

Biomass recovery of microalgae harvesting was influenced by the stirring speed as shown in Table 4. The biomass recovery efficiency of PPy/SD particles increase with increase of agitation speed from 60 to 140 rpm for *Chlorella vulgaris*. Increasing speed enhanced the dispersion and homogenized the availability of PPy/SD particles in system to be attached with microalgae cell, however after 120 rpm of stirring speed it shown declination in biomass recovery efficiency, which might had happen due to disruption of the PPy/SD particles-cell aggregates by high-speed stirring [20]. The time span also affects microalgae recovery efficiency, longer than 3 min did not improve microalgae recovery efficiency further for *C. vulgaris* (data not shown).

TABLE 4. Effect of stirring speed on recovery efficiency of microalgae.

Microalga	Recovery efficiency (%)				
	60 rpm	80 rpm	100 rpm	120 rpm	140 rpm
C. vulgaris	65.22± 1.91	77.9± 2.16	85.61± 1.88	90.82± 2.71	84.73± 2.02

Recovery of PPy/SD particles from microalgae biomass

The ability of regeneration of used PPy/SD particles material as recovery of algal biomass is an important factor to evaluate the cost effectiveness and environmental friendliness of a coagulant. The harvested microalgae cells associated with PPy/SD particles were treated with hexane in a Soxhlet apparatus operated at 80 ºC for 10 hours to extract lipid from the algal cells. Recoveries of PPy/SD particles from the residue left after extraction of lipid were performed at different concentrations (0.1–1.0M) of NaOH solution and were separated by micro-filtration. The PPy/SD particles were recollected from the filtrate and examined the percentage of recovery as well as second and third cycles of algal biomass - PPy/SD particles recovery. The recovery of PPy/SD particles was 96.8-98%, while the efficiency of biomass recoveries in second and third cycles was almost same as its original (Table 5). The efficiency of biomass recovery in second and third cycles reflects PPy/SD particles were stable at 80ºC during lipid extraction. Cassignol et al., [27] had mentioned that Polypyrrole is thermally stable till 172 ºC.

CONCLUSIONS

Saw dust particles coated with electro-conducting polypyrrole comprises a remarkable potentiality to recover high biomass from microalgae culture. PPy/SD particles provide a cost effective and environmental friendly process for biomass recovery. Low dose requirement, short settling time, high concentration factors and cell integrity of microalgae are significant advantages of PPy/SD particles over many other frequently used coagulants. The recovery of microalgae biomass also depends on the speed and time of stirring. The data for adsorption equilibrium of microalgae was found well fitted with Langmuir model and the mechanism behind the adsorption was considered to be mainly due to the electrostatic attraction between the PPy/SD particles and microalgal cells.

More than 95% of the PPy/SD particles was recovered after the extraction of lipids with the treatment of NaOH solution at pH 12 and retained almost the same microalgal biomass recovery efficiency as the newly synthesized ones, confirming the reusability of the nanoparticle for microalgae biomass recovery.

TABLE 5. Recovery and efficiency of PPy/SD.

Microalgae	First cycle		Second cycle		Third cycle	
	A	B	A	B	A	B
C. vulgaris	90.80± 2.32	98.12± 1.89	90.21± 2.11	97.11± 1.42	89.54± 2.16	96.81± 2.35

Where A = Biomass recovery efficiency
B = Recovery of PPy/SD

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REFERENCES

1. Skjånes, K., C. Rebours, and P. Lindblad, 2012. Potential for green microalgae to produce hydrogen, pharmaceuticals and other high value products in a combined process. Critical Reviews in Biotechnology, 1–44.
2. Salim, S., R. Bosma, M.H. Vermuë, R.H. Wijffels, 2011. Harvesting of microalgae by bio-flocculation. Journal of Applied Phycology, 23: 849–855.
3. Mohsin, R., Z.A. Majid, A.H. Shihnan, N.S. Nasri, Z. Sharer, 2015. Effect of Biodiesel Blend on Exhaust Emission and Engine Performance of Diesel Dual Fuel Engine. Iranica Journal of Energy and Environment, 6: 154-160.

4. Van Beilen, J.B. 2010. Why microalgal biofuels won't save the internal combustion engine. Biofuels, Bioproducts and Biorefining, 4: 41–52.
5. Uduman, N. Y. Qi, M.K. Danquah, and A.F. Hoadley, 2010. Marine microalage flocculation and focussed beam reflectance measurement. Chemical Engineering Journal, 162: 935–40.
6. Brennan, L. and P. Owende, 2010. Biofuels from microalgae-a review of technologies for production, processing, and extractions of biofuels and co-products. Renewable & Sustainable Energy Reviews, 14: 557–577.
7. Chen, C.Y., K.L. Yeh, R. Aisyah, D.J. Lee, J.S. Chang, 2011. Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production. Bioresource Technology, 102: 71–81.
8. Park, J.K., R.J. Craggs, and A.N. Shilton, 2011. Wastewater treatment high rate algal ponds for biofuel production. Bioresourse Technology, 102: 35–42.
9. Rawat, I., R.R. Kumar, T. Mutanda, and F. Bux, 2011. Dual role of microalgae: phycoremediation of domestic wastewater and biomass production for sustainable biofuels production. Applied Energy, 88: 3411–24.
10. Pragya, N., K.K. Pandey, and P.K. Sahoo, 2013, A review on harvesting, oil extraction and biofuels production technologies from microalgae. Renewable and Sustainable Energy Reviews, 24: 159–171.
11. Papazi, A., P. Makridis, and P. Divanach, 2010. Harvesting *Chlorella minutissima* using cell coagulants. Journal of Applied Phycology, 22: 349–355.
12. Aisyah, S.I., M.N.S. Norfariha, M.A.M. Azlan, I. Norli, 2014. Comparison of Synthetic and Natural Organic Polymers as Flocculant for Textile Wastewater Treatment. Iranica Journal of Energy & Environment, 5 : 436-445
13. Nassar, N.N., 2010. Rapid removal and recovery of Pb(II) from wastewater by magnetic nanoadsorbents. Journal of Hazardous Materials, 184: 538–546.
14. Benemann, J.R., 1997, Feasibility analysis of photobiological hydrogen production. International Journal of Hydrogen Energy, 22: 979–987
15. Zhang, X., and R. Bai, 2003. Surface electric properties of polypyrrole in aqueous solutions, Langmuir 19: 10703–10709
16. Ansari, R., N.K. Fahim, and A.F. Dellavar, 2009. Removal of thiocyanate ions from aqueous solutions using polypyrrole and polyaniline conducting electroactive polymers. Journal of the Iranian Chemical Society, 2: 163-171.
17. Chinnasamy, S., A. Bhatnagar, R.W. Hunt, and K.C. Das, 2010. Microalgae cultivation in a wastewater dominated by carpet mill effluents for biofuel applications. Bioresource Technology, 101: 3097-3105.
18. Widholm, J.M., 1972. The use fluorescein diacetate and phenosafranine for determining viability of cultured plant cells. Stain Technology, 47: 89–94.
19. Kang, E.T., K.G. Neoh, and K.L. Tan, 1993. X-ray photoelectron spectroscopic studies of electroactive polymers. Advance in Polymer Science, 106: 135-190.
20. Xu, L., C. Guo, F. Wang, S. Zheng, and C.Z. Liu, 2011. A simple and rapid harvesting method for microalgae by in situ magnetic separation. Bioresourse Technology, 102: 10047–10051.
21. Hena, S., 2010. Removal of chromium hexavalent ion from aqueous solutions using biopolymer chitosan coated with poly 3-methyl thiophene polymer. Journal of Hazardous Material, 181: 474–479.
22. Freundlich, H., 1907. Ueber die adsorption in Loesungen, Z. Physical Chemistry, 57: 385–470.
23. Zheng, H., Z. Gao, J. Yin, X. Tang, X. Ji, and H. Huang, 2012. Harvesting of microalgae by flocculation with poly (γ-glutamic acid). Bioresourse Technology, 112: 212–220.
24. Prochazkova, G., I. Safarik, and T. Branyik, 2013. Harvesting microalgae with microwave synthesized magnetic microparticles. Bioresour. Technol. 130: 472–477.
25. Hena, S., N. Fatihah, S. Tabassum, S. Lim, and J. Lalung, 2015. Magnetophoretic harvesting of freshwater microalgae using Polypyrrole/Fe3O4 nanocomposite and its reusability, Journal of Applied Phycology, DOI: 10.1007/s10811-015-0719-x.
26. Bai, R., and X. Zhang, 2001. Polypyrrole-Coated Granules for Humic Acid Removal. Journal of Colloid Interface Science, 243: 52–60.
27. Cassignol, C., P. Olivier, and A. Ricard, 1998. Influence of the Dopant on the Polypyrrole Moisture Content: Effects on Conductivity and Thermal Stability. Journal of Applied Polymer Science, 70: 1567–1577.
28. Ras, M., Lardon, L., Bruno, S., Bernet, N., Steyer, J. P., 2011. Experimental study on a coupled process of production and anaerobic digestion of *Chlorella vulgaris.* Bioresource Technology. 102: 200-206.
29. Oh, H.M., Lee, S. J., Park, M. H., Kim, H. S., Kim H. C., Yoon, J. H., Kwon, G. S., Yoon, B. D., 2001. Harvesting of *Chlorella vulgaris* using bioflocculant from *Paenibacillus sp. AM49.* Biotechnology, 15:1229-1234.
30. Xu, L., Wang, F., Li, H. Z., Hu, Z. M., Guo, C., Liu, C. Z., 2010. Develpoment of an efficient electroflocculation technology integrated with dispersed air flotation for harvesting microalgae. Journal of Chemical Technology and Biotechnology. 85: 1504-1507.

Persian Abstract

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چکیده

در این تحقیق یک روش برداشت ساده و سریع برای منعقد کردن و جداسازی ریزجلبک Chlorella vulgaris از سوسپانسیون رقیق به کمک رسانایی الکتریکی پلیمر پوشیده شده از خاک‌اره ارائه‌شده است. پلی پیرول (PPY) پوشش خاک‌اره به‌عنوان ماده منعقد جدیدی از طریق پلیمریزاسیون درجا از مونومر پیروز (PY) با استفاده از اکسیدان FeCl3 در محیط آبی که در آن ذرات خاک‌اره به حالت تعلیق درآمد آماده شد. پتانسیل زتای ماده منعقدشده و C. vulgaris و طیف‌سنجی فوتوالکترون اشعه ایکس (XPS) تجزیه‌وتحلیل و مشخص شد. پلی پیروز بارالکتریکی عمدتاً مثبت در محدوده pH گسترده (دو تا ده) با نقطه ایزوالکتریک ده و چهار دهم ایجاد می‌کرد درحالی‌که، کلرلا ولگاریس بار سطحی منفی از pH پنج و با نقطه ایزوالکتریک سه و هشت دهم داشت. بهترین بازده جداسازی ریزجلبک در pH معادل ده رخ داد. بازدهی برداشت بیش از نود درصد برای ریز جلبک با سرعت دوران صد و بیست دور در دقیقه ظرف مدت هفت دقیقه به دست آمد. بیشترین جذب نوری کلرلا ولگاریس بیست و هشت و هشت دهم میلی‌گرم خشک زیست‌توده / میلی‌گرم خاک‌اره پوشیده شده با PPY بود. فاکتور تمرکز به‌دست‌آمده بالاتر از سی و دو است که باعث صرفه‌جویی در انرژی و زمان برداشت میکروجلبک می‌شود و کاهش اندازه تجهیزات لازم برای آبگیری زیست‌توده را فراهم می‌کند و امکان بهبود استفاده از این میکروارگانیسم‌ها در سوخت‌های زیستی یا فرآیندهای تصفیه پساب را فراهم می‌کند.