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Statistical optimization of harvesting *Chlorella vulgaris* using a novel bio-source, *Strychnos potatorum*☆

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ABSTRACT

The present study was aimed at harvesting microalga, *Chlorella vulgaris*, by bioflocculation using seed powder of clearing nut, *Strychnos potatorum*. The research was essentially the prime step to yield a large biomass for utilising the cells in biodiesel production. Optimization of the parameters influencing bioflocculation was carried out statistically using RSM. The optimized conditions were 100 mg L^{-1} bioflocculant concentration, 35 °C temperature, 150 rpm agitation speed and 30 min incubation time and resulted in a maximum efficiency of 99.68%. Through cell viability test, using Trypan blue stain, it was found that cells were completely intact when treated with bioflocculant, but destroyed when exposed to chemical flocculant, alum. The overall study represented that *S. potatorum* could potentially be a bioflocculant of microalgal cells and a promising substitute for expensive and hazardous chemical flocculants. Moreover, this bioflocculant demonstrated their utility to harvest microalgal cells by economically, effectively and in an ecofriendly way.

1. Introduction

Microalga, a huge reservoir of lipids, is considered to be a potent biological resource for biodiesel production. Several research have proved that microalgae is clearly superior to terrestrial crops for biodiesel production due to its growing nature in waste water and produce high oil, and don't affect food chain [1]. However, harvesting and concentration of microalgae is the most challenging process in the overall process of microalgal biodiesel production and which desires commercialization [2–4]. Harvesting of microalgae usually contributes for 20–30% of the total production cost [5–7] and it is the main reason why previous attempts to produce microalgae at large scale application for biofuel have failed [8]. Many harvesting methods like centrifugation, filtration, and flocculation are employed for concentration of microalgae [9,10]. Among various methods, either used individually or in combination, flocculation technique is the most promising and cost effective [5].

Chemical substances that are commonly used as flocculants are nondegradable, could cause adverse effects to humans and their intermediate byproducts of degradation are also harmful to the ecosystem [11,12]. Nevertheless, the efficiency of chemical flocculation is dependent on pH and produces more sludge which is difficult to dehydrate [13]. Nowadays researchers focus on bioflocculation agent that is advantageous over chemical flocculant due to their biodegradability, high efficiency, nontoxicity and ecofriendliness [14,15]. Moreover, chemical-induced flocculation requires removal of excess flocculants from the medium before it can be reused [6]. Bioflocculants are made up of polysaccharides and protein materials generally produced from plants and microorganisms. Recently, Liu et al. [7] reported that harvesting of *Chlorella* minutissima UTEX2341 was done using bioflocculant isolated from *Bacillus agaradhaerens* C9. However, production of microbial bioflocculant like exopolysaccharide (EPS) for harvesting microalgae for a commercial scale usage is not economically feasible as the microorganisms produce EPS in the ranges between 3 g/L and 8 g/L [16–18] at normal fermentation conditions. Moreover, it requires many nutrients such as glucose, sucrose, and yeast extract, amino acids and sodium chloride for microbial growth and needs high amount of ethanol for isolation of

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exopolysaccharide from microorganisms [17–20] leading to extra operational cost.

In order to reduce the cost involvement, toxicity and energy utilization there is a great interest to develop economically efficient harvesting technique for microalgae. *Strychnos potatorum* seed is a potential alternate to chemical flocculants. *S. potatorum* Linn. is related to Loganiaceae family and commonly referred to as clearing nut [21,22]. It is a native of India and mainly distributed in the deciduous forests of West Bengal, central and south India. It is also found in south tropical African countries such as Malawi, Zambia, Zimbabwe, Botswana, Namibia and in Sri Lanka and Myanmar. The plant has been described as a common tree of medicinal importance in India popularly used to purify water for drinking [22].

The present study dealt with harvesting freshwater microalgae *Chlorella vulgaris* using *S. potatorum* seed powder and optimizing the influencial parameters of bioflocculation namely bioflocculant concentration, flocculation time, temperature and agitation by statistical tool, Response Surface Methodology (RSM). To best of our knowledge this is the first attempt to harvesting of microalgae using *S. potatorum* seed using RSM.

2. Materials and methods

2.1. Microalgal culture

Fresh water microalga *C. vulgaris* was obtained from Centre for Advanced Study (CAS) in Botany, University of Madras (Guindy Campus), Chennai, Tamilnadu, India. It was grown in 14 L Photo-bioreactor (PBR) using sterile Bold's Basal Medium (BBM) consisting of (g/L) $NaNO_3$ (0.25), K_2HPO_4 (0.075), KH_2PO_4 (0.175), NaCl (0.025), $CaCl_2{\cdot}2H_2O$ (0.025), $MgSO_4{\cdot}7H_2O$ (0.075), EDTA·2Na(0.05), KOH (0.031), $FeSO_4{\cdot}7H_2O$ (0.005), H_3BO_3 (0.008), $ZnSO_4{\cdot}7H_2O$ (0.0015), $MnCl_2{\cdot}4H_2O$ (0.0003), MoO_3 (0.00025), $CuSO_4{\cdot}5H_2O$ (0.0003), $Co(NO_3)_2{\cdot}6H_2O$ (0.0001) and mixing was provided by sparging air from the bottom of the PBR. The lighting was supplied by cool-white fluorescent light with an intensity of 5000 lux under 12:12 light/dark cycle. The stationary phase culture was used for the bioflocculation experiments.

2.2. Bioflocculant preparation

Fresh and healthy seeds of *S. potatorum* Linn. were collected from Shervaroy hills, Salem district, Tamilnadu, India for bio-flocculant preparation. A quantity of 15 g of seeds was sun dried and powdered using blender. The powdered seeds were stored in air tight containers and defined quantity, as per the RSM design, was autoclaved before being used for each experimental run.

2.3. Experimental design for the evaluation of bioflocculation using RSM

This investigation involved the use of Central Composite Design (CCD) of RSM and values for bioflocculation parameters were fixed according to orthogonal values (Table 1). CCD was experimented to optimize the four variables that significantly influenced the bioflocculation process. The experimental runs were designed and statistically analysed using Design Expert software (Version 8.0.7.1 Trial, Stat-Ease Inc., Minneapolis, USA). The four independent variables were evaluated at five levels (−1, −2, 0, +1, +2) with 30 experimental runs and six repetitive central points. Amount of 50 ml of *C. vulgaris* (1 g/L) was used for optimization study. The effects of bioflocculation parameters, namely bioflocculant concentration, temperature, flocculation time, and agitation at pH 7 were individually experimented and checked for bioflocculation efficiency of each run. The response obtained could be represented by a second-degree polynomial equation as:

$$Y = \beta_0 \sum_{i=1}^{n} \beta_i X_i + \sum_{i<j}^{n} \beta_{ij} X_i X_j + \sum_{j=1}^{n} \beta_{jj} X_{j^2}$$

where Y is the predicted response, β_0 is the constant, β_{ii} is the linear, β_{ij} is the second-order interaction, β_{jj} is the quadratic coefficients and X_i, X_j are the non-coded independent variables. Since number of variables is four, by substituting $n = 4$, the equation becomes,

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{14} X_1 X_4 + \beta_{23} X_2 X_3 + \beta_{24} X_2 X_4 + \beta_{34} X_3 X_4 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{44} X_4^2$$

where Y is the predicted response, β_0 is the constant, X_1, X_2, X_3 and X_4 are the input variables, $\beta_{1\text{-}4}$ are the linear coefficients, $\beta_{12\text{-}34}$ are the second-order interactive coefficients and $\beta_{11\text{-}44}$ are the quadratic coefficients.

After the set up of fixed parameters, each tube was kept in orbital shaker (Model-Technico, Honeywell Ltd., India). The initial microalgal biomass concentration in the tubes was estimated from the optical density of 750 nm (OD 750), using UV–vis Spectrophotometer (Model-SL159, ELICO Ltd., India). At a desired incubation time, the optical density of the supernatant was measured at half the height of the clarified culture. Culture broth without bioflocculant was used as control and bioflocculation efficiency was calculated as,

$$\text{Flocculation Efficiency}(\%) = \left(1 - \frac{A}{B}\right) \times 100,$$

where, $A = OD_{750}$ value of sample and $B = OD_{750}$ value of control.

On the other hand, the aluminium sulfate (0.8 g/L) commonly known as 'alum', widely used chemical flocculant for coagulation as well harvesting purposes, was employed for harvesting *C. vulgaris* at a concentration of 0.8 g/L.The cell pellets were collected from both chemical and bioflocculation processes and viability was examined under light microscope (Model-Olympus CH20i BIMF, Olympus India Pvt., India) at 100× magnification.

2.4. Cell viability test using Trypan blue staining

The viability of microalgal cells was determined by Trypan blue staining method. After harvesting of *C. vulgaris* cells with bioflocculant *S. potatorum*, 100 μl of 1% of Trypan blue solution was added to pellet, and incubated for 10 min at room temperature. The cells were then washed twice in deionized water. Broken cells appeared blue as Trypan blue solution diffused in the protoplasm region and stained the cells whereas intact, viable cells remained green, without the penetration of the stain.

Table 1
Coded values based on the factor at a time experiment for the 4 variables employed in the study.

Code	Variables	−2	−1	0	+1	+2
X_1	Bioflocculant concentration (mg L^{-1})	50	75	100	150	200
X_2	Temperature (°C)	25	30	35	40	45
X_3	Agitation (rpm)	50	100	150	200	250
X_4	Bioflocculation time (min)	10	20	30	40	50

3. Results and discussion

3.1. Response surface methodology of bioflocculation of C. vulgaris

A central composite design was developed for optimizing statistically the harvesting of *C. vulgaris* by bioflocculation using

Table 2
Design sheet with the experimental runs and their respective observed and predicted values of flocculation efficiency.

Run	Variables—Coded (Actual) values				Bioflocculation efficiency (*Y*), %	
	Bioflocculation concentration (X_1), mg L^{-1}	Temperature (X_2), °C	Agitation speed (X_3), rpm	Incubation time (X_4), min	Observed	Predicted
1	0 (100)	0 (35)	0(150)	0(30)	99.68	99.68
2	0(100)	0(35)	0(150)	0(30)	99.68	99.68
3	−1(75)	−1(30)	−1(100)	−1(20)	83.28	84.28
4	1(150)	−1(30)	−1(100)	−1(20)	87.87	87.68
5	−1(75)	1(40)	−1(100)	−1(20)	84.23	85.94
6	0(100)	0(35)	0(150)	2(50)	94.5	92.19
7	−1(75)	1(40)	−1(100)	1(40)	85.17	86.38
8	2(200)	0(35)	0(150)	0(30)	88.6	84.96
9	0(100)	−2(25)	0(150)	0(30)	85.91	84.44
10	1(150)	1(40)	1(100)	1(40)	90.38	93.59
11	0(100)	0(35)	0(150)	0(30)	99.68	99.68
12	1(150)	−1(30)	−1(100)	1(40)	83.18	86.52
13	−1(75)	−1(30)	1(200)	1(40)	83.53	85.18
14	1(150)	−1(30)	1(200)	1(40)	87.91	86.88
15	0(100)	0(35)	0(150)	−2(10)	92.8	90.21
16	−1(75)	1(40)	1(200)	−1(20)	85.05	85.92
17	−1(75)	−1(30)	1(200)	−1(20)	83.52	83.77
18	−1(75)	−1(30)	−1(100)	1(40)	83.18	83.00
19	0(100)	0(35)	−2(50)	0(30)	91.2	88.70
20	1(150)	−1(30)	1(200)	−1(20)	82.34	85.34
21	0(100)	0(35)	0(150)	0(30)	99.68	99.68
22	0(100)	0(35)	0(150)	0(30)	99.68	99.68
23	1(150)	1(40)	1(200)	−1(20)	89.47	90.33
24	0(100)	0(35)	2(250)	0(30)	91.43	89.04
25	−1(75)	1(40)	1(200)	1(40)	88.18	89.06
26	−2(50)	0(35)	0(150)	0(30)	78.29	77.04
27	1(150)	1(40)	−1(100)	−1(20)	89.61	92.17
28	0(100)	0(35)	0(150)	0(30)	99.68	99.68
29	0(100)	2(45)	0(150)	0(30)	96.23	92.81
30	1(150)	1(40)	−1(100)	1(40)	92.31	92.74

Strychnos seed powder. The responses as flocculation efficiency (%) at different experimental runs under parameters namely bioflocculation concentration, temperature, agitation speed and incubation time are represented in Table 2. An overall second order polynomial equation by multiple regression analysis was obtained for the flocculation (*Y*) as represented below:

$$Y = +99.68 + 1.98X_1 + 2.09X_2 + 0.084X_3 + 0.49X_4 + 0.71X_1X_2 - 0.46X_1X_3 + 0.032X_1X_4 + 0.12X_2X_3 + 0.43X_2X_4 + 0.67X_3X_4 - 4.67X_1^2 - 2.76X_2^2 - 2.70X_3^2 - 2.12X_4^2$$

Table 3
ANOVA for the response surface quadratic model of bioflocculation of *C. vulgaris*.

Source	Sum of Squares	df	Mean Square	*F* Value	*p*-value
Model	1066.45	14	76.18	11.26	<0.0001
X_1-concentration	94.21	1	94.21	13.93	0.0020
X_2-temperature	105.13	1	105.13	15.54	0.0013
X_3-agitation	0.17	1	0.17	0.025	0.8768
X_4-time	5.87	1	5.87	0.87	0.3663
X_1X_2	8.05	1	8.05	1.19	0.2925
X_1X_3	3.32	1	3.32	0.49	0.4942
X_1X_4	0.016	1	0.016	0.0024	0.9615
X_2X_3	0.24	1	0.24	0.036	0.8523
X_2X_4	2.97	1	2.97	0.44	0.5178
X_3X_4	7.25	1	7.25	1.07	0.3169
X_1^2	598.27	1	598.27	88.45	<0.0001
X_2^2	209.56	1	209.56	30.98	<0.0001
X_3^2	200.37	1	200.37	29.62	<0.0001
X_4^2	123.17	1	123.17	18.21	0.0007
Residual	101.46	15	6.76		
Lack of fit	101.46	10	10.15		
Pure error	0.000	5	0.000		
Cor total	1167.91	29			

where, *Y* is the flocculation efficiency, X_1 is bioflocculant concentration, X_2 is temperature, X_3 is agitation speed, X_4 is incubation time respectively.

The goodness of fit of regression equation (R^2) developed could be measured by adjusted determination coefficient. Regression analysis determines the significance of the experimental model of bioflocculation. The R^2 value of 0.9131 and adjusted R^2 of 0.8320 shows that the model could be significant predicting the response and explaining 95% of the variability in the model. The statistical significance of the equation was evaluated by F-test and ANOVA (analysis of variance) which showed that the model was statistically significant at 95% confidence level ($p < 0.05$). ANOVA reported the model *F*-value of 11.26 which indicated that the model is significant (Table 3). *P*-value denotes the importance of each coefficient, helping in understanding the interactions among the variables. The most significant factors of this model are X_1, X_{12}, X_{22}, X_{32} and X_{42}. Values of *p* less than 0.0500 indicate that the model terms are significant whereas values greater than 0.1000 indicate the model terms are not significant. The model also depicted the statistically non significant lack of fit ($p > 0.05$), indicating that the responses are adequate for employing in this model.

Three dimensional response surface plots represent regression equations and illustrate the interactions between the response and experimental levels of each variable. These plots let us locate the optimum levels of each variable for the maximum bioflocculation efficiency to harvest the highest amount of microalgal cells. Fig. 1 illustrates the response surface plots and represent the pair wise interaction of the four variables. Higher interaction between concentration and temperature resulted in large bioflocculation.

From this optimization study, the optimal values of concentration, temperature, agitation speed and incubation time were found as 100 mg L^{-1}, 35 °C, 150 rpm and 30 min respectively. The maximum efficiency was estimated to be 99.68% which is in complete agreement with the prediction of the model (99.68%).

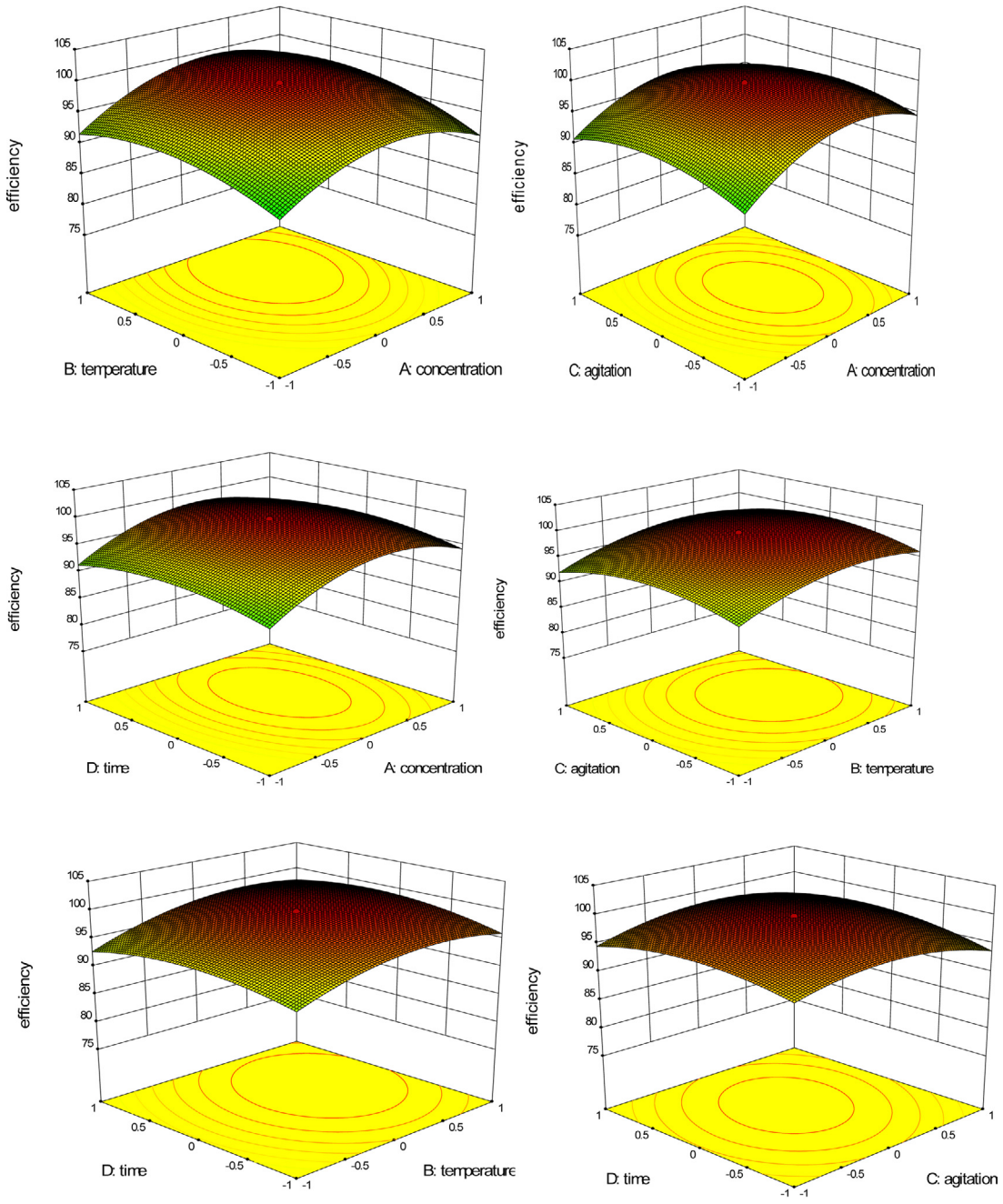

Fig. 1. 3D response surface plots depicting the effects of four independent variables: Concentration, Temperature, Agitation and Time on Bioflocculation efficiency.

The validation of the model was done by carrying out three experiments in the optimised conditions for harvesting by bioflocculation. The mean observed value obtained was 99.24, which was in good agreement with the predicted response (99.37).

3.2. Effect of bioflocculant concentration

Noteworthy effect was observed with varying bioflocculant concentrations on harvesting of microalga *C. vulgaris* by bioflocculation process. Low concentration of *S. potatorum* seed powder shows little effect on the removal of biomass achieving only 78.29% with 50 mg/L. However, increasing concentration of bioflocculant there was an increase in harvesting efficiency (Fig. 1). The maximum harvesting of *C. vulgaris* was obtained 99.68% at a bioflocculant concentration of 100 mg L^{-1}, and decreased sharply when the bioflocculant reached beyond the optimum level.

After bioflocculation of the microalgae cells by *S. potatorum*, the cell aggregates settled at the bottom of the test tubes at optimum concentrations, but microalgal cells floated in the medium when the bioflocculating agent was used in higher concentrations. Higher dosages of the bioflocculant did not show any effect on flocculating *C. vulgaris*. This is due to the competition of the floc formation and the formation of excess coagulant residue since all microalgae particles had already formed colloids which created destabilisation [23,24]. This current finding was not in agreement with result reported by Riano et al. [13], showed the maximum bioflocculation efficiency of 92% with chitosan as flocculant at a concentration of 214 mg/L and 35.4% with less amount 5 mg/L of bioflocculant.

Teixeira et al. [12] studied on harvesting of *C. vulgaris* using *Moringa oleifera* seed flour and reported that the maximum flocculation efficiency was found to be 1 g/L with increase in pH to 9.2. But in our current study maximum bioflocculation efficiency was observed with very less amount of bioflocculant concentration without increasing the pH. Similarly, without artificially increasing pH, Ignacio de Godos et al. [23] also reported that chitosan was able to flocculate 98% of microalgae–bacteria consortium consisting of *S. obliquus, C. sorokiniana, Chlorococcum* sp. and the *Chlorella*. Since, *S. potatorum* seed powder contains strychnine which is responsible for the high flocculation efficiency at neutral pH [25] and hence it is widely used for domestic water purification in most part of Tamilnadu state, India [26].

3.3. Effect of temperature

Temperature played a vital role in flocculation process. The maximum bioflocculation was occurred at temperature 35 °C. This is apparently due to the collision of cells caused during increasing mobility at higher temperature, leading to flocculant-microalgae interactions and hence producing effective aggregates [1,27]. This phenomenon was clearly described by Pan et al. [28] i.e. in chemical kinetics, at higher temperature the suspended particles move faster and frequency of collision is also increased which leads to increase in the rate of reaction. Increasing the number of collisions increases the number of possible interactions that can occur, which in turn improves the bioflocculation rates. The increasing mobility resulting from temperature increase relates to the molecular mobility of the flocculant molecules which results in an increase in bioflocculant–microalgae interactions per time, hence productivity and recovery are elevated [1]. However, a rapid decrease in bioflocculation efficiency was experienced when the temperature was raised above 35 °C. This finding was in agreement with the results reported by Uduman et al. [1] which showed that the flocculation rate of *Chlorococcum* sp. was decreased when the temperature reached above 40 °C and polymer flocculants used for harvesting. This was due to the susceptibility of microalgae cells at higher temperature causing cell death finally resulting in decreased agglomeration.

3.4. Effect of agitation

Agitation is one of the important factors in harvesting microalgae by flocculation. Agitation effect was analysed in the ranges of 50–250 rpm and the maximum bioflocculation efficiency was observed at agitation speed of 150 rpm, which was sufficient for all of the microalgae cells to be adsorbed by the *S. potatorum* seed powder. Harvesting efficiency was not significant at low agitation speed (Fig. 1). However, when the agitation speed was increased further, the harvesting percentage of microalga decreased at a speed of 250 rpm. This observation was in agreement with Ahmad et al. [29]. This phenomenon is caused by the restabilization of the cells at high mixing speed [30]. The influence of agitation speed over bioflocculation efficiency observed in the present work followed the same tendency reported by Riano et al. [13]. These authors studied that chitosan could be a strong bioflocculating agent used to harvest *C. vulgaris, Microcystis* sp. and *Acutodesmus obliquus*. They experienced that agitation speed of 131 rpm showed the highest flocculation of 92% and the flocculation efficiency fell when the agitation speed was increased to 600 rpm producing only 79%.

Another report done by Zheng et al. [31] confirmed that agitation really influences the flocculation process. This team studied on polymeric phosphate chloride (PPAC) as a flocculant to treat wastewater and they observed that at high agitation speed (600 rpm), the flocculation efficiency was not improved. High-speed mixing tends to break the flocs, causing the coagulated cells to be redispersed and introduced again into the medium [29]. Some researchers have recommended rapid mixing followed by slow mixing during the flocculation process. However, after reaching optimal flocculating dosage, no significant difference in the size of flocs and the settling velocity was observed by researchers [32].

3.5. Effect of bioflocculation time

The interaction between the bioflocculation time and the harvesting of microalgae is illustrated in Fig. 1, which showed that *S. potatorum* seed powder needed a optimal flocculation time of 30 min to flocculate 99.68% of the *C. vulgaris* cells. The percentage of microalgae cells removed was lower, with only 89.7% harvested at shorter mixing time of 30 min. The less harvesting rate was caused by the decrease in contact between the microalgal cells and *S. potatorum* seed powder. However, this reaction time is very less when compared to results shown by Papazi et al. [33]. This team achieved the maximum flocculation efficiency of 80% using chemical for *C. minutissima* after 3–4 h of reaction time with the optimal concentrations of 0.75 and 0.5 g/L for sulfate and chloride salts, respectively. This finding indicated the bioflocculant *S. potatorum* seed powder was more advantageous than the chemical flocculants by reducing the time for harvesting microalgae.

In general, the bioflocculation time depends on the size of the floc. An increased floc size was observed to increase the "free" settling velocity compared to the individual particles that do not form flocs [29]. Additionally, it has been shown that when the flocs settle faster, the quality of the removal is better. *S. potatorum* seed powder promotes faster aggregation of microalgal cells through the formation of bridges between the dispersed cells, allowing the formation of particles of a sufficient size that settle faster. As the bioflocculation time increased from 30 to 90 min, the total number of collision will increased and the possibility of microalgal cells and bioflocculant particles collide each other increased, allowing flocculation and adsorption to occur [29]. This could be caused

Table 4
Efficiency of various chemical and bioflocculants to harvest microalgae.

Method	Microalgae	Habitat	Efficiency (%)	Reference
Bioflocculation with *Strychnos potatorum* seed powder	*C. vulgaris*	Fresh	99.68	Current study
Flocculation with non-ionic polymer Magnafloc LT-25	*Chaetoceros calcitrans*	Marine	80	Knuckey et al., 2006 [35]
Flocculation with non-ionic polymer Magnafloc LT-25	*Tetraselmis suecica*	Marine	80	Knuckey et al., 2006 [35]
Flocculation with $AlCl_3$	*Chlorella minutissima*	Marine	90	Papazi et al., 2010 [33]
Flocculation with cationic polymer 71303	*Chlorococcum* sp.	Marine	89.9	Uduman et al., 2010 [14]
Flocculation with cationic starch	*Parachlorella* kessleri SAG 2787	Fresh	80	Vandamme et al., 2010 [21]
Bioflocculation with *T. suecica*	*N. oleoabundans*	Marine	46.2	Salim et al., 2011 [18]
Bioflocculation with seeds of *Moringa oleifera*	*Chlorella vulgaris*	Fresh	87	Teixeira et al., 2012 [25]
Bioflocculation with γ-PGA	*Nannochloropsis oculata* LICME 002	Marine	90	Zheng et al., 2012 [36]
Bioflocculation with *Paenibacillus* sp. AM49	*Chlorella vulgaris*	Fresh	83	Oh et al., 2001 [37]

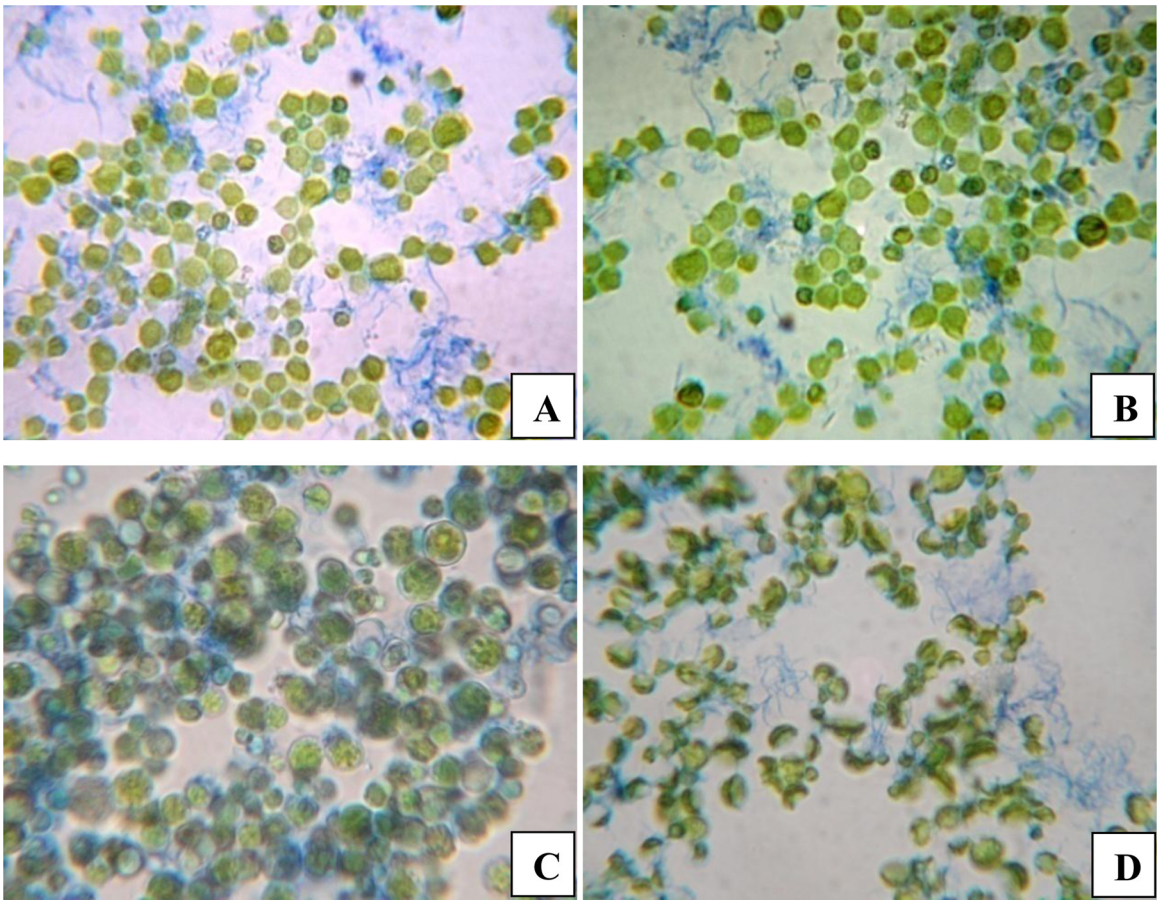

Fig. 2. shows the viability and damages of *C. vulgaris* cells after harvesting. Cells harvested with *S. potatorum* seed powder no stain uptake (A), no cell destruction—standby with *S. potatorum* seed powder for 2 h after harvesting (B), cells harvested with alum uptake the stain and appeared in blue colour (C), standby with alum for 2 h after harvesting showing complete cell destruction (D).

by the bridging mechanism that occurs in the removal process [34]. This finding concluded that increased mixing time may cause increased adsorption. The current study had proved that the use of this seed powder would apparently the best bioflocculant to harvest the microalgal cells and Table 4 shows the comparative chart of harvesting efficiency of different flocculants used to flocculate microalgae.

3.6. Cell viability test

Cell viability test is an important study in the process of harvesting microalgae for biodiesel production. The prime fact in this process is that the flocculants which is used for harvesting of microalgae should not destruct the microalgae. Because if the microalgal cells are disrupted by flocculants, it leads to release of intracellular inclusions including lipids into the culture medium which would very difficult to extract lipid and need enormous amount of solvents. When the culture was harvested with bioflocculant, *S. potatorum* seed powder, the cells remained intact without any damage and there was no stain uptake by cells after harvesting and even after standby for 2 h (Fig. 2A and B), whereas when alum was used as flocculant for harvesting microalgae, cells were readily stained by Trypan blue after harvesting (Fig. 2C) and completely destroyed at standby after 2 h (Fig. 2D).

4. Conclusion

This research dealt with the efficiency of seed powder of *S. potatorum* on flocculating *C. vulgaris* by RSM. Optimal conditions were found to be 100 mg L^{-1} bioflocculant concentration, 35 °C temperature, 150 rpm agitation speed and 30 min incubation time yielding a maximum efficiency of 99.68%. As the seeds were able to flocculate at neutral pH effectively, no manual alteration of pH was done and also cells remained viable. Thus the seeds could be an advantageous and a novel bioflocculant in algal technology and be helpful in rich harvesting of microalgae for cost effective production of biodiesel from algal lipids.

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