Journal of Environmental Science and Technology

ISSN 1994-7887

OPEN ACCESS Journal of Environmental Science and Technology

ISSN 1994-7887
DOI: 10.3923/jest.2021.35.41

Research Article
Impact of Growth Conditions on Indigenous Algal Biomass Production

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Abstract

Background and Objective: Population explosion and urbanization are the major cause of the energy crisis. Conventional fossil fuel is a non-renewable and unsustainable source of energy. To fulfil the demands of the growing population, sustainable energy sources became a crucial need. Algae-based biofuel production has turned into an appealing approach as it is one of the most reliable energy sources. The present work explored the optimization of growth conditions of analgal consortium collected from a waste water drain. **Materials and Methods:** The collected biomass was identified and subjected to different ranges of pH, temperature and light intensity in the laboratory. The growth rate was recorded and presented as a descriptive stat. **Results:** The identification results revealed the prevalence of cyanobacterial strains in the collected consortium. The experimentation showed substantial growth of consortium at the temperature range of 25-30°C and neutral pH was found suitable for constant growth of biomass. Direct sunlight showed a negative influence on biomass production as fluctuations in light intensity cause stress in algae. The high light intensity of 2060 lx showed improvement in the growth of biomass. **Conclusion:** Determination of ideal conditions for wastewater algae in the present study exposed the field of utilization of wastewater algae for various vital purposes. This can specifically helpful in techno-economic improvements in the field of algal exploitation or consumption. The knowledge of ideal growth conditions of algae can be helpful to enhance the bioremediation process and biofuel production.

Key words: Energy crises, wastewater drain, algal consortium, optimization, biodiesel, phycocyanin, fossil fuel, wastewater algae

Citation: Kaur, H., A. Rajor and A.S. Kaleka, 2021. Impact of growth conditions on indigenous algal biomass production. J. Environ. Sci. Technol., 14: 35-41.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The population explosion, industrial development, growing pollution and energy crisis pave the way for a challenge to researchers and technologists to find a substitute source of energy. Several kinds of research are being conducted to bring an alternative energy source that can overcome the energy shortage[1]. The negative ecological influences of fossil fuel use are provoking worldwide searches for sustainable fuels. Algae-based biofuel is considered one of the most reliable energy sources.

Algae are autotrophic organisms that contain chlorophyll and other pigments. These pigments help to trap light energy from the Sun. In the photosynthesis process, algae can produce organic molecules such as fats/lipids, which can be used to yield biodiesel[2,3]. Moreover, algal species are being used to remove hazardous pollutants from the wastewater and leftover can be recycled as manure or food additives for cattle. Several algal extracts are used in cosmetics, pharmaceutical products and preserved food articles[4]. Some of the algal species namely *Dunaliella*, *Spirulina*, *Chlorella*, *Euglena*, *Schizochytrium*, etc. are rich sources of pigments (carotenoid and phycocyanin), lipids, antioxidants, vitamins (vitamin B, C and E) and medicines[5]. Biodiesel production from algal cultivation is a great prospect as it can serve as a sustainable source of energy however it has itself some limitations too.

One of the major limitations is associated with determining the optimum environmental conditions for the growth and accomplish the ideal surroundings for continuing the exponential growth of algae to yield high oil content[6]. It is difficult to sustain *in vitro* conditions of algal cultures in outdoor fields because fluctuations in temperatures, light intensity and other factors may cause stress in algae. Competition from offensive species is also another problem of algal cultivation in natural conditions. Due to a stressful environment, biomass production may decrease[7]. Consequently, the net oil productivity or bioremediation efficacy will decrease due to reduced growth. However, it is possible to overcome this limitation by providing ideal conditions after in-depth knowledge of growth kinetics[8]. Ideal growth conditions are species-specific in the case of algae. The biomass production is reliant upon several factors such as abiotic factors (temperature, pH, water quality, minerals, carbon dioxide, photo period and light intensity), biotic factors (cell density and cell fragility), mechanical factors (mixing, gas bubble size, distribution and mass transfer)[9,10]. The step of the optimization process helps to design the bioreactor in which essential requirements such as nutrients, physical factors, etc. can be provided for high algal biomass production. This process can be helpful to increase the industrial productivity of algal biomass used for various purposes[11]. The prime thirst of the present study is to evaluate the optimum conditions for algal consortium collected from wastewater drain.

MATERIAL AND METHODS

Study area: The present study was carried out in the laboratory of Science and Technology Entrepreneur Park (STEP) at Thapar University, Patiala, India from December, 2018-April, 2019.

Site description: Algal samples were collected from a wastewater drain namely Badi Nadi/Patiala River (30°20'4.92''N 76°25'33.96''E). Badi Nadi or Patiala River is the most important subsidiary rivulet that joins the Ghaggar River and it is a 71.08 km long wastewater drain that carries mainly wastewater from Patiala city and several adjacent towns and villages.

Algae collection: The collection of the algal consortium was carried out by following standard methods given by APHA (2012)[12]. Nonmetallic samplers and polythene zipper bags were used to collect algal samples. Samples were transported to the lab and then rinsed with tap and deionized water to remove debris/extra salts before its use.

Growth media and cultivation: Inoculation of the consortium to flasks was done over a flame in the chamber of laminar airflow (Thermodyne, India). Before inoculation surface of the chamber was sterilized with rectified spirit and by UV irradiation. Algal samples were cultivated in a growth room in which 28°C temperature was maintained. Sterilized BG-11 liquid media was used to grow an algal consortium[13]. Culturing was done in autoclaved, 250 mL Erlenmeyer flask (Borosil, India). Flasks were kept in a growth cabinet under cool white fluorescent tubes emitting light with an intensity of approx. 2000 lx at the temperature of 28$\pm$2°C Fig. 1. The cultures were maintained by changing the media in flasks after 7 days to keep the algal mass at the exponential growth phase. Cultures were hand-shaken twice a day to keep the biomass inhomogeneous conditions. Sub culturing was carried out to obtain the required algal mass for further experimentation. The cultures were harvested by centrifugation at 4000 rpm for 15 min and dewatered living algal pellets were used for the experiments. Standard microbiological methods were followed to identify algal species. Identification was done based on morphological characters[14].

Fig. 1: Algal culturing in laboratory conditions

Experimentation: Algal optimization was done by subjecting the algal consortium at different conditions like pH (6, 7 and 8) temperature (25, 28 and 30°C) and light intensity (650 lx, 2060 lx and sunlight). The temperature of the growth rooms was measured by a standard thermometer (Zeal, England). Light intensity was measured by using a lux meter and pH was measured by a pH meter (Hanna, India). The first calculation was made on the seventh day of inoculation. The algal mass was centrifuged and weighted after dewatered by blotting paper. Weight was measured in each week of the experiment. Again, the same algal mass was inoculated in the same media and the experiment was carried out for 21 days. The growth rate was evaluated based on the increase in fresh weight of algal mass[15].

RESULTS AND DISCUSSION

The growth of algal mass is regulated by several environmental factors. Temperature, pH, light intensity, quality of light, duration of photo period, mixing, nutritional composition are major influencing factors. The present study aimed to optimize the collected algal consortium in different environmental factors such as pH, temperature and light intensity. Each experiment was conducted as triplicates.

Identification of algal consortium: Algal consortium collected from Badi Nadi (Patiala River) was identified up to genus level. The studied consortium belongs to the genus *Oscillatoria*, *Nostoc*, *Chlorella*, *Nitzschia* and *Merismopedia* (Table 1).

Table 1: Identified genus from algal consortium

Genus	Phylum
Oscillatoria	Cyanophyta
Nostoc	Cyanophyta
Merismopedia	Cyanophyta
Nitzschia	Ochrophyta
Chlorella	Chlorophyta

Table 2: Descriptive stat for growth of algal biomass

Parameter	Mean (g)	Standard deviation
pH		
6	0.65	0.03
7	0.72	0.06
8	0.55	0.03
Temperature (°C)		
25	0.76	0.07
28	0.6	0.02
30	0.87	0.11
Light intensity		
625lx	0.6	0.08
2060 lx	0.79	0.15
Sunlight	0.34	0.1

Statistical analysis: A descriptive statistic summary is given in Table 2 in which mean and standard deviation values are presented. After three weeks of growth, the average biomass was recorded as 0.65, 0.72 and 0.55 g at pH 6, 7 and 8, respectively. At temperature ranges of 25, 28 and 30°C, the mean growth was observed as 0.76, 0.60 and 0.87 g, respectively. The average biomass growth at a light intensity of 650 and 2060 lx was recorded as 0.6 and 0.79 g, respectively. The biomass exposed to direct sunlight showed negative growth and the average biomass was recorded as 0.34 g after three weeks of the experiment.

Effect of pH on biomass production: The pH of the medium significantly affects the metabolic reactions of algae. Most of the algal species cannot grow at very low or at very high pH. It can grow only at optimum pH[16]. The effect of pH on the growth of the algal consortium was determined by using different pH levels such as 6.0, 7.0 and 8.0 and 0.5 g of the initial weight of algal biomass was taken. Considerable growth of algal mass was observed at all tested pH levels. Rapid growth was observed at pH 6.0 and 7.0 and slow and continuous growth was recorded at pH 8. (Table 3). The comparative influence of pH on algal growth is presented in Fig. 2 which demonstrated that the highest biomass yield was recorded at pH 7.

Sharma *et al.*[17] found that the algal consortium showed maximum production of biomass, lipids and chlorophyll content at pH 7. Shruthi and Rajashekhar[18] observed four cyanobacterial species that showed maximum biomass production at a pH range of 6.5-7.5. Spilling *et al.*[19] found that at pH 7.5 lipid production was higher as compared to pH 10. The growth rate was not significantly different at both pH levels. Similar findings on pH effect at algal biomass production were observed by Munir *et al.*[15] and Zhu *et al.*[20].

Effect of temperature on biomass production: Temperature is another regulatory factor for algal growth and it directly affects biochemical reactions such as photosynthesis. In the current study, different temperature ranges were used to optimize the algal growth. The initial weight of algal biomass 0.5 g was used in this experiment and tested temperatures were 25, 28 and 30°C. At all three different temperatures, substantial growth was observed (Table 4). After a growth period of three weeks, maximum biomass was observed at a temperature of 30°C i.e. 0.853 g. At a temperature of 25°C, an increasing (stable) growth trend was noticed and biomass weight of 0.804 g was observed. Minimum growth was observed at 28°C that gave a biomass weight of 0.601 g. However, the weight of biomass produced at three different temperatures was not significantly different. It indicated that the algal consortium survived at a temperature range of 25-30°C (Fig. 3).

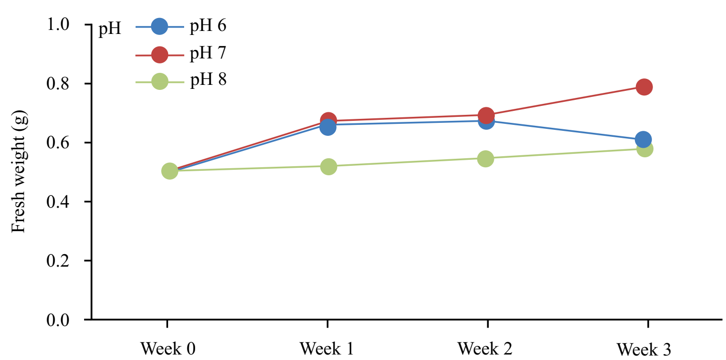

Fig. 2: Comparative growth of algal consortium at different pH

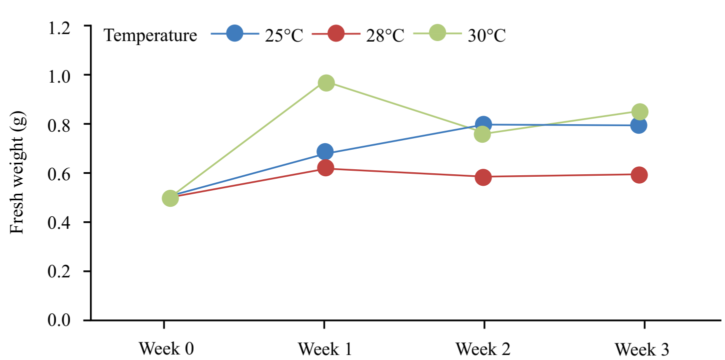

Fig. 3: Comparative growth of algal consortium at different temperatures

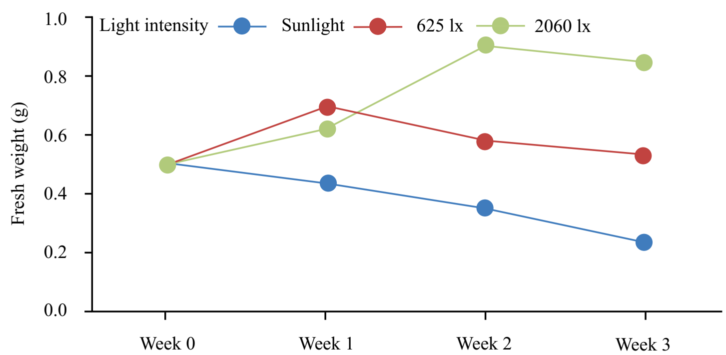

Fig. 4: Comparative growth of algal consortium at different light intensities

Table 3: Effect of pH on growth of the algal consortium

	Fresh weight (g)		
pH	Week 1	Week 2	Week 3
pH 6	0.661	0.674	0.611
pH 7	0.673	0.695	0.791
pH 8	0.519	0.547	0.579

Initial weight of each sample was 0.5 g, data was recorded for three replicates for each experiment

Table 4: Effect of temperature on growth of the algal consortium

	Fresh weight (g)		
Temperature (°C)	Week 1	Week 2	Week 3
25	0.677	0.795	0.804
28	0.619	0.584	0.601
30	0.978	0.765	0.853

Initial weight of each sample was 0.5 g, data was recorded for three replicates for each experiment

Table 5: Effect of light intensity on growth of the algal consortium

	Fresh weight (g)		
Light intensity (lx)	Week 1	Week 2	Week 3
625 lx	0.691	0.578	0.531
2060 lx	0.622	0.907	0.849
Sunlight*	0.438	0.35	0.233

Initial weight of each sample was 0.5 g, data was recorded for three replicates for each experiment, *Fluctuating light intensities

Han *et al.*[21] showed that 30°C was the ideal temperature to achieve high biomass of *Chlorella pyrenoidosa.* Csavina *et al.*[22] recorded high growth of *Amphora* sp. at temperature 30°C. Kitaya *et al.*[23] observed the highest growth rate of *Euglena gracilis* at a temperature range of 27-31°C. Deng *et al.*[24] observed that a marine green algae *Chaetomorpha valida* showed a healthy growth at a temperature range of 17-29°C and a range of 21-29°C was considered a suitable temperature for its consistent growth. Singh and Singh[9] reviewed that most of the algal strains showed the highest growth at a temperature range of 20-30°C.

Effect of light intensity on biomass production: Light intensity is a very important factor because the photosynthesis process directly depends upon light. Oxidative stress induced by a very low or a very high light intensity than the optimum limit can reduce the growth of algae[25]. In the present study, algal cultures were made to grow under two different ranges of light intensities available in the growth room. These intensities were 650 and 2060 lx and 0.5 g of the initial weight of algal biomass was taken. A discontinuous trend of growth was observed at both intensities (Table 5). A maximum biomass yield of 0.849 g was found at the intensity of 2060 lx. Reduced biomass was observed after the first week at a light intensity of 650 lx and the weight of biomass was recorded as 0.531 g. Significant variations were recorded in biomass production that depicted the considerable effect of light intensities on algal growth (Fig. 4). In another experiment, algae did not show growth in direct sunlight that might be due to the fluctuating light intensities that cause stress in algal biomass[26].

The results of biomass production to light intensity were following several studies such as Shuxia *et al.*[27] also found a considerable growth of two algal species viz., *Phaeocystis globosa* and *Thalassiosira rotula* at a light intensity of 2960 lx. Yeesang and Cheirsilp[28] found that a microalgae strain identified as *Botryococcus* PSU showed healthy growth at a light intensity of 2442 lx. Rai *et al.*[29] observed that *Chlorella* sp. showed maximum growth at a light intensity of 2700 lx as compared to 3300 lx. Nzayisenga *et al.*[30] also recorded increased biomass and lipid production with an increase in light intensity.

The healthy growth of algae in laboratory conditions indicated that algae native to wastewater drains can be explored for various purposes such as in the food industry, water treatment and biodiesel production.

CONCLUSION

The present research work was focused on measuring the optimum growth conditions of indigenous algal consortium collected from a wastewater drain. The predominance of genera that belong to Cyanophyta was observed in the present study. So it is an indication that blue-green algae can grow well in wastewater. The outcomes of the current work showed that examined consortium can grow in the temperature range of 25-30°C. The neutral pH gave maximum growth of biomass and high light intensity showed better growth as compared to low intensity. On the other hand, direct sunlight showed negative effects on biomass growth.

SIGNIFICANCE STATEMENT

This study discovers the ideal conditions for wastewater algal consortium. It can be used to increase industrial production and consequently can expand profitability. This study will help the researchers to get the pre-knowledge about growth conditions of algal biomass that can be helpful to enhance the process of bioremediation and biofuel production in the research field. In this way, algal strains native to wastewater give an immense scope to be used as a substitute source of energy that is the most unexplored area of research.

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