

Full Length Research Paper

Growth of *Scenedesmus dimorphus* in different algal media and pH profile due to secreted metabolites

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In this study investigation was made to evaluate the effects of different algal media components to get optimized cell count of *Scenedesmus dimorphus*. Five different fresh water algal media such as Bold's Basal Medium (BBM), M4N medium, BG-11 medium, N-8 medium and M-8 medium were used for culturing *S. dimorphus* in flask culture. A set of environmental factors including light, temperature, air flow rate and nutritional components was standardized to obtain the highest productivity of 0.1406 g/L with specific growth rate of 0.10483/day. This study designates the bold basal medium as advantageous one for *S. dimorphus* and also reveals that production of metabolites by the same algal strain depends mostly on the nature of constituents of media and might have different influence on the pH.

Key words: *Scenedesmus dimorphus*, bold basal medium, algal growth.

INTRODUCTION

To meet the existing energy and environmental issues, renewable energy has been designated as a sustainable solution (Amin, 2009; Hallenbeck and Benemann, 2002). It has been documented that more than 80% of the energetic resources has been utilized to achieve the existing status of advancement on this planet (Huesemann, 2006). Algae as a source of oil/fuel has been documented as preferred on oil producing terrestrial

crops. In addition to oil/fuel, algae are also well known for producing polyunsaturated fatty acids, which have been used as feed for fish and other animals (Harlioğlu, 2012; Narejo and Rahmatullah, 2010; Spolaore et al., 2006). Microalgae as a source of biofuel/renewable energy have been documented for significant environmental and commercial importance. Microalgae are not only sources of fuel, food for humans and animals, but are also the

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sources of a wide range of chemical compounds used in industry, food technology, and pharmaceuticals as well (Bruton et al., 2009). Photosynthetic microalgae are potential candidates for utilizing excessive amount of CO₂, since these organisms are capable of fixing CO₂ to produce energy and chemical compound upon exposure to sunlight (Neenan et al., 1986). Microalgae have high growth rates and tolerance for varying environmental conditions. Microalgae can be grown in arid and in semi-arid regions with poor soil quality where woody or herbaceous crops cannot be grown. Saline water from aquifers or the ocean can be used for growing microalgae. Such water has few competing uses and cannot be used for agriculture, forestry, or as potable water. The yield of biomass per hectare from microalgae is three to fivefold greater than the yield from typical crop plants (Bischoff and Bold, 1963).

Microalgae cultivation is gaining importance for its application in fuel and feed sectors in the world. A range of documented algal media can be used to cultivate specific algal strain for maximum growth and strategies can be designed to obtain the targeted products optimally. A number of algal media to culture freshwater algae have been reported, but the productivity is different from strain to strain. This study focuses on using five different media to culture fresh water algae *Scenedesmus dimorphus*. These media include Bold's Basal Medium (BBM), M4N medium, BG-11 medium, N-8 medium and M-8 medium (Eliasson et al., 1999; <http://web.biosci.utexas.edu/utex/mediaDetail.aspx?mediaID=26>). The source of carbon in all is carbon dioxide for photoautotrophic growth except BG-11 medium. The aim of this study is to determine the best medium for *S. dimorphus* in the batch photo bioreactors. Here, the study was designed to investigate the most suitable media under optimized influencing factors for the significant growth of *S. dimorphus* at lab scale. At the same time a critical investigation was made on nature of metabolites production from same strain but in different media with dissimilar nutrients or concentration of nutrients to influence the pH, a newly designed flocculant was also used to harvest the biomass for maximum biomass recovery.

MATERIALS AND METHODS

Growth media

All the chemicals used were of analytical grade unless pointed out clearly. Bold's Basal Medium (BBM) was prepared using distilled water, and the pH was adjusted (6.7 ± 0.3) with 5 N sodium hydroxide and 5 N hydrochloric acid (Bischoff and Bold 1963; Rowley, 2010). M4N medium and BG-11 Medium were prepared according to reported media recipes (Mandalam and Palsson, 1998) and N-8 and M-8 Media were prepared according to reported media recipes (Guillard and Rytner, 1962).

Algae strain

S. dimorphus is in the Chlorophyta family and was provided by Algaetech International Sdn Bhd Malaysia on agar Petri plates. Algae are cultivated first by transferring to test tube in BBM media. The test tubes are placed in a well-lit window with temperature 28 to 30°C until the medium turns green, signaling adequate algae growth. They were transferred to the batch PBR (Duran bottle 2 L size), where a larger volume (900 ml) of medium was used for higher biomass.

Growth of algal strains in batch system

The batch system, shown in Figure 1 was used for studying culture performance. In this system, 2 L Duran bottles were used as batch reactors and sealed with cap stoppers with two stainless tubes through which air was fed, exhausted and screwed by plastic cover. Air flow through polyvinylchloride pipes connected with 0.2 µm membrane filters and connected between stainless steel tubes and air pumps. The air feed tube was kept immersed inside the growth container and angled at the bottom of the container to allow mixing, to prevent sedimentation of the algae, to ensure that all cells of the population are equally exposed to the light and nutrients, and to improve gas exchange between the culture medium and the air. Two cool white fluorescent lamps were employed as the light source of growth with an average light intensity of 1.3 ± 0.05 KLux. The volumetric flow rate of air was 1.5 L/min. Bioreactor temperature was monitored at ambient laboratory conditions as 30 ± 2°C.

Effect of different media on *S. dimorphus* growth rate

Cultures were subjected to five different media of different chemical compositions, BBM medium, M4N medium, GB-11 medium, N-8 medium and M-8 medium. All the media were in broth form, growth was monitored through optical density (OD) and cell count using microscope. Simultaneously, five batch photobioreactor (Scott Duran bottles) having 2 L capacity containing 900 mL of each medium and 100 mL *S. dimorphus* were subjected to evaluate the effect of different media on *S. dimorphus*. All media in the duran bottles were pre sterilized in autoclave at 121°C for 20 min before inoculation. The cultures were incubated at room temperature 30 ± 2°C under continuous light illuminated with cool fluorescent lamp (the light intensity was 1.3 ± 0.05 KLux). High quality chemical constituents were used for preparation of media with maximum accuracy (0.0001 g) in weighing by using Electronic balance, Precise, XT220A. Observations of algae growth was carried out daily in all media. The initial cell concentration was 5.3 x 10⁶ cell/mL. To avoid settling, and for accelerating the growth process, air supply with constant volumetric flow rate 1.5 L/min (1.5 vvm) was used. Growth was followed through optical density during 15 days which was recorded by using spectrophotometer at wavelength of 730 nm.

Specific growth rate of *S. dimorphus*

Specific growth rate is a measure of number of generations (the number of doublings) that occur per unit of time in an exponentially growing culture. The exponential (straight line) phase of growth was carefully determined and specific growth rate was obtained using Equation (1) (Chisti, 2007).

$$\mu = \ln(N_t/N_0)/t_t - t_0 \quad (1)$$

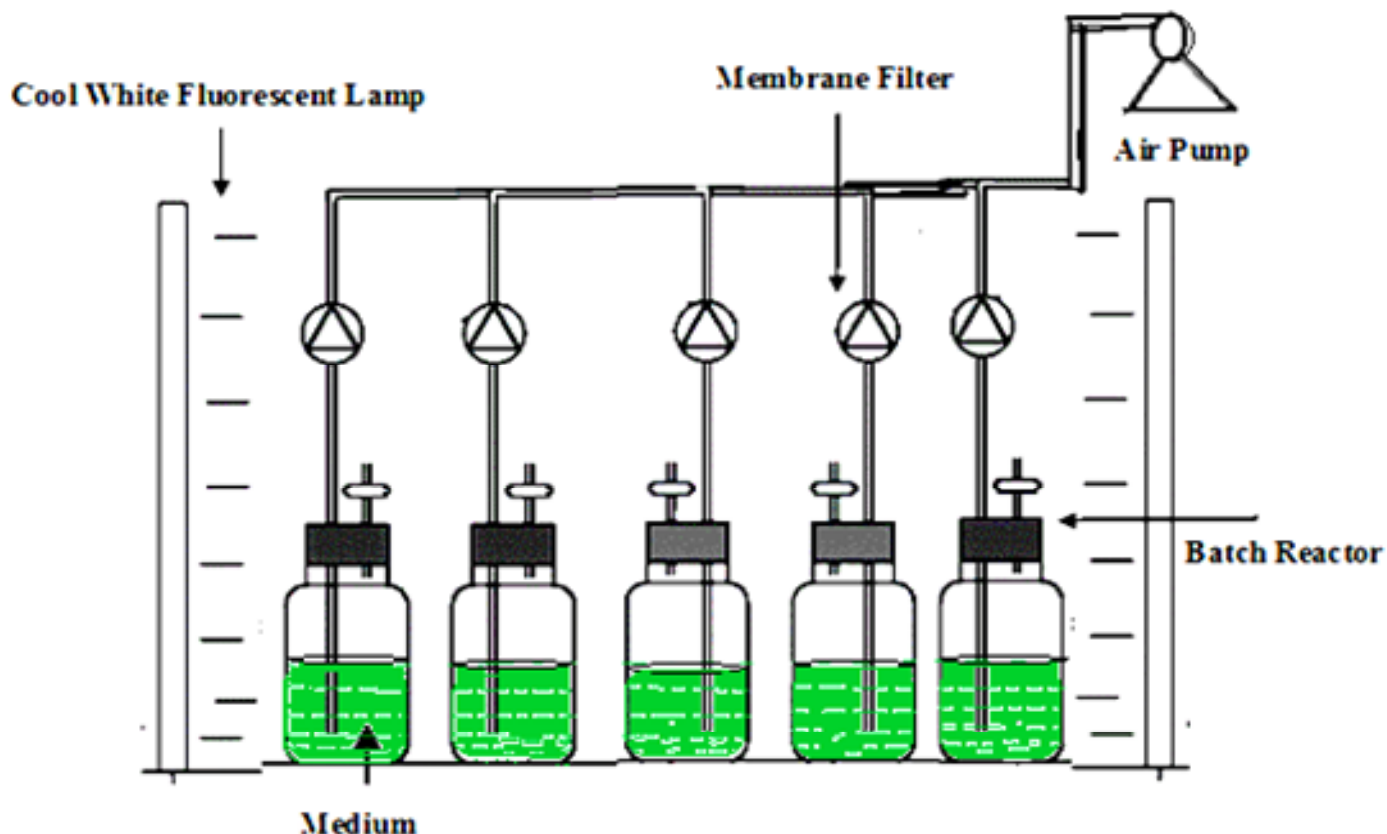


Figure 1. Schematic diagram of batch photobioreactor system.

N_0 is number of cells at the start of log phase, N_t is number of cells at the end of log phase. T_0 is Starting day of log phase, T_t is Final day of log phase.

CO₂ fixation (g/L.d)

Biomass and carbon production can be calculated based on fact that the microalgae contain 53.55% carbon (Reddy, 2002), some reports said micro algal biomass contains approximately 50% carbon by dry weight which presents that 1 kg of dry algal biomass utilize about 1.83 kg of CO₂ (Dragone et al., 2010; Reddy, 2002). The percentage of carbon fixed based on input can be calculated by using Equation (2).

$$C\% = \frac{\left(\frac{C\text{ (g/L.d)}}{C\text{ (g/L.d)}}\right)}{\left(\frac{C\text{ (g/L.d)}}{C\text{ (g/L.d)}}\right)} \times 100 \quad (2)$$

Biomass recovery using a newly designed flocculant

A flocculant was prepared using palm oil industry waste (waste activated bleaching earth). The flocculant was in possession of aluminium and silica contents (unpublished data UKM, Biotechnology Lab). The composition of flocculant was two separate solutions of 350 ppm aluminium in 1N HCl and 400 ppm silicon in 1N NaOH. Different concentration of both parts of

flocculant 1 to 5% (w/v) (with a unit difference of 1) were added together at room temperature (25 to 28°C) to each 500 ml beaker containing 400 ml of algal culture making both solutions finally neutralized, then biomass removal was monitored at 2, 4 and 6 h intervals.

In order to characterize harvesting of algal cells (as a result of coagulation/flocculation) by a new flocculant prepared in our laboratory as a solution form, the batch experiments were done using a six Scott duran bottles 250 mL filled with 200 mL of *S. dimorphus* collected after stationary phase. The duran bottles allow comparison of five different doses of flocculant to determine the required dose for adequate removal of suspended microalgae and the sixth bottle was used as a control. Doses used in these experiments to achieve removal of freshwater algae were 1, 2, 3, 4 and 5% (v/v), and the last one (control) without any flocculant.

In this process, it is essential that the flocculant was added by slow mixing to allow good contact between the small flocs and to agglomerate them into larger particles. The pH was measured before and during the flocculation period, also cell concentration and optical density at 730 nm was recorded. The biomass removal percentage was calculated during the flocculation by using Equation (3).

$$\% \text{ Removal} = \frac{\text{Initial cell number} - \text{Final cell number}}{\text{Initial cell number}} \times 100 \quad (3)$$

So, Equation (3) could be compacted to this formula.

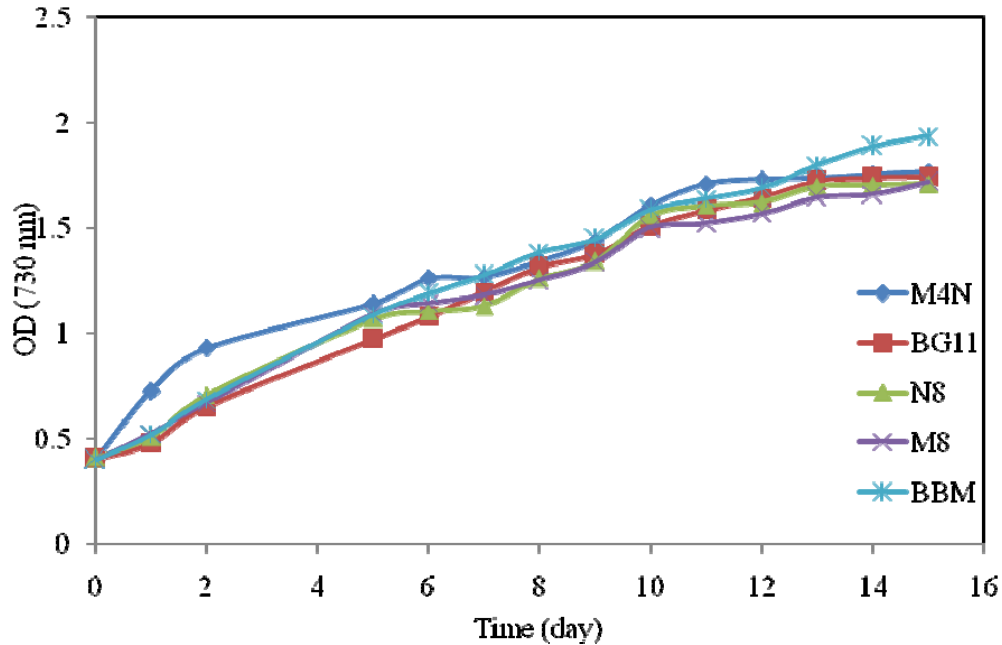


Figure 2. Growth of *S. dimorphus* in different media.

$$\% \text{ Removal} = \frac{\text{Cellremoval}}{\text{Initialcell number}} * 100 \quad (4)$$

Cell removal efficiency percentage (%RE) was calculated using the following Equation (5) (Fogg, 1966).

$$\% \text{ RE} = \left[1 - \frac{\text{Final fluorescence}}{\text{Final fluorescence of control}} \right] * 100 \quad (5)$$

Where; Final fluorescence is optical density after flocculation process at limited time. Final fluorescence of control is optical density for the control sample at same time.

RESULTS AND DISCUSSION

Effect of different media on growth rate

Effect of different nutrient composition on growth pattern forms the objective of present work that aimed at selecting the best medium for *S. dimorphus*. Five runs with various culture media were carried out to select a suitable medium for cell growth of *S. dimorphus*. It can be seen in Figure 2 that *S. dimorphus* was growing faster (in 15 days) in Bold's Basal Medium (BBM) medium as compared to the other media. M4N and BG-11 came after BBM, while M-8 and N-8 came at the final as arranged. The growth curve did not show lag phase and it demonstrates that there was a quick adaptation of *S. dimorphus* to all media. pH was not controlled and the changes were monitored during the growth period as

shown in Figure 3. Nature of metabolites secreted by the algae was not examined but their relation with pH was observed and evaluated. It was observed that M4N is the only medium which did not allow any abrupt change in pH and slowly increased the pH after 7th day of cultivation. In other words, algae in M4N medium were not secreting alkaline or acidic metabolites to affect the pH like algae in other media. *S. dimorphus* secreted alkaline metabolites in all other media and increased the pH during first five days and then got stable up to 15 days. This behaviour shows that the same strain can be influenced differently by different media constituent to produce metabolites of different nature.

The absorbance of the sample was measured using a spectrophotometer and correlated to calculate the dry weight or the number of cells per unit volume. The empirical equation for the calibration curve showed a linear relationship between optical densities and dry cell weight (g/L), represented by Equation (6). Also, a linear relationship between optical densities and biomass concentrations (cell*E+6/L) is represented by Equation (7).

$$y = 1.0885x \quad (6)$$

$$y = 13.055x \quad (7)$$

The biomass productivity, specific growth rate, optical density, cell concentration and CO₂ fixation were

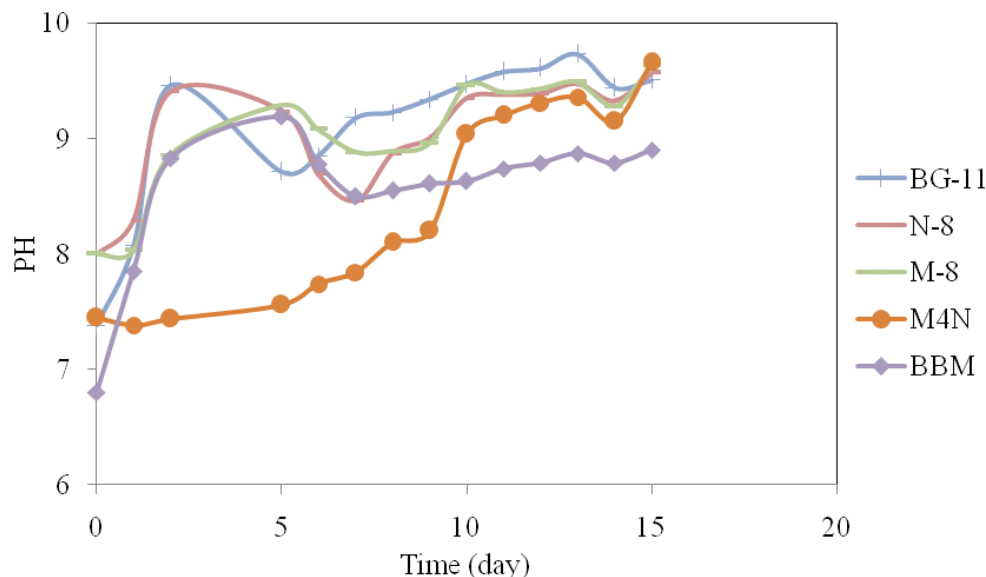


Figure 3. pH profile, while growing *S. dimorphus* in different media.

Table 1. Results of growth rate in culturing media.

Medium	BBM	M4N	BG11	N8	M8
Specific growth rate (μ)/day	0.10483	0.09781	0.09693	0.09492	0.09687
Final optical density (OD)	1.937	1.765	1.742	1.711	1.719
Final cell number (cell*E+6/L)	25.288	23.042	22.742	22.337	22.442
Biomass productivity (g/L.d)	0.1406	0.12808	0.1264	0.1242	0.1247
CO ₂ fixation (g/L.d)	0.2573	0.2344	0.2313	0.2273	0.2282

estimated for all media as shown in Table 1, based on the equations (1), (6) and (7). The tabulated values show the good comparison between all checked media which indicate that a bit better growth rate has been seen in the BBM medium.

The best result for growth of *S. dimorphus* was obtained when BBM was used (Table 1). The biomass productivity was 0.1406 g/L.d. Mass of C per day entering the system was 3.4339 g/day. Microalgae contain 50% carbon, so the amount of carbon production per day in the system was 0.0703 g/day. Thus by using equation (2), the percentage of carbon fixed based on input is:

$$C\% = \frac{(0.0703 \frac{\text{g C product}}{\text{day}})}{(3.4339 \frac{\text{g C input}}{\text{day}})} \times 100 = 2.047\%$$

The theoretical carbon percentage found is less than 5 to 35% of the carbon fixed by marine phytoplankton and immediately lost from the cells as excreted organic matter (Yun et al., 1997). It may be due to high air flow rate used

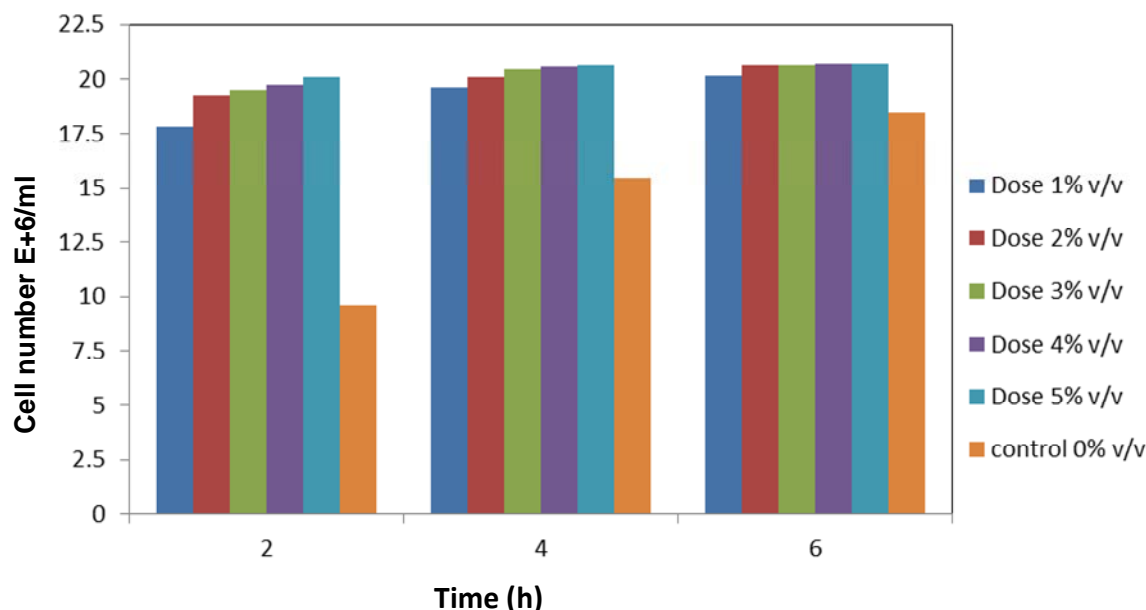
and most of carbon lifted from the system quickly with low dissolved amount, but it was best to keep culture on agitation and prevent our strain from settling. Reddy (2002) reported the carbon fixation based on the input 46.20 gC/day was approximately 3.65% for the flat-plate photobioreactor (Reddy, 2002). Yun et al. (1997) found that 0.624 g CO₂/L.d fixation from flue gas in wastewater medium at 15% (v/v) CO₂ was supplied to the air-adapted inoculum and at light intensity approximately 8 Klux, while in this study it was found 0.258 g CO₂/L.d in BBM with air only (Osborne, 2009).

Biomass recovery using a novel flocculant

The results for biomass recovery, removal percentage, and removal efficiency percentage are tabulated in Table 2. The results were in the trends to increase the biomass recovery by increasing the dose of flocculant from 1 to 5% (v/v). The maximal removal achieved at 5% (v/v) during 2 h, while more effective removal can be obtained

Table 2. Biomass recovery, biomass removal percentage and removal efficiency percentage at different doses during time.

Time (h)	2			4			6		
Flocculant (% v/v)	Cell (E+6/mL)	Removal (%)	Efficiency (%)	Cell (E+6/mL)	Removal (%)	Efficiency (%)	Cell (E+6/mL)	Removal (%)	Efficiency (%)
0	9.60	46.27	0.00	15.50	74.70	0.00	18.48	89.04	0.00
1	17.80	85.78	54.38	19.66	94.75	67.55	20.17	97.18	15.84
2	19.30	93.01	84.38	20.10	96.88	87.09	15.84	20.61	99.33
3	19.50	93.98	89.06	20.48	98.69	88.08	20.66	99.57	68.32
4	19.75	95.18	89.84	20.59	99.20	90.40	20.67	99.63	75.25
5	20.10	96.87	92.19	20.66	99.55	92.38	99.82	78.22	18.48

**Figure 4.** Biomass recovery during time with different doses of flocculant.

at 1% (v/v) by extending the period to 6 h as shown in Figures 4 and 5, where the initial cell concentration was 20.75×10^6 cell/mL. The removal percentage was calculated by using Equation (4) and plotted per each dosage of flocculant. The results showed the maximal removal percentage that can be achieved at 5% (v/v) in 2 h (96.87%), but better removal can be achieved with 1% (v/v) dose after 6 h (97.18%) as shown in the Figure 5.

Also, the highest cell removal efficiency percentage (% RE) was obtained at dose 5% (v/v) 92.19% after 2 h, while the lowest (% RE) was obtained at dose 1% (v/v) 54.38% after 2 h.

Conclusion

The findings revealed that the Bold basal medium (BBM)

is the optimal suitable medium for culturing *S. dimorphus* due to the comparatively high productivity of biomass (0.1406 g/L.d) at temperature 30°C, free air volumetric flow rate 1.5 L/min, and under continuous light intensity 1.3 ± 0.05 KLux.

Regarding metabolite production, some valuable findings can be expressed here as, the metabolite production from the same strain may vary to express different pH in different media. It was observed that the metabolites produced from *S. dimorphus* were mostly neutral in the beginning while growing in M4N but the other media presents some alkaline nature of metabolites during all growth period.

Variation in the pH of culture depends on the nature of metabolites secreted in response to the nutrients in different media but not due to the nature of algal strain.

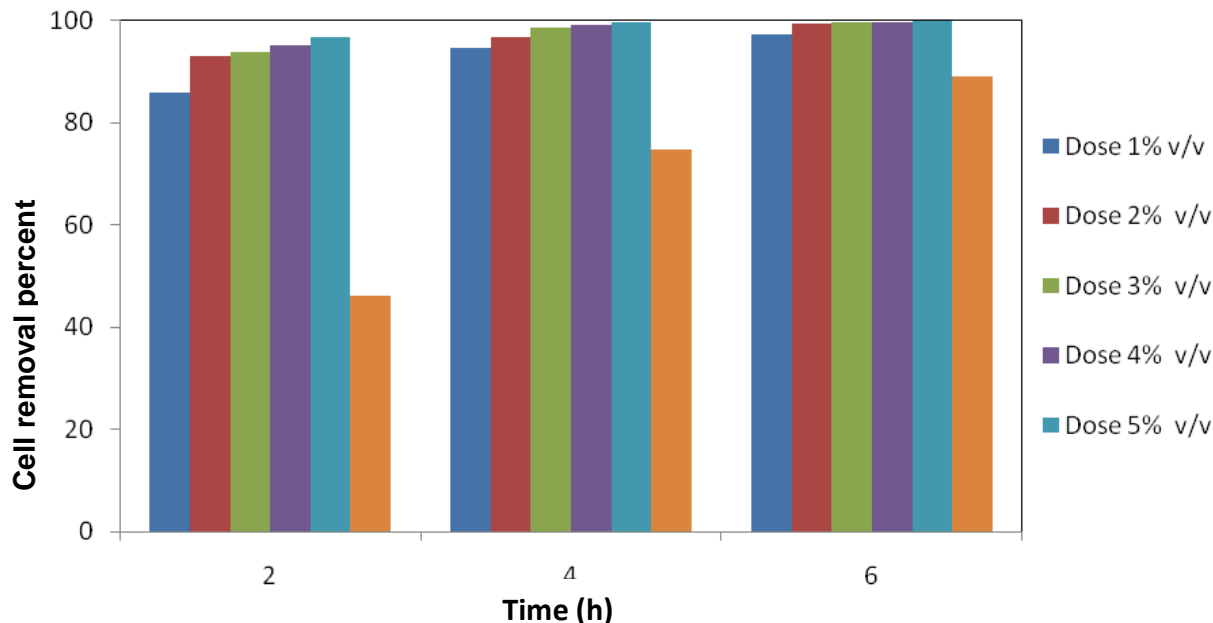


Figure 5. Removal percentage of biomass/algae using different doses of flocculant with time.

Conflict of interests

The author(s) have not declared any conflict of interests.

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