Utilizing Industrial Effluents as a Growth Medium for Microalgal Culture: The Effect of LED Lights on Biomass Enhancement

Dr. Allika Sailaja[1], Dr.Manisha Bachachav[2], Arpita Dhanawade[3], Dr.Minal Wani[4]

1. Department of Biotechnology, Sinhgad College of Engineering, Vadgaon, Pune-41.
2. Sinhgad College of Science, Vadgaon, Pune-41.
3. Dr D. Y. Patil Biotechnology and Bioinformatics Institute, Pimpri, Pune.
4. Dr D. Y. Patil Biotechnology and Bioinformatics Institute, Pimpri, Pune.

Abstract

Microalgae are unicellular aquatic organisms which grow in the presence of CO_2 and light to create biomass. Lipids, protein, and carbohydrates are frequently found in abundance in microalgae. Many of them are grown for use as feed, food, and biofuel. Cultivation of microalgae on a large scale has certain disadvantages such as expensive nutrient supply, contamination by foreign microbes and high investment in downstream processing.

The rise in industrial effluent pollutants is a serious threat to biodiversity. Harmful chemicals, metals and nutrients add on to water pollution. A cost-effective method for removing nutrients and metals from wastewater is to use wastewater as a medium for microalgae culturing. To cultivate microalgae, a sizable amount of land is needed. This issue is resolved by using microalgae cultivation and wastewater treatment ponds simultaneously. The method of pretreatment, the species of algae used, and the properties of the wastewater all affect how much biomass is produced by microalgae culture. (madhuraya R .et .al)

In this study, we have experimented with the growth of microalgal species in dairy effluent, fertilizer effluent and sugarcane industry effluent. Parameters such as light, pH and temperature affect the growth of biomass. The experiments were conducted in three cycles: to check the growth of microalgae in synthetic media (zarrouk's media) under sunlight and tube light, to determine the growth of microalgae in the industrial effluents at various concentrations under Sunlight, to determine the growth of microalgae at different wavelengths of light.

The results of these experiments concluded that microalgae culture in synthetic media in the presence of sunlight showed greater yield. The lower concentrations of industrial effluents show successful growth of mixed algal species indicating a cost-effective method to cultivate microalgae. From cycle 3, in comparison to LED lights, the highest biomass yield was shown by green light.

Keywords

Microalgae, wastewater treatment, zarrouk's media, industrial effluents.

Introduction

Microalgae are tiny, eukaryotic, unicellular or multicellular organisms which can produce glucose via photosynthesis and release oxygen. Their biomass contains a lot of protein, vitamin B12, β-carotene, unsaturated fatty acids, and other minerals. They also contain good amounts of fat, which marks them as a good source of biofuel and their rapidly decomposable nature marks them to be a good source of biofertilizer.

A wide range of persistent organic pollutants from industrial effluents is responsible for ecological disruption, climate change, groundwater depletion, melting ice caps, and ozone layer depletion due to photochemical oxidation. (1) These environmental disputes have led to global warming, which calls for experts to concentrate on the effects of pollution and develop strategies to lessen them. India barely controls 4% of the world's freshwater resources despite having 18% of the world's population. In reaction to the projected catastrophic water shortage, these facts illustrate why people seek to recycle and reuse wastewater. (2) Even though it is required to remove most nutrients from wastewater before discharging it into bodies of water and open land, this is still frequently not done, especially in developing nations. (3) Wastewater must therefore be cleaned before being released into bodies of water. Phytoremediation is a technique by which algae and cyanobacteria absorb CO_2 from the atmosphere and remove fertilizers and xenobiotics from waste. (4)

Microalgae engineering has gained a lot of attention recently since different microalgae species significantly reduce CO_2 and greenhouse gas emissions through photosynthesis. Several essential factors, including pH, temperature, light accessibility, CO_2, O_2, and, most crucially, the concentration of nutrients, affect the ability of algae to grow in wastewater. As the world's population grows, so does the need for energy, which ultimately drives up the price of energy. (6) Because of their rapid development and low land requirements, photosynthetic microorganisms can produce biomass more effectively than other types.

Algal cultivation is technically possible, but there may also be some disadvantages. While production potential and harvesting methods are given a lot of attention in algae research, the expense and necessity for nutrients are frequently disregarded as a source of worry. (7) Among the obstacles to the creation of high algal biomass are: Expensive nutrient supply in the process, Contamination with other microorganisms, especially bacteria, which often exists in large-scale algal cultivation, downstream process demand with nearly 30% of the entire investment, a large amount of water required for the cultivation of microalgae. (6)

Depending on the species, microalgae can be grown in freshwater, brackish water, or even seawater. Studies of microalgae in freshwater media that have already been conducted have little bearing on future large-scale algal production for a variety of uses (7,8). This is because fresh water is scarce throughout the world, and its use for growing microalgae will undoubtedly compete with its current production of food and fodder (8,9).

These expenses can be decreased or eliminated by using nutrient-rich industrial or agricultural wastewater as a medium for cultivation. As a result, it has been suggested that using waste streams from various industrial processes (dairy, textile, sugar, and agriculture) for microalgae cultivation could help to reduce costs.

Rigid restrictions on the discharge of final industrial effluents into water bodies have been implemented due to environmental concerns. Numerous organic and inorganic substances found in industrial wastewater have the potential to pollute the environment. A novel biotechnological method for treating wastewater with microalgae is both effective and environmentally friendly. Because they take in nutrients, microalgae thrive in the effluent that is rich in nutrients and then turn them into biomass. The liquid waste stream from the dairy industry has a sizeable organic component, which contains significant amounts of protein, nitrogen, phosphorus, dissolved carbohydrates, and nutrients. Dairy effluent contains organic waste, which poses a serious environmental risk due to its high COD and BOD levels, as well as the difficulties posed by rapid putrefaction. The source of the lab-grown algae was discovered to be fertilizer company effluent, which on average contained phosphates, nitrates, and sulphates. Urea, free ammonia, phosphates, iron, zinc, sulphate, and nitrate were among the ingredients in fertilizer effluent. Microalgae may develop in effluents and produce valuable biomass while removing organic matter and minerals needed to build the biomass. Sugarcane effluent, the primary by-product of sugarcane ethanol plants, is an acidic, dark brown liquid that is rich in organic compounds (Glycerol, lactic acid, sugars), nitrogen, phosphorus, and ions (e.g., K^{+}, $Ca2^{+}$, Mg^{2+}). Even though this wastewater is frequently used to irrigate crops, continuous use of it can cause several changes in the fertility of the soil and lower crop yields. The use of sugarcane effluent for the production of microalgal biomass has previously been suggested, but studies have shown that using it at very high concentrations could inhibit the growth of microalgae.(19) Microalgae cultivation in dairy effluent has several advantages, including the ability to extract high-value products like lipids, proteins, and carbohydrates for use in the fuel, pharmaceutical/nutraceutical, and chemical industries, as well as the ability to produce biomass using organic carbon, nitrogen, and minerals without the need for additional nutrients.

The purpose of this study was to determine whether untreated industrial effluents can be used as a source of food for large-scale microalgal growth. The objective of this study is to the growth of microalgal species in various industrial effluents and optimize growth parameters such as temperature and pH. The study also aims to analyze algal growth characteristics under different LED lights of varying illuminance.

Materials and methods

For the growth of microalgal studies three different industrial effluents were collected. The dairy effluent was collected from the outlet of a local sweets industry in Jamshedpur, Jharkhand. The fertilizer effluent was collected from the outlet of the Shraddha seed and fertilizer industry from Pune, Maharashtra. The sugarcane waste was gathered from a Sugar factory outlet in Sangli, Maharashtra. The pond near the Aditya Birla Hospital in Thergaon, Pune (fig.1) is where the mixed microalgae cultures were gathered. All the samples were refrigerated in a cold room with having temp between 3-5°C to prevent contamination.

Fig 1: pond sample from a water source near Aditya Birla Hospital, Pune, Maharashtra.

The pond water sample was examined under a light microscope to identify algal strains. They were identified using published algal monograms, morphological studies, and information about their mode of reproduction. (fig.2). The following strains namely- Spirogyra, Closterium navicular, Stigeoclonium, Oscillatoria subbrevis, Melosira, Diatom were found when observed under light microscope. (20,21)

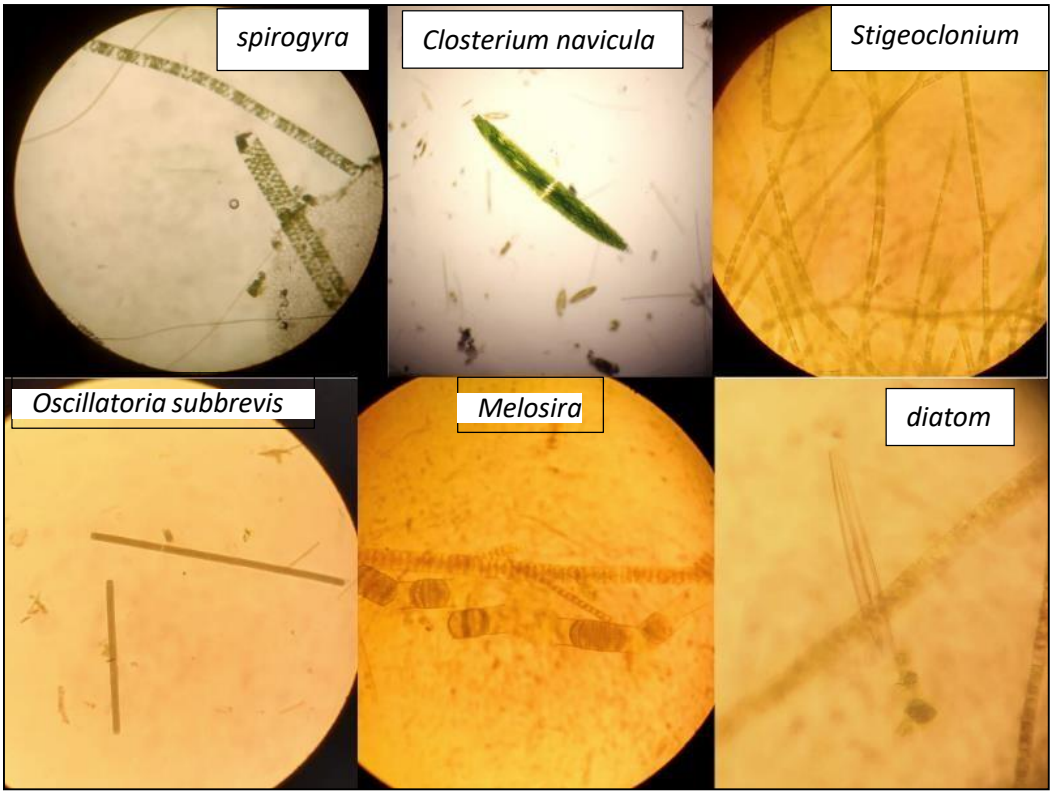

Fig 2: algal species identified in the pond sample using a Light microscope.

Algal cultures were grown in Zarrouk's medium in the current study as a synthetic media. The components of Zarrouk’s medium are provided in Table No. 1. The synthetic media was autoclaved at 121°C for 15 minutes and allowed to cool. The final pH adjustment was made to 8. The media was inoculated with the algal sample and successive subculturing was done for future studies.

Sr No	Chemical Compounds	Concentration g/L
1	NaCl	1g
2	$CaCl_2.2H_20$	0.04g
3	K_2SO_4	1g
4	K_2HPO_4	0.5g
5	$MgSO_4.7H_2O$	0.2g
6	$FeSO_4.7H_2O$	0.01g
7	EDTA	0.08g
8	Distilled Water	1 L

Table no1: composition of Zarrouk's media

Experimental setup: The effluents were inoculated with microalgal culture in separate conical flasks. To compare optimal growth, the microalgal cultures were allowed to grow for 4 weeks nonstop at normal room temperature under tube light and the same study was also done under sunlight. To check various factors related to the growth of microalgae in dairy, sugarcane and fertilizer industry effluent, the experiments were carried out in three distinct cycles, which are listed below.

Cycle 1: To check the growth of microalgae in synthetic media under 2 different Light sources. (Sunlight & Tube light) (fig.3)

2 Erlenmeyer conical flask consisting of 500 ml each of culturing media was inoculated with algae sample & was kept for observation for a period of 4 weeks.

Fig 3(a): algal culture in synthetic media under sunlight

Fig 3(b): algal culture in synthetic media under tube light

Synthetic media	Wet weight (g)	Dry weight (g)
Zarrouk's media	6.56g	4.074g

Table no 3: microalgal biomass produced in synthetic media

Cycle 2: To determine the growth of microalgae in the industrial effluents at various concentrations under Sunlight. (fig 4 a,b,c)

The effluent was diluted at various concentrations to check the optimum growth of microalgae in them, table no 2.

Concentration (%)	Distilled water (ml)	Effluent (ml)	Total Volume (ml)	Dilution Factor
0.25	487.5	12.5	500	40
0.5	475	25	500	20
1	450	50	500	10
2	400	100	500	5

Table no 2: Concentrations of effluents

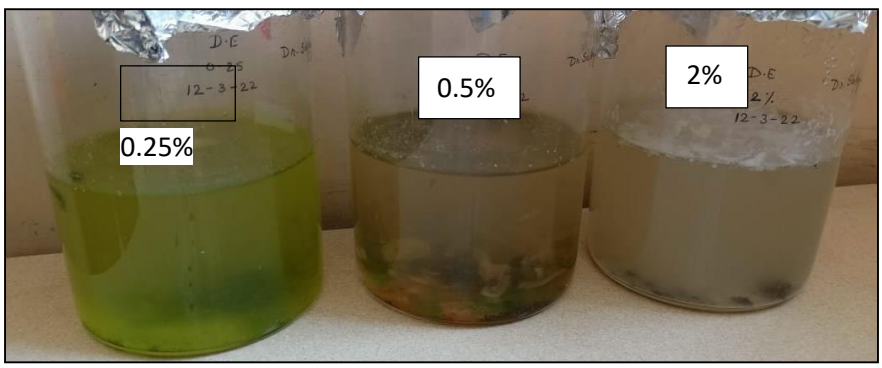

Fig 4 (a)

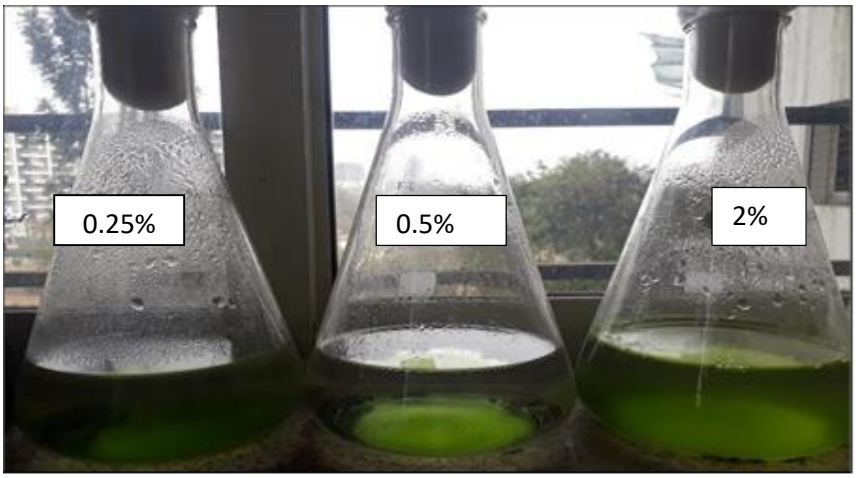

Fig 4 (b)

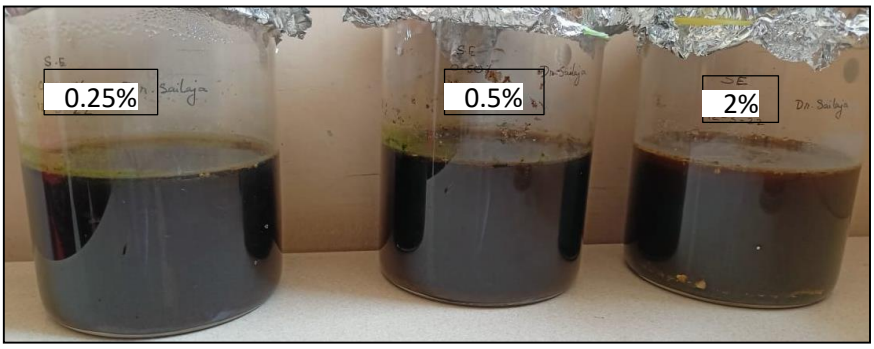

Fig 4 (c)

Fig 4: algal biomass in industrial effluents at different concentrations in the presence of sunlight
(a) dairy effluent
(b) fertilizer effluent
(c) sugarcane effluent

Dilution factors	Effluents	Wet weight (g)	Dry weight (g)
0.25	Dairy	1.07	0.18
	Fertilizer	5.2	3.67
	Sugarcane	1.5	0.38
0.5	Dairy	3.5	0.35
	Fertilizer	4.23	2.78
	Sugarcane	2.4	1
1	Dairy	1.83	1.12
	Fertilizer	4.58	2.32
	Sugarcane	1.56	0.61
2	Dairy	6.26	3.39
	Fertilizer	3.71	1.74
	Sugarcane	2.84	2.3

Table No 4: Biomass produced in different concentrations of effluents

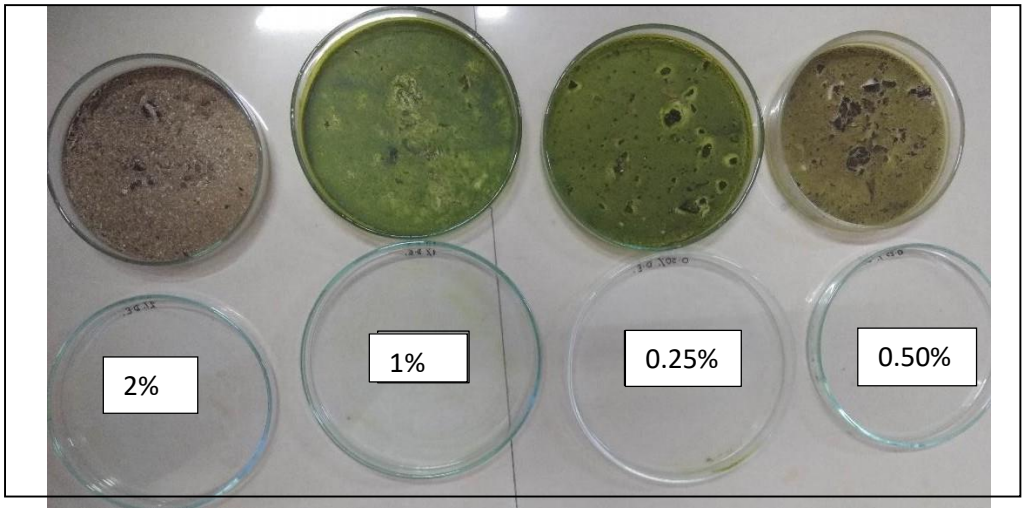

Fig 6 (A) dried biomass produced in dairy effluent at different concentrations.

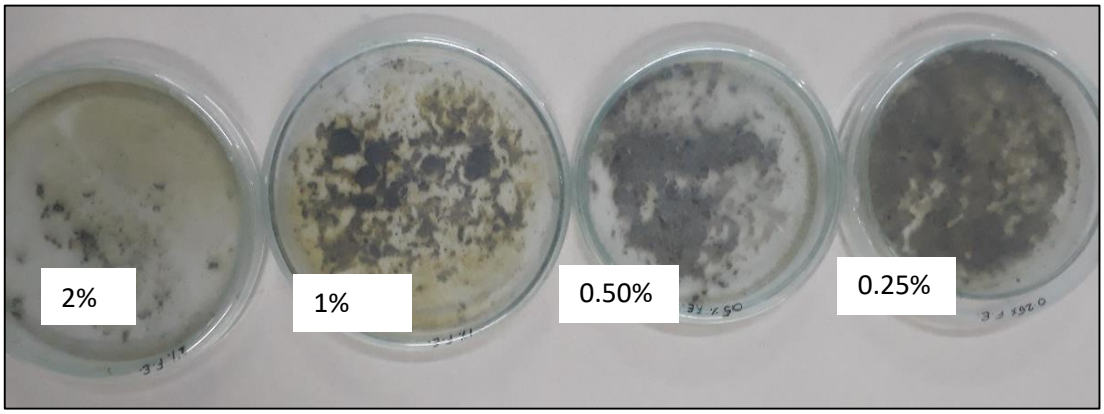

Fig 6 (B) dried algal biomass obtained from fertilizer effluent at different concentrations.

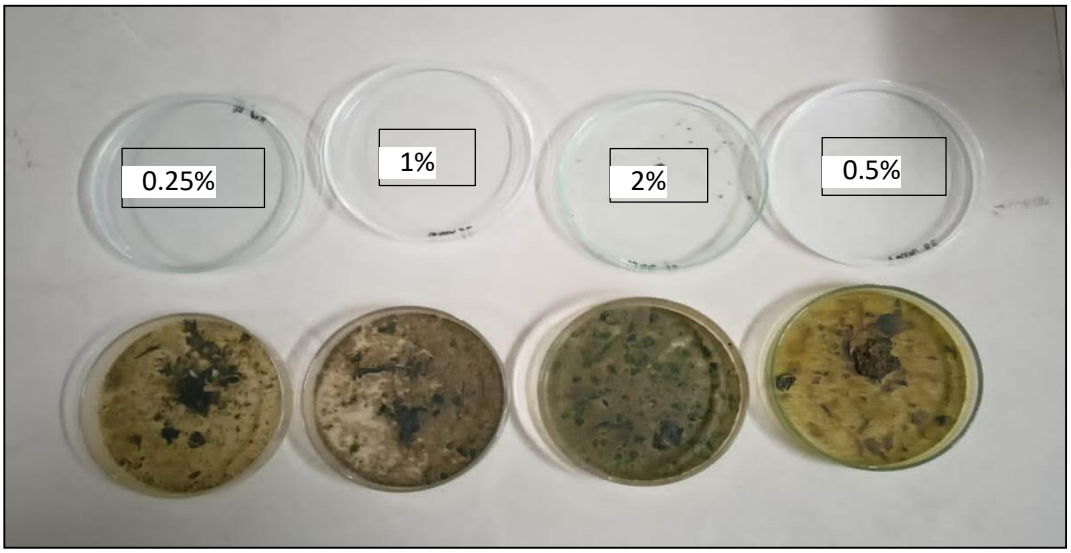

Fig 6 (C) dried algal biomass obtained from sugarcane effluent at different concentrations.

Cycle 3: Determine microalgae growth at different wavelengths of light.

Since LEDs have a narrow band wavelength and use little power, they are regarded as the best light source for the growth of algae. The growth cycle in this cycle was aided by the use of four different coloured LED lights, including white, green, yellow, and red. To eliminate any interference from outside light, the experiment was set up in a pitch-black space. A digital Lux meter was used to gauge each LED light's brightness. Centrifugation was used to separate the algal biomass for 10 minutes at 7000 rpm. (fig 5) Calculations were made for the wet and dry weights (dried at 90^0 C until a constant weight was observed).

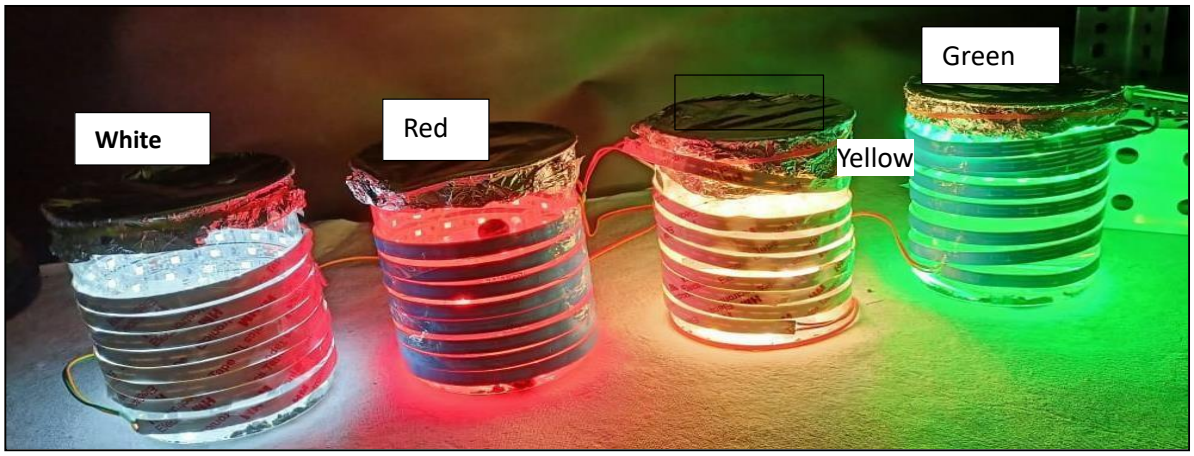

Fig 5: algal growth in different LED lights

	Biomass obtained in Industrial effluents					
Lights	I		II		III	
	A	B	A	B	A	B
Green	5.54	0.832	0.97	0.41	3.776	0.88
Yellow	6.465	0.454	0.84	0.11	5.85	0.317
Red	5.742	0.489	1.26	0.10	4.09	0.294
White	2.98	0.329	0.68	0.12	2.165	0.326

Table no 5: biomass produced under LED lights

*I = dairy effluent; A= wet weight in grams

II= fertilizer effluent; B = dry weight in grams

III= sugarcane effluent

Results

1. Effect of light

To determine the best conditions for growth, a mixed culture of the previously mentioned lake's algae was initially tested. For three weeks, the ability of Zarrouk's media to produce algae under both tube and natural light was tested. After careful observation over the course of those weeks, it was determined that, despite the source of light and nutrition offered, the culture placed under tube light(fig. 3b) had perished due to the lack of an ideal temperature in the culture room. The other culture was exposed to sunlight(fig.3a), and growth was seen, proving that all the requirements were met.

2. Effect of pH

The dissolved carbon dioxide in the culture affects pH. A decline in potential hydrogen indicates a decline in photosynthetic activity. A rise in this parameter, (fig7.) on the other hand, indicates growth in microalgae. For their photosynthesis, microalgae consume carbon. As a result, the culture will contain less CO_2 and have a higher pH. The pH rise caused by the expanding algae also causes phosphorus precipitation and ammonia removal from the air, and it may also have a disinfectant effect on the wastewater.

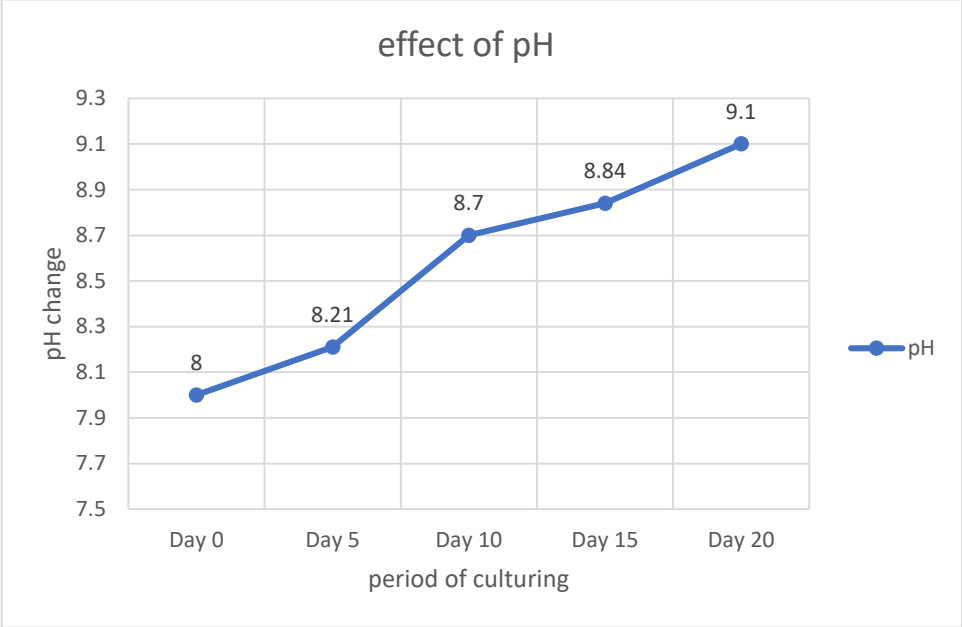

Fig7: average pH change observed in synthetic media

3. Algal growth in industrial effluents

With no conditioning or pH adjustments, the industrial effluents were used in place of the synthetic media to begin a new cycle. The mixed culture could react to the affluent and produce biomass in this medium under both mixotrophic and heterotrophic cultivation. Growth was seen for various effluent concentrations, including 0.25%, 0.5%, 1%, 2%, and 3%. The purpose of these dilution factors was to provide an overall estimate of which concentrations would comply with the necessary conditions for better algal biomass production. For each concentration, the pH(fig.9) was monitored parallel at regular intervals. One set of the concentrations was placed outside in the sunlight, and the other set was prepared and placed inside the culture room under tube lighting. No growth was seen for the culture that was placed in the culture room under a tube light, which was a consistent observation. By the start of the third week, it was evident that the 3% concentration cultures were no longer viable because they were unable to handle the presence of too many nutrients and experienced nutrient shock. The findings demonstrated that microalgal growth increased with decreasing wastewater concentration, reaching its maximum at a concentration of 0.25% in dairy wastewater(fig.8). For the fertilizer effluent and sugarcane industry effluent maximum growth was seen at 2% concentration.

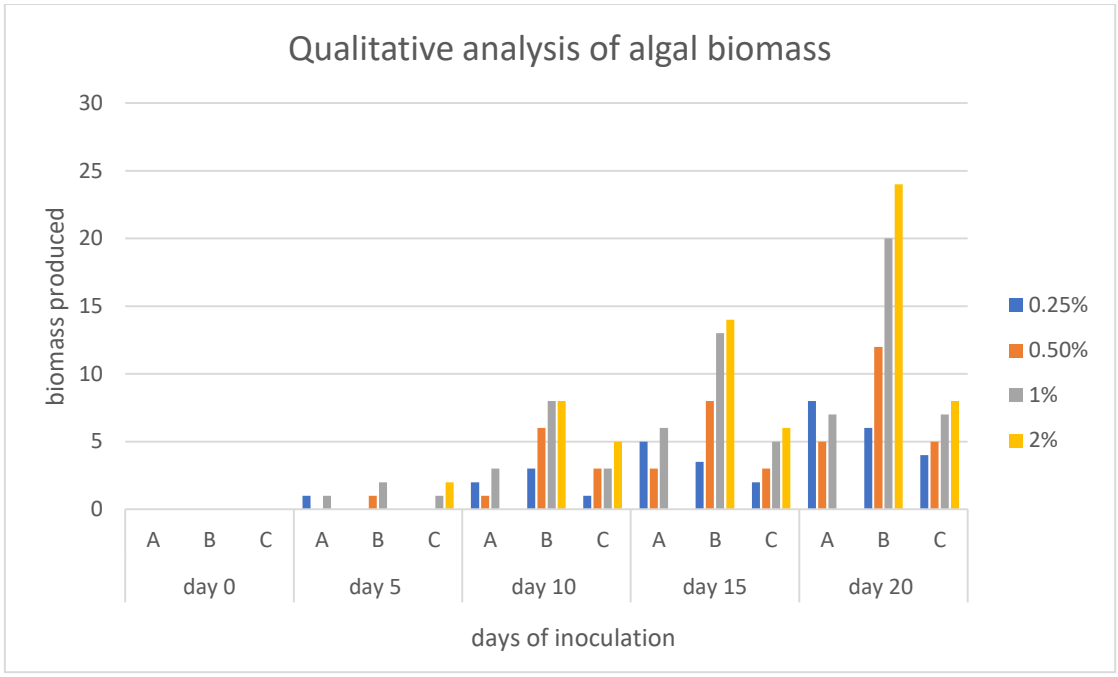

Fig 8: algal culture growing in effluents under sunlight
*(A= dairy effluent, B= fertilizer effluent, C= sugarcane effluent)

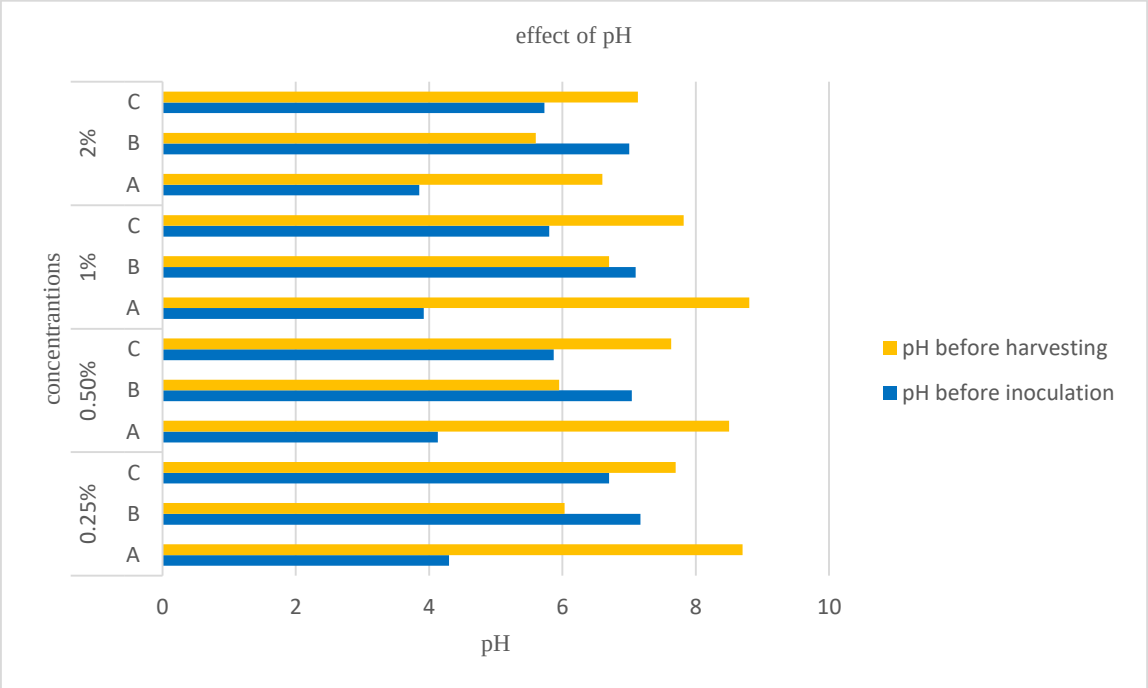

Fig 9: pH before and after cycle for different concentrations of effluents.
*(A= dairy effluent, B= fertilizer effluent, C= sugarcane effluent)

4. Algal growth under LED lights

Based on previous cycles, it was observed that temperature and concentrations are among the most important factors in algal growth. Because the temperature was improperly maintained, the cultures grown under tube light showed no signs of development. As a result, a set-up with four different coloured LED lights with different wavelengths was taped around the culture container and the algal growth was studied.

The illuminance of the light was also noted, providing yet another graphical dataset (fig.10) illustrating the variation in algal biomass growth seen in various light spectrums. The number of photons incident per unit area of a material is the measure of radiation intensity for visible light. The energy of the photons that make up the visible light radiation is correlated with the intensity of light. The number of photons incident per unit area of material causes an increase in light intensity. This is called illuminance. The relationship between intensity and light wavelength is inverse. (table no 6)

$E=hf=hc/\lambda$

Where E= Energy of a photon; h = Planck's constant; C= speed of light; λ= wavelength of light.

Light source	Wavelength (nm)	Illuminance (lux)
Sunlight	600	1538
Tube light	550	1270
Green	537.5	1440
Yellow	580	1344
Red	665	553
White	539	224

Table no 6: relation between wavelength and light intensity for various light sources

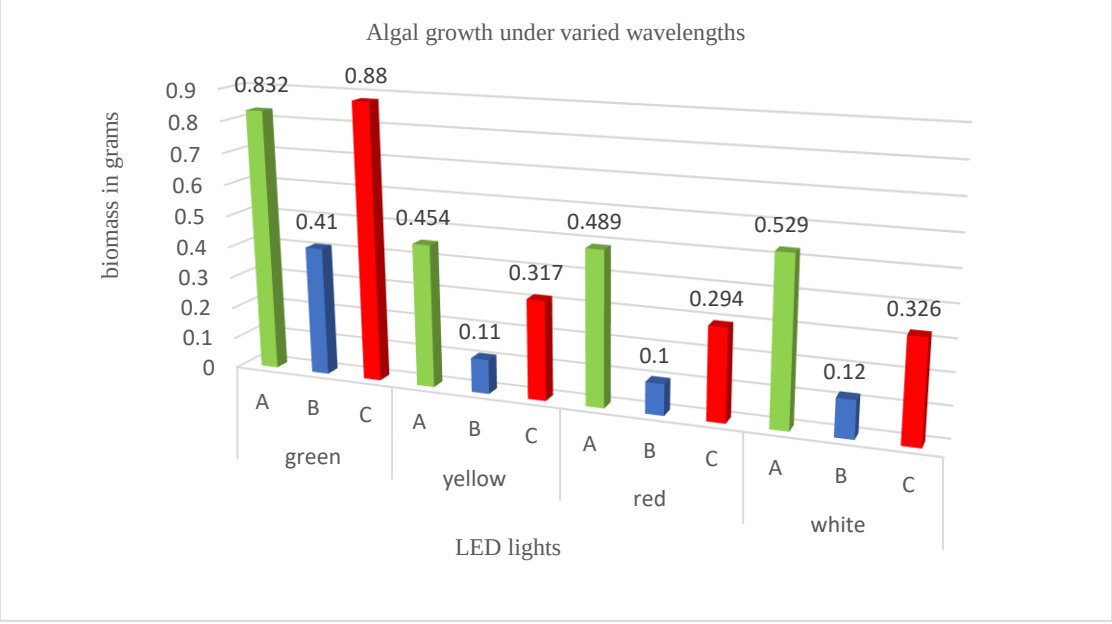

Fig 10: growth of algal biomass at varied wavelengths of LED lights

*(A= dairy effluent, B= fertilizer effluent, C= sugarcane effluent)

The ideal wavelength needed for algal growth was tested using a LUX meter. Also in order to prevent interference between the various wavelengths of light used in the study, the flasks were individually wrapped in white chart paper. Since the majority of microalgal species grow between 490nm and 720 nm, four distinct coloured LED lights with wavelengths (green-520-555nm, yellow-570-590nm, red -630-700nm) were chosen to examine the growth of algal

biomass. The maximum growth of biomass was observed between 500 and 570 nm under the green LED.

Light source	Wavelength (nm)	Illuminance (lux)
Sunlight	600	1538
Tube light	550	1270
Green	537.5	1440
Yellow	580	1344
Red	665	553
White	539	224

Table no 6: relation between wavelength and light intensity for various light sources

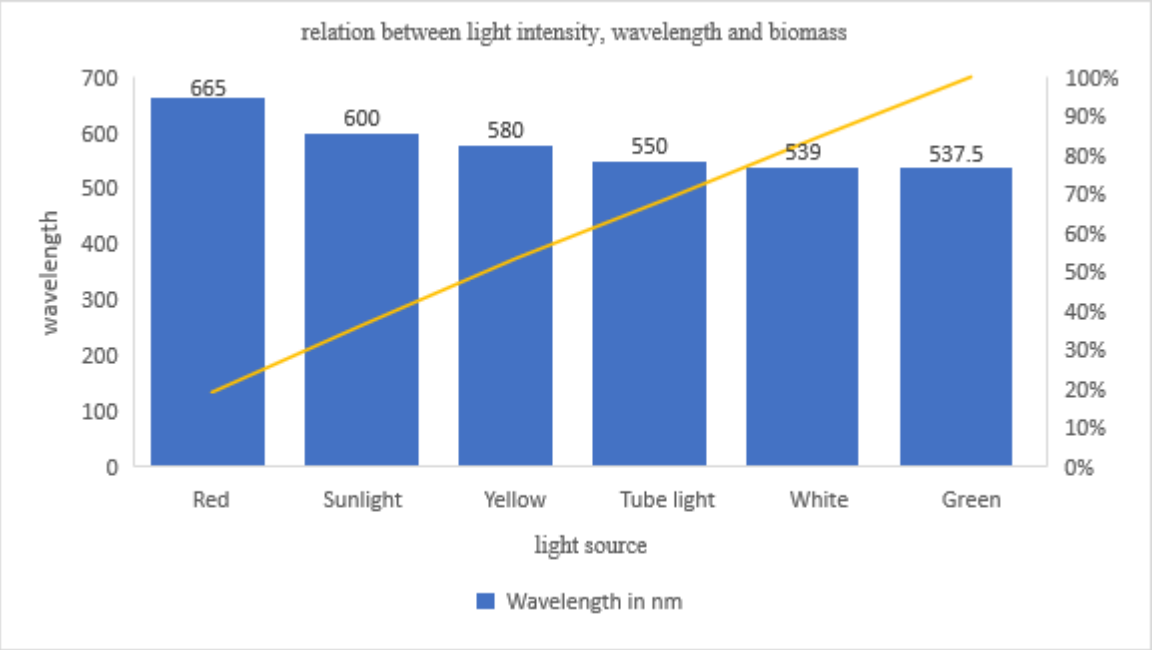

Fig 11: relation between light intensity, wavelength and biomass for industrial effluents.

From fig. 11, we can hypothesize that for mass culture cultivation, strongly absorbed light (red) leads to more oversaturation and is suboptimal for biomass production (only 10%), whereas weakly absorbed light (green, yellow) maximizes productivity. Analysis by Emerson and Lewis'(24) and Tanada (25) for the action spectra of microalgal photosynthesis revealed that once green light with a wavelength of 500–600 nm is absorbed, it is utilized with high efficiency.

Discussions

In the present work, studies showed that the algal biomass production was influenced by the culture technique used as well as physical factors like temperature, light intensity, pH, and scale of operation. Three distinct cycles that are correlated with one another were used to experiment. Cycle 1 was performed to determine the growth of microalgal biomass using synthetic media under two different light sources- natural sunlight and tube light. The experiment showed that sunlight had the potential to produce greater algal growth than the tube light source. The more

sunlight that enters the flask, the better the algae's chances of survival because they need more light energy.

In cycle 2, algal growth was observed in 3 different industrial effluents viz; Sugar industry, dairy industry and fertilizer industry. Growth of algal biomass was observed at different concentrations of industrial effluents (0.25%, 0.5%, 1%, 2%). Two sets of this experiment was done, one under sunlight and the other under tube light as light sources. It was found that when effluent concentrations were reduced, the algal biomass yield was high. The higher biomass yield raises the possibility that wastewater could be a practical method for growing microalgae. This cycle proved that using various effluents as a growth medium could be successful and reduce the cost of microalgae cultivation. Contaminants and dangerous metals may be removed from wastewater using microalgae-based bioremediation. The process also generates algal biomass, which can be converted into additional bioproducts with higher value.

In the third cycle, microalgal strains were inoculated in various beakers that were covered with LED lights of various wavelengths, including green (520-555nm), yellow (570-590nm), red (630-700nm), and white LED light used as a control during this cycle. It was perceived that algae are phototropic organisms that need a specific wavelength of light to grow to their full potential.

In comparison to other LED lights, green LED lights at 500 to 570nm caused a higher algal biomass production which weighed 832g (table no 7). The mixed culture strains used in this experiment could have converted more of the light energy of a particular wavelength into chemical energy through photosynthesis.
From the present work, it can be concluded that using industrial effluents as a growth medium was feasible in achieving good amounts of algal biomass and could lower the cost of microalgae production using conventional methods.

Future Scope

Additional experiments can be carried out involving larger effluent volumes and the growth of algal biomass can be studied. Also rather than studying the growth of mixed algal cultures in industrial effluents, the study of the growth of biomass of individual microalgal strains can be a viable alternative and can promise better results.

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