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Microalgal Harvesting considering *Moringa* and Watermelon Seeds as Natural Flocculants

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Abstract

Microalgae are aquatic microorganisms that are capable of producing their food through multifarious photosynthetic activities, utilizing carbon dioxide and nutrients as the building blocks to produce biologically complex compounds. Commercially valuable compounds, such as biodiesel, β- carotene and omega-3 fatty acids, are highly sought-after by large energy, agricultural and nutraceutical entities. Harvesting represents approximately 25 to 60% of the total cost of production. Using a natural flocculant reduces energy consumption and makes the algal products, such as omega-3 fatty acids and β- carotene more marketable for feed and animal consumption. Rapid 10.0 mL test tube assessments and batch experiments using Phipps and Bird jar test method were conducted to determine the optimal dosages for the two natural flocculants derived from *Moringa oleifera* and *Citrullus lanatus* (watermelon). A reference chemical flocculant (alum) was also used in these experiments. Preliminary results showed that alum and *Moringa* were more effective than *Citrullus lanatus* (watermelon). Further experiments need to be conducted considering lower concentrations of watermelon seed powder, as the initial experiments showed concentrations greater than 12.0 g/L resulted in decreasing efficacy.

KEYWORDS: Algal production, flocculation, coagulation, dewatering

Microalgae are aquatic microorganisms that are capable of producing their food through multifarious photosynthetic activities, utilizing carbon dioxide and nutrients as the building blocks to produce biologically complex compounds. These valuable compounds, particularly lipids, are highly sought-after by large energy, agricultural and nutraceutical entities. Diversification of an economy and energy resources is especially important in a time of economic recession, when Trinidad and Tobago is so heavily dependent on oil to secure economic stability and growth. Most of the existing research is being done by China, United States and European countries, such as the Netherlands. Very little is being done in Trinidad and Tobago and the Caribbean region to explore algal bio-resources.

The challenge arises when a dense harvestable yield is reached and these suspended cells are to be separated from the aquatic media that they are contained in.   Harvesting costs are typically high and account for 20% to 60% of the total cost, as the mass fractions in culture are generally low and microalgal cells possess a negative charge that keep them in a dispersed, colloidal state. Harvesting typically consists of a two-stage process. The first stage involves bulk harvesting, where the aim is to have total solid matter that ranges between 2-7% using flocculation, flotation or gravitational sedimentation (Chen et al. 2011). The second stage is thickening and involves concentrating the sludge or slurry. Centrifugation and filtration are energetically expensive and are not feasible at the industrial scale.

The flocculation process is commonly used in the wastewater, biotechnology and algal industries. Flocculation refers to the process whereby an agent is added to facilitate bonding of particles, whereby larger aggregates settle and are easier to separate from the aquatic media. To facilitate flocculation of microalgal suspensions, a number of chemical flocculants may be used (Lee et al. 2015). There are both organic and inorganic flocculants. Inorganic or cationic flocculants, such as aluminium- based alum neutralizes the negative surface charge. Alum was used as a reference coagulant in this study, due its known high efficacy with many microalgal species. Many nutraceutical and agricultural entities prefer natural feeds and concerns may arise with the use of alum application as there is an increased risk of aluminium contamination. Natural solutions are always market preferable.

Flocculation is very pH dependent (US EPA 2019). Other properties that affect the separation of microalgae from water are inclusive of size, shape, specific gravity, motility, growth phase, the presence of extracellular organic matter (EOM) concentration and composition, and appendages (Udom et al. 2013). Prior to flocculation, microalgae are also capable of restoring their negative surface charge by transferring ions across their cell membranes. To facilitate flocculation of microalgal suspensions, a number of chemical flocculants may be used (Lee et al. 2015). There are both organic and inorganic flocculants. Inorganic or cationic flocculants, such as aluminium-based alum neutralizes the negative surface charge. Alum was used as a reference coagulant in this study, due its known high efficacy with many microalgal species. Many nutraceutical and agricultural entities prefer natural feeds and concerns may arise with the use of alum application as there is an increased risk of aluminium contamination. Natural solutions are always market preferable.

Utilizing local, natural flocculants may also be beneficial as there may be a decreased reliance and importation of chemical flocculants. In addition, there may be additional stimulation of the agricultural economy for an increased demand for these non- consumable products. Natural flocculants derived from *Moringa oleifera* and *Citrullus lanatus* (watermelon) seed flour were used in wastewater treatment in parts of sub-Saharan Africa (Udom et al. 2013; Muhammad et al. 2015). Both *Moringa oleifera* and *Citrullus lanatus* (watermelon) were locally available. The aim of this study was to investigate the efficacy of the flocculation process considering three flocculants: *Moringa oleifera* and *Citrullus lanatus* (watermelon) and alum.

METHOD

Bioprospecting and initial growth

Samples of microalgal cultures were collected at multiple sites across Trinidad and Tobago. The collected samples were then lab grown in UTEX photobioreactors (PBR) using a defined media and a low irradiance intensity of approximately 40 μmol m^{-2} s^{-1}. pH was maintained between 6.5 and 8.5. Nitrate MR, Cadmium Reduction Method 8171 in the HACH DR/2800 Spectrophotometer and Reactive (Orthophosphate) Method 8048, were conducted using the HACH Spectrophotometer (DR/2800) every week.

Growth at the pilot-scale

When the algal biomass in the UTEX photobioreactors (PBR) reached approximately 1.0g /L, aliquots of 10 mL of algal cultures were added to 100 mL of media in modified upcycled 1.5 L plastic bottles that were used as photobioreactors. Incremental volumes were added weekly until the operating volume of 1.0L was reached. An air system comprising of a Boyu air compressor (Model ACQ-004), aquarium tubing (3.18 mm diameter), and air stones were used to aerate the cultures. The air pressure in the bottles was regulated using plastic air valve T-connectors.

Preparation of flocculants

Seeds from *Moringa oleifera* and *Citrullus lanatus* (watermelon) were sourced locally and extracted. The *Moringa oleifera* seeds were then sundried for two weeks, while the *Citrullus lanatus* seeds were placed in a preheated oven at 80°C and were left to dry for 24 hours. Then both seeds were similarly prepared using an electrical osterizer food processor. A mortar and pestle were then used reduce the seed grain size into a powder form. The *Citrullus lanatus* seeds were similarly prepared.

Testing the efficacy of the flocculants

Jar tests were conducted according to the Phipps and Bird jar test procedure (APHA et al. 1915). The microalga suspension was collected from the pilot-scale photobioreactors. The pre-weighed coagulants were then added to each beaker containing 500mL microalga suspension. This mixture was then rapidly stirred using NUOVA and CIMAREC 2 stir plates, at $7.0*10^3$ rpm for 90 seconds. The beakers were then allowed to settle and observed after 1 minute, 90 minutes and 24 hours. After the 24-hour interval, a known volume of the supernatant for each beaker was collected with a syringe and was filtered through a pre-weighed SIMSII membrane filter paper, with a diameter of 47mm and a pore size of 0.45 µm, using a vacuum pump and a vacuum filter. The optimal efficacy of the flocculation process or solids settling was determined by examining the differences in total suspended solids (TSS) in the initial suspension and final effluent, when the three flocculants were added (Table 1).

TSS was calculated using the following equation (USEPA, 2019):

$$\text{TSS (mg/L)} = \left(\frac{(A-B)}{sample\ volume\ (mL)} * \frac{1000mL}{L}\right) \quad \text{(Equation 1)}$$

Where: *A*= mass of filter paper + residue, *B*= mass of filter paper before residue

Table 1: Concentration of flocculant considered for optimal flocculation

Concentration considered (g/L)	Flocculant		
	Alum	**Moringa**	***Citrullus (watermelon seeds)***
	0.0	**0.0**	**0.0**
	4.0	-	-
	6.0	**6.0**	-
	8.0	**8.0**	-
	-	**10.0**	-
	-	-	**12.0**
	-	-	-
	-	-	**16.0**
	-	-	**20.0**

RESULTS AND DISCUSSION

The microalgae culture had an initial TSS of 1.6 g/L. Microalgae would settle on itself if given sufficient time, evident by the negative controls having a mean success of 68.4% (Table 2). These flocculation harvesting experiments were conducted over a 24-hour period, but if given further time, the degree of flocculation should even further increase. In an industrial setting, the pros and cons need to be weighed for the increased waiting period and any further yield. These preliminary results also showed the alum and *Moringa* were the more effective (97.0%) flocculants than *Citrullus (watermelon seeds)*. In addition, the efficacy was further reduced with increasing concentrations of *Citrullus (watermelon seeds),* suggesting that the optimal concentration is at a lower concentration than what was investigated. The experiment should be repeated using lower concentrations. A study conducted by Muhammad et al. 2015, which tested the efficiency of watermelon seed flour in its application to water treatment, it was found that a dose of 0.1 g/L at a pH of 7 was ideal in removing turbidity from water gathered at the Gubi dam, Bauchi state, to the North-East of Nigeria. Further experiments have to be expanded to consider optimization and the use of lime, as alkaline conditions promote autoflocculation.

Table 2: Summary of Mean Flocculation Efficacy

Concentration considered (g/L)	**Flocculant**		
	Alum	***Moringa***	***Citrullus (watermelon seeds)***
0.0	68.4	68.4	68.4
4.0	73.4	-	-
6.0	78.0	94.2	-
8.0	96.0	91.9	-
10.0	97.0	96.7	-
12.0	-	-	85.7
14.0	-	-	-
16.0	-	-	51.8
20.0	-	-	53.6

CONCLUSION

Powder derived from *Moringa oleifera* and *Citrullus lanatus* (watermelon) seeds were produced. Preliminary investigations to consider these as microalgae flocculants. Results suggest that alum and *Moringa* are more effective in the raw state as a flocculant than watermelon seeds. Moderate flocculation efficiency success (68.4%)was observed when using no flocculant in the negative control. Further experiments need to be conducted to consider at a wider flocculant concentration, experimentation duration and pH range.

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