Original Research Article

Harvesting of *Chlorella variabilis* Biomass Using *Moringa oleifera* Seed-Induced Sedimentation.

Abstract

Harvesting cell biomass from microalgae cultures is capital intensive and represents a significant percentage of the total production cost. Although many synthetic chemicals have been used to induce sedimentation of microalgae cultures, depending on the use of the harvested biomass, the use of natural flocculants is preferred. The efficacy of using *Moringa oleifera* seed powder, cold water extract, and autoclaved cold water extract of *Moringa oleifera* seeds to induce sedimentation of *Chlorella variabilis* cells were investigated. In all the three cases, the rate of sedimentation increased with increase in the concentration of the *M. oleifera* seed used. In comparison with seed powder, use of cold water extract resulted in significant decrease in the sedimentation rate ($p < 0.05$). However, more than 60% sedimentation was achieved by addition of extract from 10 g/L seed and incubating for only 30 minutes. The extract was autoclaved without significant decrease in the efficacy of sedimentation ($p > 0.05$). More can 70% sedimentation of *Chlorella variabilis* culture with an optical density of 3.5 was achieved in 30 minutes by addition of autoclaved extract from 7 g/L seed. This is considered sufficient for harvesting biomass from many microalgae cultures.

Key words: *Chlorella variabilis*, *Moringa oleifera*, seed powder, cold water extract, harvesting of microalgae, biomass sedimentation

1. INTRODUCTION

Cultivation of microalgae has been increasing steadily due to the various useful applications they offer in wastewater treatment [1-5], biodiesel oil production [6-12] as well as in production of antioxidants [13-16]. Microalgae are also used in soil bioremediation [17], production of single cell protein [18, 19] and carbon dioxide fixation [20]. Microalgae are also used to purify water and treat effluent from dyeing industries [21, 22]. Although, microalgae have these various applications, the cost of harvesting the microalgal biomass after cultivation is capital intensive and represents significant percentage of the total production costs [23]**.** Several methods have been developed for harvesting microalgae biomass and these include filtration of the culture [24], centrifugation [25], microbial flocculation [26], floatation [27] or by sedimentation [25]. Natural sedimentation is hardly enough for harvesting microalgae biomass for various applications and there is usually a need

to add some flocculants. The use of various inorganic and organic flocculants have been investigated and these include metal salts such as Aluminium sulfate, Aluminium chloride, Ferric chloride and Ferric sulphate [23, 28, 29], and Polyethylenoxide [29]. Papazi *et al*., [30] also tested the ability of 12 salts to sediment *Chlorella minutissima* cells in culture. Among all these flocculants, natural organic flocculants are preferred because they are environmentally friendly and some of them are edible. Some authors have worked on the use of organic flocculants such as chitosan [28, 31, 32, 33] and even microbial flocculant [26]. Seeds of *Moringa oleifera* have been extensively investigated as flocculants in water treatment and removal of dye effluent from industries [22, 34]. Recently some researchers have reported the use *M. oleifera* seeds in various forms to harvest microalgae due to its inexpensiveness, availability and non-toxicity. Teixeira and Teixeira [35] used seed cake, seed flour and extract from cake and flour to flocculate *Chlorella vulgaris*. Hamid et al., [36] compared the potentiality of *M. oleifera* seed flour, protein powder and alum to flocculate *Chlorella sp. cells* for the purpose of harvesting them. Udom et al., [37] compared the effectiveness of various flocculants (alum, ferric chloride), cationic polymer (Zetag 8819), anionic polymer (E-38), *Moringa oleifera* and *Opuntia ficus-indica* cactus) for harvesting microalgae grown in semi continuous culture in a photobioreactor under natural light. They investigated the cost effectiveness of each flocculating agent. Hamid *et al.*, [36] harvested microalgae from aquaculture waste water as a phytoremediation method using *M.oleifera*. In most of these previous experiments, either rigorous extraction steps were used or the pH of the media were adjusted to either highly alkaline [39] or acidic level. These added to the cost of harvesting and the method of pH adjustment is not suitable for continuous culture operations where only a fraction of biomass is harvested, and the residual biomass serve as inoculums for the subsequent operation.

In the present study, the ability of *M. oleifera* seed powder, filtrate from cold aqueous suspension of seed powder and autoclaved filtrate were compared for their ability to flocculate *Chlorella variabilis* cells without any pH adjustment.

2. MATERIALS AND METHODS

2.1 Materials

Moringa oleifera pods were harvested from the Botanical Garden, Depratment of Plant Science and Biotechnology, University of Nigeria, Nsukka. *Chlorella variabilis* NIES-2541 stock culture was obtained from the Department of Microbiology University of **Nigeria,** Nsukka.

2.2 Preparation of *Moringa oleifera* seed

The seeds were removed from the pods and the outer shells were removed by hand. Only healthy seeds were selected and used for sedimentation experiments. Three sets of dry seeds of *Moringa oleifera* were prepared namely: (a) powdered seed, (b) powdered seeds were

soaked in cold water for 30 minutes, and the extract was filtered through cheese cloth, and (c) the extract obtained from (b) was autoclaved for 20 minutes at 121 ^{O}C.

2.3 Sedimentation with powdered seed.

Chlorella variabilis NIES-2541 stock was maintained in BG11 medium. The stock culture was revived and cultured in BG 11 medium under photoautotrophic condition for two weeks in 500 mL Erlenmeyer flasks. The cultures were mixed by intermittent manual shaking three times daily. The culture was illuminated at an intensity of 100 μmolm^{-2}s^{-1} using a 32-W white bulbs (ASTRA NU-PARK, CHINA). Three grams of dry *M. oleifera* seeds were ground to fine powdery paste with mortar and pestle. The powder was suspended in distilled water to a concentration of 50g/L. Various volumes corresponding to various concentrations(1-5g/L) of the *M. oleifera* suspension was added into labeled test tubes. Corresponding volumes of algal biomass with optical density of 5.2 at 680 nm were dispensed into each test tube to make a total volume of 10 mL. The mixture was inverted severally to mix and then allowed to stand undisturbed on a test tube rack. One milliliter sample was withdrawn from the upper layer of each test tube every 30 minutes for a period of 180 minutes. At the end, each sample was diluted with 9 mL of distilled water and the optical density was read at 680 nm. Each experiment was performed three times and the average values were plotted.

2.4 Sedimentation with cold water extract of moringa seed

Three grams of dry *M. oleifera* seeds were ground to fine powdery paste with mortar and pestle. Two grams of the powder was suspended in 40 mL of distilled water inside 100 mL conical flask and manually shaken intermittently for 30 minutes to extract the active ingredients. The suspension was filtered through a double folded cheese cloth and various volumes (0.2 to 1.0 mL) of the clear supernatant were dispensed into labeled test tubes. Appropriate volumes of fully grown *C. variabilis* culture (9.8 - 9 mL) with an optical density of 5.2 were dispensed into the corresponding labeled test tubes. Each test tube was inverted gently several times to mix. The mixture was allowed to stand undisturbed for 180 minutes. One milliliter sample was withdrawn from the top of each test tube every 30 minutes for a period of 180 minutes. At the end, each sample was diluted with 9 mL of distilled water and the optical density read at 680nm. Each experiment was performed three times and the average values were plotted.

2.5 Sedimentation with autoclaved *M. oleifera* seed filtrate.

Three grams of dry *M. oleifera* seeds were ground to fine powdery paste with mortar and pestle. Two grams of the powder was suspended in 40 mL of distilled water inside 100 mL conical flask and manually shaken intermittently for 30 minutes. The suspension was filtered through a double folded cheese cloth and the filtrate was autoclaved at 121^{o}C for 20 minutes. After cooling to room temperature, various volumes (0.2 to 1.0 mL) of the autoclaved filtrate was dispensed into labeled test tubes. Appropriate volumes of fully grown *C. variabilis* culture (9.8 - 9 mL) with an optical density of 3.5 was dispensed into the corresponding test

tubes and inverted gently several times to mix. The mixture was allowed to stand undisturbed and one milliliter sample was withdrawn from the top of each test tube every 30 minutes for a period of 180 minutes. At the end, the samples were diluted with 9ml of distilled water and the optical density read at 680nm. Each experiment was performed three times and the average values were plotted.

2.6 Percentage sedimentation

The percentage of *Chlorella variabilis* NIES-2541 cells sedimented by different concentrations of the filtrate or powdered *M. oleifera* seeds after 30 minutes incubation was calculated using the formula:

Percentage sedimentation = $I\ OD_{680} - FOD_{680} / I\ OD_{680} \times 100$

Where I OD = Initial optical density of the algal culture used
F OD = Final optical density of the algal culture after incubating for 30 minutes with *M. oleifera* seed extract or powder.

2.7 Statistical analysis

All the experiments were performed in three replicates and the results were presented as means of the three values. Analysis of Variance (single classification) was used to test for significance differences among the treatments

3. RESULTS AND DISCUSSIONS

Various concentrations of powdered *Moringa oleifera* seeds were either used directly (powdered) or mixed with 20 mL of distilled water, extracted for 30 minutes under shaking, and filtered. The effects of addition of the powder or filtrate to the culture broth on sedimentation of *Chlorella variabilis* NIES-2541cells are shown in Figure 1. The results showed that the rate of cell sedimentation, as measured by decrease in the optical density of the upper phase, was dependent on the concentration of the *M. oleifera* seed powder/filtrate. When 1 g/L of the powder was added directly, the optical density decreased from 5.2 to 2.1 in 180 minutes. However, by increasing the concentration to 5 g/L, the sedimentation rate increased significantly and the optical density decreased to 1.02 after 90 minutes. In other words, about 80% of the *Chlorella* cells can be harvested through sedimentation by adding 5 g/L *M. oleifera* seed powder to the culture. However, since the powder sediments with the cells, separation of the seed powder from the cells can impose a technical challenge. Thus the effect of adding filtered extract to the culture broth on cell sedimentation was investigated. As shown in Figure 1, addition of filtrate also induced flocculation, and thus sedimentation of the cells in concentration dependent manner. The optical density decreased from 5.2 to 2.1 (about

60% decrease) when extract from 5 g/L seed was added. Although, the percentage sedimentation obtained in the present experiment was lower than that of other workers [35, 39] the extraction procedures used here and extraction time were different. The algal species were also not the same and the medium pH was not adjusted in the present experiment. The moisture content and particle size of the *Moringa* seed powder were not also the same with that of other workers.

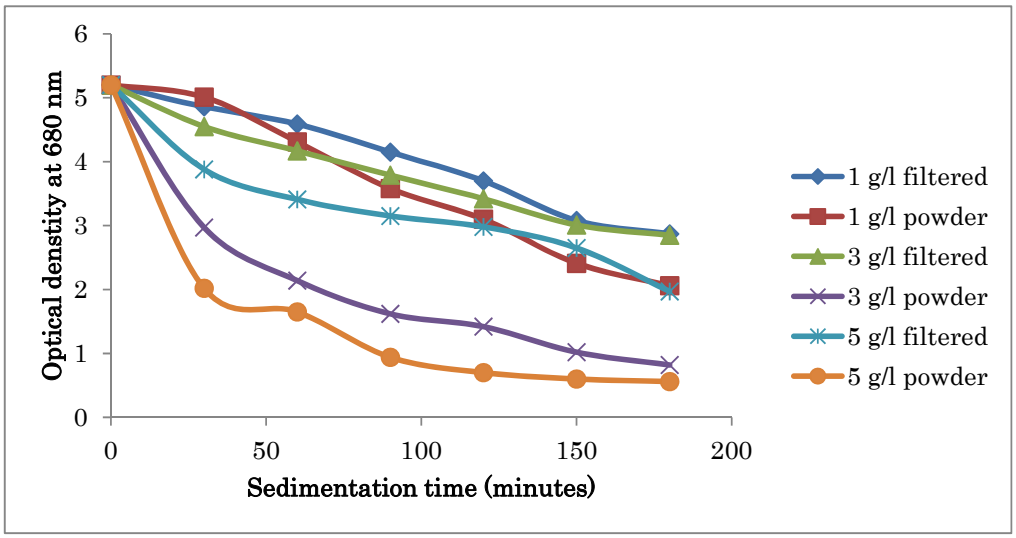

Figure 1. Effect of various concentrations of powdered and filtered *M. oleifera* seed extract on sedimentation of *Chlorella variabilis* cells. The various concentrations of the seed powder or filtrates were added to the culture in test tubes. They were properly mixed and allowed to stand. The optical density (absorbance) of the upper layer was measured at time intervals.

The effects of higher concentrations of the *M. oleifera* seed powder and extracts on cell sedimentation were investigated and the results are shown in Figure 2. The rates of sedimentation were also concentration dependent. However, increasing the *M. oleifera* seed powder concentration from 6 g/L to 10 g/L, did not result in any significant difference ($p > 0.05$) in the amount of sedimented cells after 90 minutes of incubation. More than 80% sedimentation was obtained in the cultures treated with *M. oleifera* seed powders higher than 6%. When filtrates of *M. oleifera* seed extracts were used, 37%, 54%, and 62% sedimentations were obtained for 6g/L, 8 g/L and 10 g/L, respectively. These were lower than the corresponding values obtained when *M. oleifera* seed powders were used. However, it is important to note that by adding extract from 10 g/L *M. oleifera* seed powder to *Chlorella variabilis* culture and prolonging the incubation time to 180 minutes, as high as 80% of the cells sedimented and thus efficiently harvested.

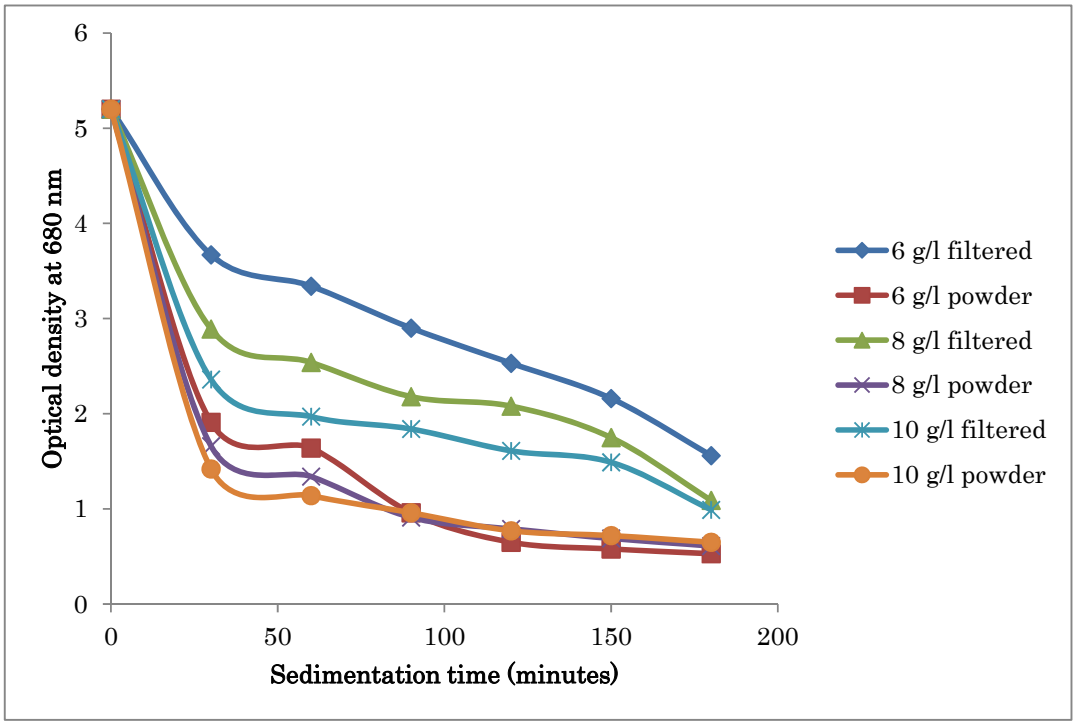

Figure 2. Effect of concentrations of powdered and filtered *M. oleifera* seed extract on sedimentation of *Chlorella variabilis* cells. The experimental procedure is as explained for Figure 1.

A comparison of the percentage sedimentation of *Chlorella variabilis* culture after 30 minutes treatment with *M. oleifera* seed powder and filtrate is shown in Figure 3. For the short incubation time of 30 minutes, about 74% of the cells can be harvested by addition of 10 g/L of *M. oleifera* seed powder. However, with 5 g/L, only about 60% of the cells sedimented after 30 minutes of incubation. In the case of extract, there was almost linear relationship between the filtrate concentration and percentage cell sedimentation after 30 minutes. It is worthy to note that addition of extract from 10 g/L resulted in 56% sedimentation. Although, the use of extract in place of powder resulted in a significant decrease in the sedimentation ($p>0.05$) for all the concentrations tested, the advantage of using the extract is that there is no need for separation of the seed debris from the cells after sedimentation. Although *M.oleifera* seed is edible and has been reported to have many therapeutic values, depending on the intended microagae cell usage, it may be very necessary to separate the seed debris because of the possible effects of *M. oleifera* seed powder on the taste, and activities of the harvested cells. On the other hand, the seed debris after the extraction can potentially be used as feed and food additives. In this study, extraction was done for only 30 minutes with cold water. The extraction yield can be increased by increasing the extraction time, as well as using other treatments such as hot water or other solvents. The use of organic solvents such as ethanol and ethyl acetate may result in a significant increase in the extraction yield. However, it will add to the cost of extraction and the solvents must be

evaporated before use, thus adding to the complexity and cost of the process.

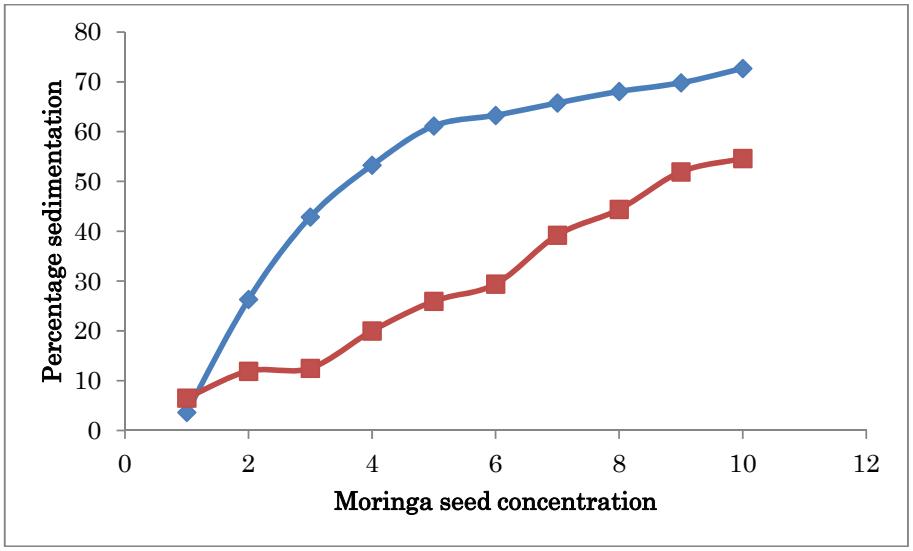

Figure 3. Comparison of the effects of *M. oleifera* seed powder and filtered seed extract on percentage sedimentation of *Chlorella variabilis* cells after 30 minutes of incubation. The percentage sedimentation was calculated as explained in subsection 2.6.

The above results have shown that the percentage sedimentation (amount of cells harvested) can be increased by increasing the concentration of the *M. oleifera* seed or prolonging the sedimentation time. The choice would depend on the type of microalga cell. Increasing the concentration of the *M. oleifera* seed will increase the harvesting cost and the economic feasibility of using very high concentration of the seed depends on the value of the microalgae. On the other hand, prolonging the sedimentation time reduces the culture time if artificial light is used or if the harvesting is done in the day time. However, for open door cultures utilizing solar light, the harvesting can be done at night. Nevertheless, the stress of sedimentation on the cells must be considered. This depends on the type of cells, and there is a need to evaluate the sensitivity of the target cells to long time sedimentation.

In the course of this study, it was found that the extracts were easily contaminated by molds during storage at room temperature. Thus, the effect of autoclaving the extract on the efficacy of sedimentation was investigated. The results showed that the compound responsible for the sedimentation is heat stable and addition of the autoclaved extract resulted in efficient sedimentation of *Chlorella variabilis* cells. As shown in Figure 4, with an initial optical density of 3.5, addition of autoclaved *M. oleifera* seed extract resulted in the sedimentation of the cells in concentration dependent manner. After 60 minutes of sedimentation, the optical densities of the cultures treated with autoclaved extracts from 1 g/L, 3 g/L and 5 g/L decreased to 2.5, 2.2, and 0.9, respectively. However, there was no significant difference in the optical density of the cultures treated with autoclaved extracts from 7 g/L and 10 g/L. In both cases, the optical density decreased to about 0.52.

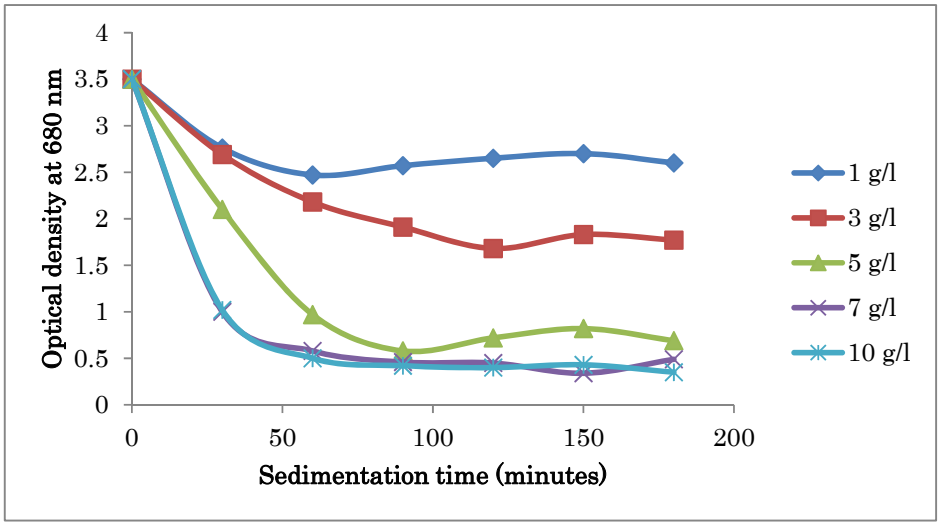

Figure 4. Effect of autoclaved *Moringa oleifera* seed filtered extract on sedimentation of *Chlorella variabilis* cells. The experimental procedure was as explained for Figure 1.

The dependence of the percentage sedimentation on the concentration of the seeds used for extraction is shown in Figure 5. The percentage sedimentation increased almost linearly with increase in the concentration of the seeds used for extraction up to 7 g/L. Although the initial cell concentration (OD = 3.5) was lower than the concentration used in Figure 1 (5.2), it is important to note that even with the autoclaved extracts, the sedimentation rates were very high. With extracts from 7 g/L, more than 70% of the cells in a culture with optical density of 3.5 sedimented in 30 minutes. This is very significant since it is not necessary to harvest all the cells during microalgae cultivation. The residual cells may serve as the seed for the next batch of culture. In fact, depending on the cells and the culture condition, it is recommended that only about 50% of the cells should be harvested at a time. When too much cells are harvested, the culture will experience another lag phase leading to poor light utilization efficiency.

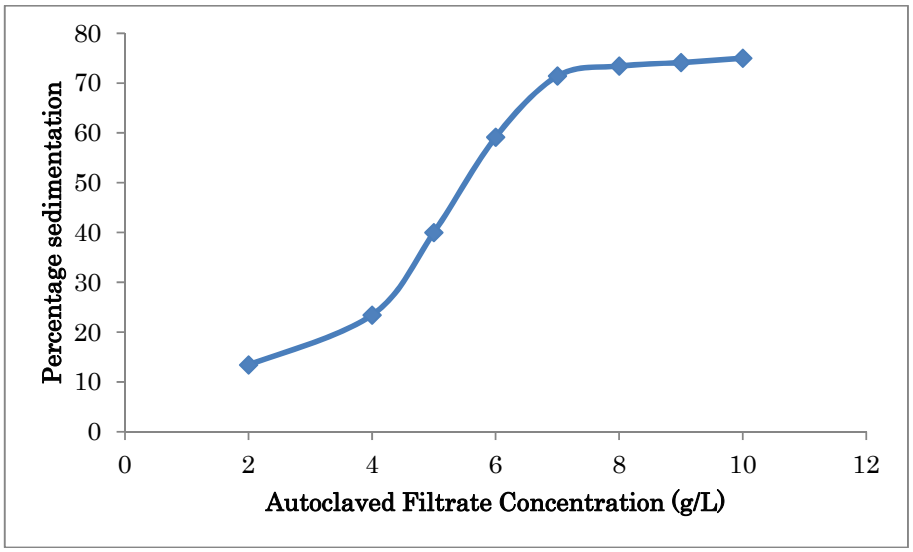

Figure 5. Effect of autoclaved filtered *M. oleifera seed* extract on percentage sedimentation of *Chorella variabilis* cells after 30 minutes of incubation. The percentage sedimentation was calculated as explained in subsection 2.6.

4. Conclusion

Morinag oleifera seed powder was very efficient in sedimentation of *Chlorella variabilis*, and thus can be used to harvest the cells from the culture broth. Replacing the seed powder with filtered cold water extract of the seed resulted in decrease in the sedimentation rate but high percentage sedimentation was still achieved by increasing the concentration and prolonging the treatment time. Further optimization of the extraction processes requires a better knowledge of the nature of the active ingredients. Okuda et al., [40] reported that the flocculation ingredients are proteins while Bichi [41] noted that they are polyelectrolites. However, the present study suggests that the flocculation-inducing compound in *M. oleifera* seed is apparently heat-stable since autoclaved filtrate of the seed extract was still very efficient in cell sedimentation.

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