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Application of Different Techniques to Harvest Microalgae

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ABSTRACT: The appropriate technologies for microalgae harvesting are vital to enhanced economic feasibility prior for biofuel production from microalgae biomass. Efficient harvesting technique is the biggest challenge that needs to be overcome for commercialization of microalgae based biofuel. Furthermore, the small size of microalgae, the same density of cells and growth medium, negative surface charge of microalgae as well as their growth rate are the factors that contribute to the needs of frequent harvesting compared to other plant. The aim of this study is to compare the efficiency of three harvesting methods, namely sedimentation, centrifugation, and magnetic separation. Three different locally isolated microalgae species from palm oil mill effluent (POME) that were used in this study are *Chlorella* sp. UKM2, *Coelastrella* sp. UKM4 and *Chlamydomonas* sp. UKM6. Based on the results, centrifugation showed the best performance with 98% harvesting efficiency for *Chlorella* sp. UKM2 using rotational speed of 7000 rpm for 5 minutes. The harvesting of *Chlamydomonas* sp. UKM6 by sedimentation using alum gives the lowest efficiency which is 76% after 8 hours. This is due to the selectivity of alum that perform more effective for freshwater microalgae such as *Chlorella* sp. UKM2 (8%) and *Coelastrella* sp. UKM4 (81%) compared to *Chlamydomonas* sp. UKM6. For magnetic separation using iron oxide nanoparticles (IONPs), *Chlorella* sp. UKM2 and *Coelastrella* sp. UKM4 showed 94% of harvesting efficiency, when provided with 500 mg/L of IONPs. This study indicates that centrifugation is the best method for microalgal biomass harvesting due to its high efficiency and most economical technique.

KEYWORDS: *Chlorella, Coelastrella, Chlamydomonas*, Microalgae, Harvesting

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INTRODUCTION

Most of valuable products have been produced from microalgae biomass. Nowadays, microalgae biomass is the front-runner for renewable energy sources. This third-generation feedstock is capable to produce numerous valuable products, such as biodiesel, bio-ethanol and bio-hydrogen. Furthermore, microalgae are superior compared to other plants due to their ability to produce higher biomass (Besson & Guirand, 2013 and Farooq *et al.*, 2015).

It is vital to have an economic, scalable, and environmental friendly technology to harvest microalgal biomass in order to achieve a feasible economy in commercializing microalgae. Based on previous studies, 20-30% of the microalgal biomass cost consists of the cost of harvesting (Grima *et al.*, 2003; Mata *et al.*, 2010 & Verma *et al.*, 2010). Therefore, choosing a suitable harvesting technique is crucial to make the process more economic. Two important elements, namely the characteristics of microalgae including cell size, shape, and density as well as their growth condition (Show and Lee, 2014). There are five techniques that are commonly used in harvesting, namely sedimentation, centrifugation, flocculation, flotation, and filtration (Besson & Guiraud, 2013; Hu *et al.*, 2013; Prochazkova *et al.*, 2013; Salim *et al.*, 2013; Wang *et al.*, 2015). The aim of this study is to investigate the performance of three different techniques to harvest microalgal biomass, namely, sedimentation, centrifugation and separation using magnetic particles.

METHODOLOGY

Cultivation of microalgae

In this study, Bold Basal Media (BBM) was used as the cultivation media for three species of locally isolated microalga; *Chlorella* sp. UKM2, *Coelastrella* sp. UKM4 and *Chlamydomonas* sp. UKM6. Table 1 shows the ingredients for BBM in 1 L solution.

Table 1. Ingredients for BBM stock solution

Ingredients	per 1 L	Ingredients	per 1 L
$NaNO_3$	25.0 g	Trace elements solution	
$MgSO4.7H_2O$	7.5 g	$ZnSO_4.7H_2O$	8.82 g
NaCl	2.5 g	$MnCl_2.4H_2O$	1.44 g
K_2HPO_4	7.5 g	MoO_3	0.71 g
KH_2PO_4	17.5 g	$CuSO_4.5H_2O$	1.57 g
NaCl	2.5 g	$Co(NO_3)_2.6H_2O$	0.49 g
		H_3BO_3	11.42 g
		EDTA	50.0 g
		KOH	31.0 g
		$FeSO_4.7H_2O$	4.98 g
		H_2SO_4	1.0 mL

All ingredients were added to make 1 L of stock solution. BBM was sterilized prior for microalgae cultivation in 1 L conical flasks with 30% (v/v) of inoculum size as well as an adequate supply of light and air.

Determination of microalgae biomass

The suitable harvesting time and maximum growth rate, μmax by each microalga species was determined based on their growth profile. The harvesting process will be conducted when the growth of microalgae reached the end of exponential phase, where the highest biomass was produced. The growth rate for each species are different, hence the growth profiles were plotted based on the reading of optical density, OD. Two mL of samples for each species was taken for OD measurement at the wavelength of 650 nm (OD650). Meanwhile, microalgae biomass produced was determined based on the total solid (TS) method by Sluiter *et al.* (2008).

Microalgae harvesting technique

1. Sedimentation

The harvesting efficiency was conducted based on the performances of sedimentation with and without flocculant. For sedimentation without flocculant, 500 mL of cultured microalgae was transferred into the separation funnel as shown in Figure 1. For sedimentation with flocculant, the same amount of cultured microalgae were transferred into a beaker with 5% (v/v) of 1 M alum ($Al_2(SO_4)_3$). Then, the mixture was stirred using magnetic stirrer for 1 min before it was transferred into the separation funnel for 8 hours. OD_{650} were taken at the bottom of sediment every hour to measure the harvesting efficiency.

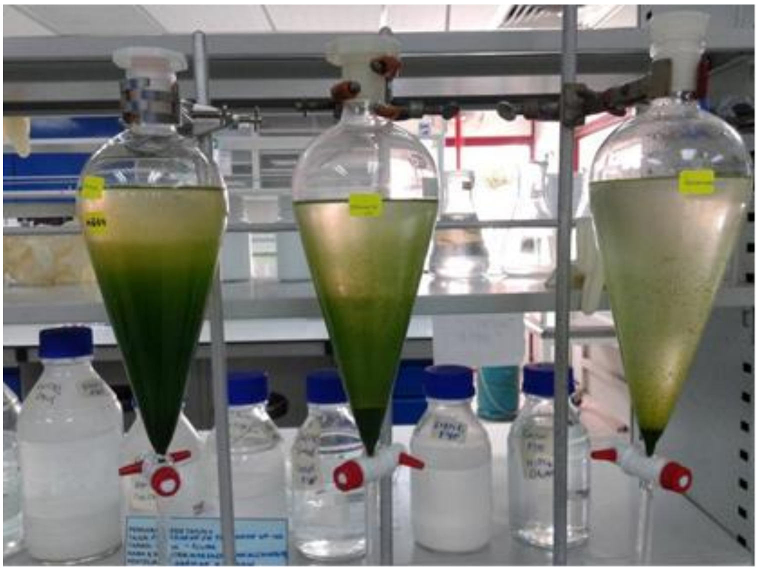

Figure 1. Sedimentation process with and without alum.

2. Centrifugation

Table-top centrifuge was used in this study with 4 different rotational speed; 1000 rpm, 3000 rpm, 5000 rpm and 7000 rpm for 5 min each. Thirty five mL of sample was withdrawn and transferred into 50 mL centrifuge tube. OD_{650} was taken before and after centrifugation to measure the harvesting efficiency.

3. Magnetic separation using nanoparticles

Iron oxide nanoparticles (IONPs) were used to separate the microalgal biomass from the cultivation media with the aid of neodymium magnet (NdFeb) as shown in Figure 2. The effect of IONPs was studied using 5 different concentrations; 25 mg/L, 50 mg/L, 150 mg/L, 300 mg/L and 500 mg/L.

(a) (b)

Figure 2. (a) Iron oxide nanoparticles (IONPs); (b) neodymium magnet (NdFeb).

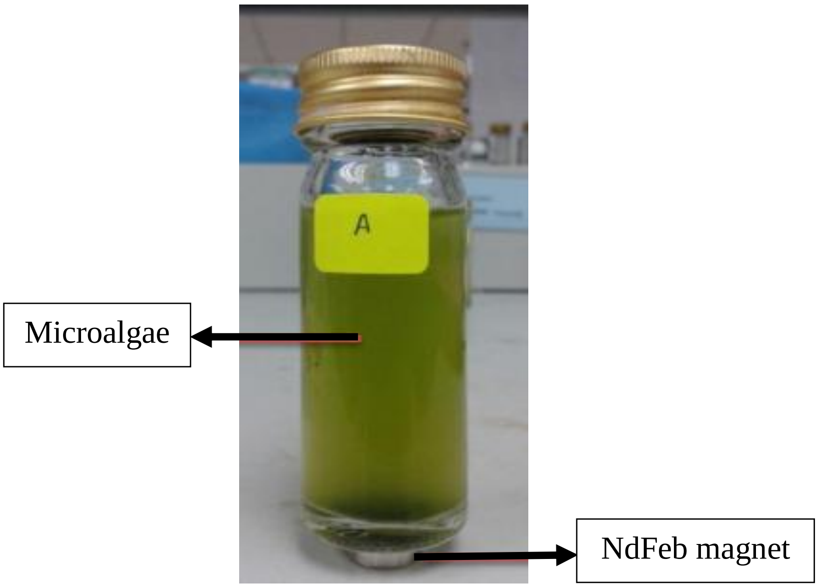

Figure 3. Magnetic separation by IONPs.

Harvesting efficiency calculation

The measurement of harvesting efficiency for all techniques was determined using the equation below:

$$\text{Harvesting efficiency (\%)} = \frac{(D1 - D2)}{D1} \times 100$$

Where D1 and D2 are the readings of optical density at 650 nm before and after harvesting, respectively.

Economical method analysis

The economical method analysis was done based on the study by Japar *et al.* (2017). Table 2 shows the criteria involved in choosing the most economical harvesting method.

Table 2. Criteria for economical harvesting method.

Criteria	Score	Description
Harvesting efficiency	1 – 2	Less than 50%
	3 – 4	50% – 90%
	5	More than 90%
Operational cost	1 – 2	Require high energy consumption
	3 – 4	Require minimum energy consumption
	5	No energy consumption
Logistic cost	1 – 2	High secondary costs such as costs of recovery and maintenance
	3 – 4	Low secondary costs such as costs of recovery and maintenance
	5	No secondary costs involved

Impact on the environment	1 – 2	Usage of chemicals with high toxicity and gives negative impact to the environment
	3 – 4	Usage of chemicals with no toxicity or pollutant
	5	No usage of chemicals
Harvesting time	1 – 2	More than 5 hours
	3 – 4	1 hour – 5 hours
	5	Less than 1 hour

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RESULT AND DISCUSSION

Growth profile and biomass production

The harvesting efficiency is at maximum when the biomass produced was at its highest (Hu *et al.*, 2013). Hence, the growth profile was used to determine the suitable harvesting time.

The growth of each microalgae were measured for 10 days as shown in Figure 4. There are few factors that affecting the production of biomass and microalgae growth rate such as microalgae species, light sources, cultivation media, air and nutrient (Blair *et al.*, 2014). Based on Figure 5(a), *Chlorella* sp. UKM2 produced the highest biomass of 1.46 g/L after 9 days of cultivation. For *Coelastrella* sp. UKM4 and *Chlamydomonas* sp. UKM6, the highest biomass produced was after 8 and 7 days of cultivation, respectively with 1.27 g/L. Hence, from the growth profile, the suitable harvesting time for *Chlorella* sp. UKM2, *Coelastrella* sp. UKM4 and *Chlamydomonas* sp. UKM6 are in 9th, 8th and 7th day, respectively.

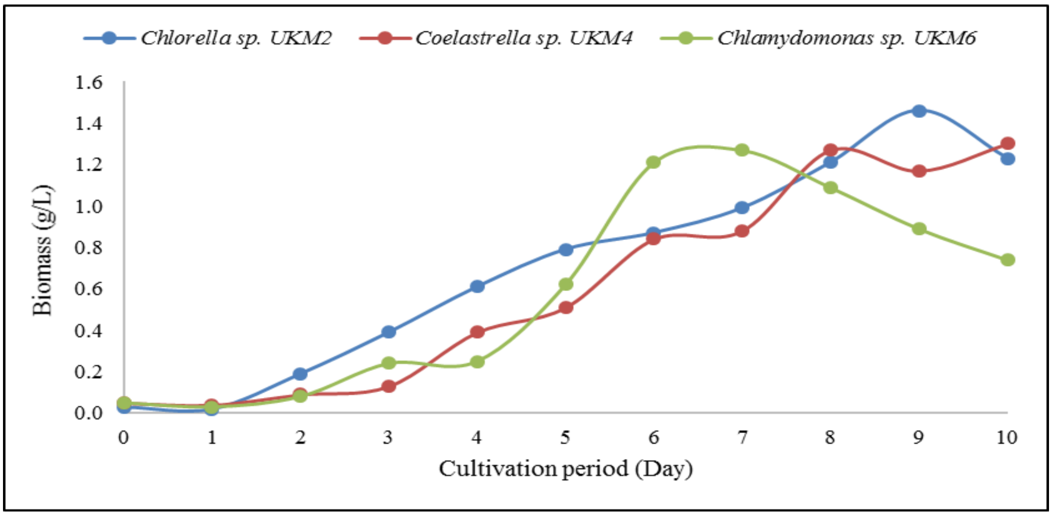


Figure 4. Growth profile of three locally isolated microalgae

Harvesting efficiency

1. Sedimentation

In sedimentation, solid and liquid are separated by gravitational force (Salim *et al.*, 2013). There are few factors that affecting the rate of deposition and biomass harvesting efficiency such as density and size of microalgae, temperature, light intensity, and time (Mariam *et al.* 2015). According to Grima *et al.* (2003), sedimentation with flocculant is the most effective method due to its ability to achieve >80% harvesting efficiency. This study compared the harvesting performances of sedimentation with and without flocculant.

Based on Figure 5, sedimentation without flocculant was able to harvest 27%, 25% and 21% of *Chlorella* sp. UKM2, *Coelastrella* sp. UKM4 and *Chlamydomonas* sp. UKM6, respectively. The harvesting efficiency for each microalga were increased more than 3-fold for *Chlorella* sp. UKM2 (88%), *Coelastrella* sp. UKM4 (81%) and *Chlamydomonas* sp. UKM6 (76%) with addition of alum. All species shows different harvesting efficiencies considering they have different characteristics, such as, size, shape, motility, and biomass production rate. All these factors could affect the efficiency and reaction in flocculation process (Lal & Das, 2016).

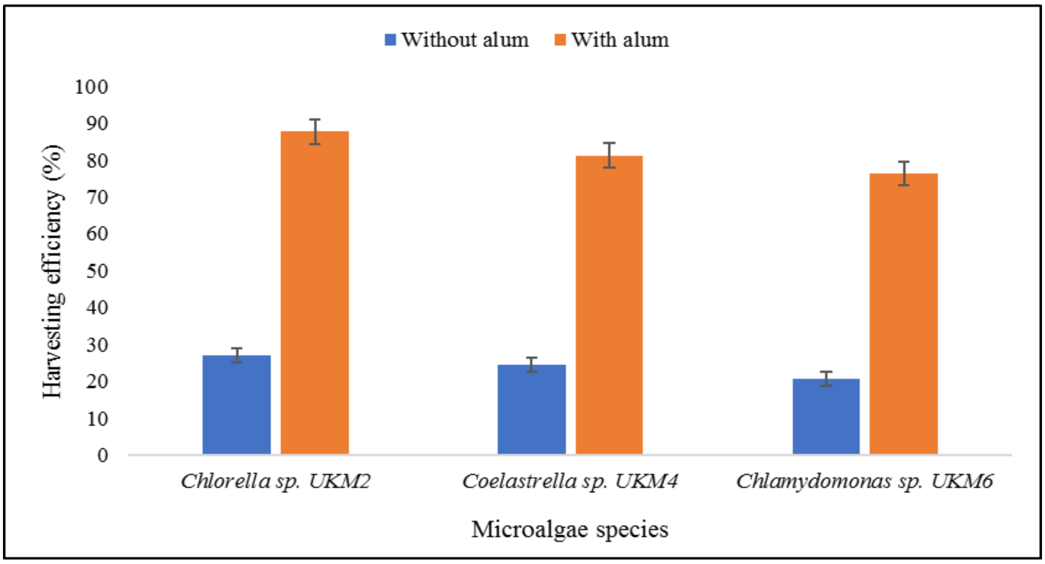


Figure 5. Harvesting efficiency of sedimentation.

Furthermore, the increasing harvesting efficiencies with addition of flocculant were due to the increasing density of microalgae as a result of the attachment of flocculant (positive charge) to the surface of the cell (negative charge). According to Sharma *et al.* (2013), alum is the best flocculant for freshwater microalgae especially *Chlorella* and *Coelastrella* due to the stability of their cell surface. Table 3 shows the comparison of harvesting performance using sedimentation technique.

Table 3. Comparison of harvesting efficiency using sedimentation.

Species	Sedimentation time (hr)	Harvesting efficiency (%)	Reference
***Chlorella* sp.**	5	90	Sharma *et al.* (2013)
***Chlamydomonas* sp.**	-	80	Delrue at al. (2015)
***Chlorella* sp. UKM2**	8	88	This study
***Coelastrella* sp. UKM4**		81	
***Chlamydomonas* sp. UKM6**		76	

2. Centrifugation

The efficiency of microalgal biomass harvesting by centrifugation is depends on the rotational speed used (Mariam *et al.*, 2015) and can be achieved up to >95% (Uduman *et al.*, 2010).

Based on Figure 6, the highest harvesting efficiency of 98%, 96% and 90% can be achieved using 7000 rpm for *Chlorella* sp. UKM2, *Coelastrella* sp. UKM4 and *Chlamydomonas* sp. UKM6, respectively. Lower efficiencies are achieved when 3000 rpm and 5000 rpm are being used to harvest *Chlamydomonas* sp. UKM6.

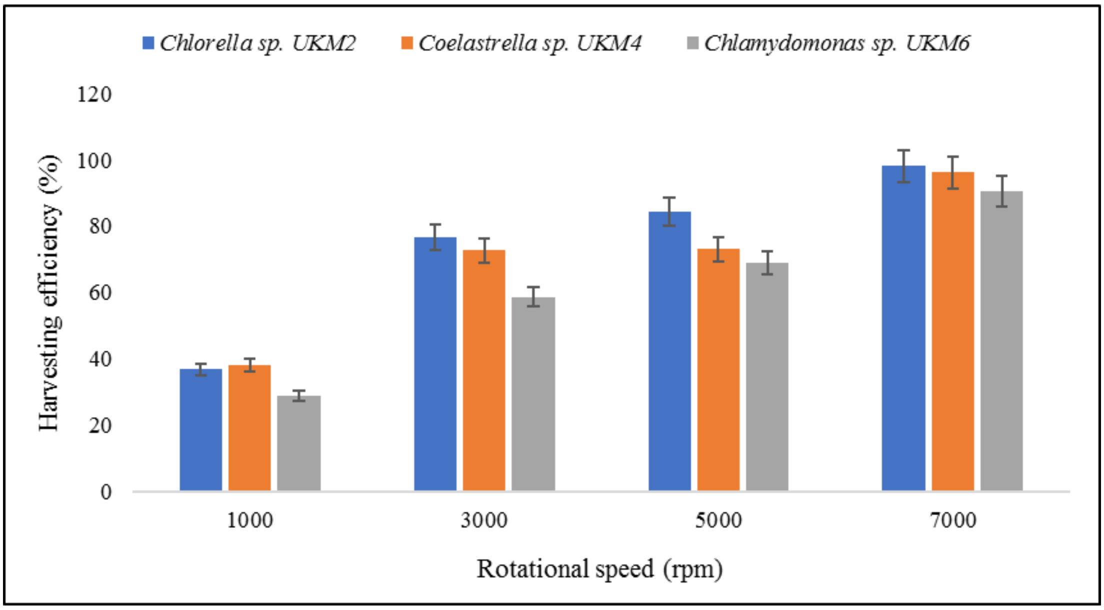


Figure 6. Harvesting performance of three microalgae species by centrifugation.

Wu (2010) reported that the difference in density and size of microalgae cells are also affecting the performance of harvesting process. Cells with larger size are harder to harvest and led to higher rotational speed and energy requirement, altogether. Table 4 shows the different sizes of the three microalgae species where *Chlamydomonas* sp. have the largest cell compared to *Chlorella* sp. and *Coelastrella* sp.

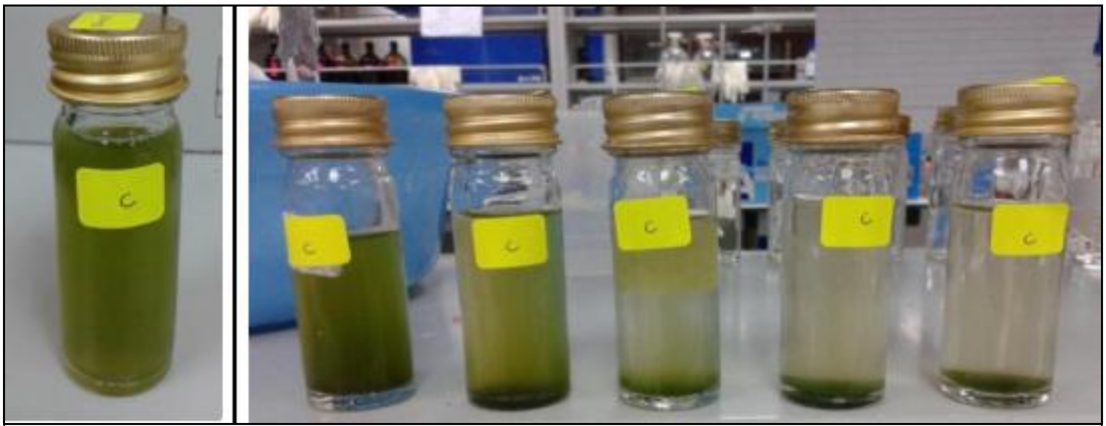
Table 4. Microalgae size (MCC, 2016)

Species	Size (μm)
***Chlorella* sp.**	2 – 10
***Coelastrella* sp.**	5 – 10
***Chlamydomonas* sp.**	10 – 30

3. Magnetic separation using nanoparticles

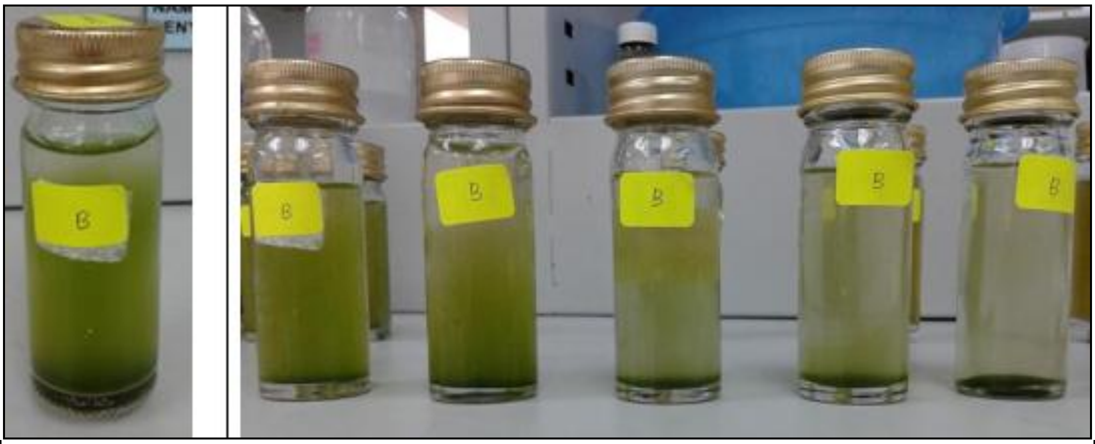
Prochazkova *et al.* (2013) reported that particle surface modification, magnetic particles concentration and pH of the surroundings also play very important roles in microalgal biomass harvesting. Hence, this study compared the effect of different concentration of IONPs in harvesting process as shown in Figure 7. The higher IONPs concentration used resulted in the higher harvesting efficiency can be achieved.

According to Wang *et al.* (2015), the harvesting efficiency will be increasing as the dosage increased until its reached the maximum separation efficiency. The harvesting efficiency will remain the same although the dosage is increased (Hu *et al.* 2013). At a lower dosage of IONPs, the magnetic particles are unable to adsorb the microalgae cells or vice versa and led to lower harvesting efficiency.



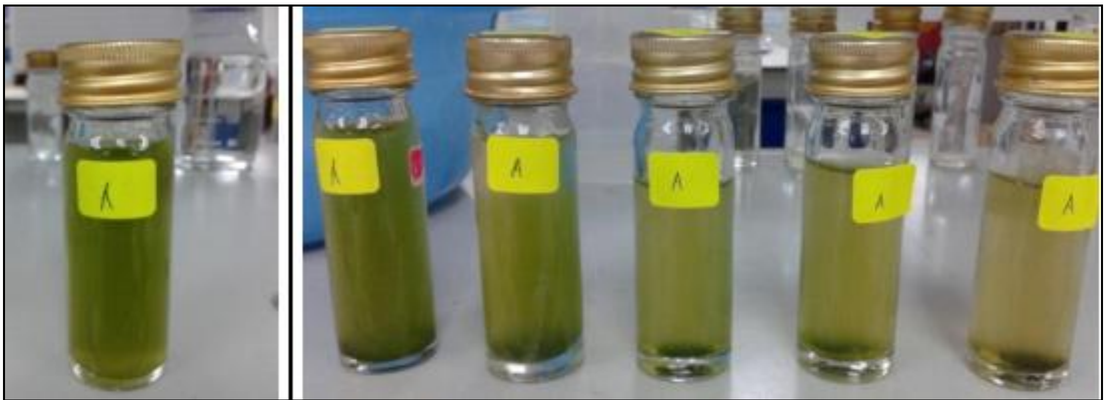
Control 25 mg/L 50 mg/L 150 mg/L 300 mg/L 500 mg/L

(a) Magnetic separation of *Chlorella* sp. UKM2



Control 25 mg/L 50 mg/L 150 mg/L 300 mg/L 500 mg/L

(b) Magnetic separation of *Coelastrella* sp. UKM4



Control 25 mg/L 50 mg/L 150 mg/L 300 mg/L 500 mg/L

(c) Magnetic separation of *Chlamydomonas* sp. UKM6

Figure 7. Magnetic separation using IONPs.

The comparison of harvesting performance using magnetic particles is shown in Figure 8, where *Chlorella* sp. UKM2 and *Coelastrella* sp. UKM4 are successfully harvested using 500 mg/L IONPs with 94% efficiency. For *Chlamydomonas* sp. UKM6, 82% of biomass is harvested using 150 mg/L IONPs, where the interaction between magnetic particles and microalgae cell are at the optimum condition. The harvesting efficiencies are the same when using 3000 mg/L and 5000 mg/L IONPs.

Based on the study reported by Hu *et al.* (2013), microalgae with small cells have higher surface area, hence more magnetic particles are needed to achieve the maximum separation efficiency.

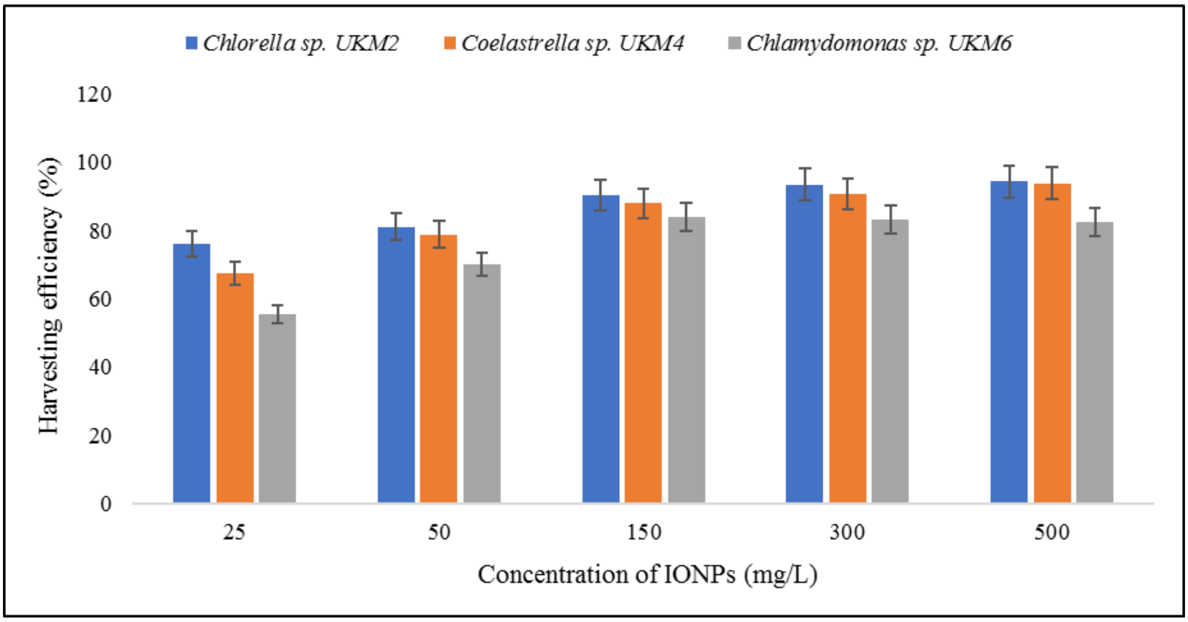


Figure 8. Comparison of harvesting performance using different concentration of IONPs.

4. Economical method analysis

Commercialization of microalgal biomass for production of valuable products, such as biofuel, bioethanol, nutrient supplement, and fertilizer, require a harvesting technology that are economically feasible and efficient. Based on the criteria in Table 2, the comparison of harvesting techniques that were used in this study is shown in Table 5. Harvesting using centrifugation gives the highest score of 21/25, followed by magnetic separation (20/25), sedimentation with flocculant (18/25) and sedimentation without flocculant (17/25). Mariam *et al.* (2015) also reported that centrifugation is one of the most economical and efficient technique. Although this technique requires high energy, the time needed to harvest microalgal biomass is shorter compared to other techniques and there is no chemical involved that can give negative impact to the environment.

Table 5. Comparison of harvesting techniques.

Criteria	Sedimentation without flocculant	Sedimentation with flocculant	Centrifugation	Magnetic separation
Harvesting efficiency (5)	1	4	5	5
Operational cost (5)	5	4	2	3
Logistic cost (5)	5	4	4	3
Impact on the environment (5)	5	2	5	5
Harvesting time (5)	1	4	5	4
Total (25)	**17**	**18**	**21**	**20**

CONCLUSION

There are few factors that affecting the efficiency of harvesting microalgal biomass including the morphology of microalgae and their growth condition. Three species of locally isolated microalgae were used to compare different technique of harvesting, namely sedimentation with and without flocculation, centrifugation, as well as magnetic separation using nanoparticles. Sedimentation without any addition of flocculation gives the lowest harvesting efficiency with 27%, 25% and 21% of *Chlorella* sp. UKM2, *Coelastrella* sp. UKM4 and *Chlamydomonas* sp. UKM6, respectively. On a side note, centrifugation proved to be the most efficient technique with 98%, 96% and 90% of *Chlorella* sp. UKM2, *Coelastrella* sp. UKM4 and *Chlamydomonas* sp. UKM6, respectively using 7000 rpm for 5 minutes.

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