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Optimization of Novel Symbiotic Bacteria in Algae Growth

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Abstract

In this study, a novel symbiotic bacterium (*Stenotrophomonas maltophilia)* was isolated from algae culture and cocultured with the abundant species *Chlorella pyrenoidosa* and *Scenedesmus abundans* in 3 N BBM+V medium under aseptic conditions. Furthermore, an optimization study was carried out to maximize the growth of the algae biomass. The independent parameters used to determine the bacterial inoculum concentration, pH of the medium, aeration rate of the culture system and other known parameters were temperature, light intensity, inoculum volume of algae and culture time. Thus, the effect of the bacterial inoculum concentration was studied by varying the concentration by 0, 2, 4, 6, 8 and 10%. The initial pH of the medium was changed by changing the medium pH via buffer solutions. The culture system aeration rate was the foremost important factor for determining the actual outcome of the product. Therefore, to determine the influence of the aeration rate in the system, different ranges of volumetric oxygen rates were tested: 90, 80, 70, 60, 50, 40, and 30%. The results showed that the optimal values for maximum biomass production were 8% bacterial inoculum concentration, 7% pH of the medium, and 90% aeration rate.

Keywords: Algae, *Chlorella pyrenoidosa*, *Scenedesmus abundans*, *Stenotrophomonas maltophilia*, Symbiotic, Lipid

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Introduction

The world's energy demand is currently the most important factor. Fossil fuels are typically used to meet this demand, but excessive use of these resources causes depletion and environmental problems. Evolving hazards from fossil fuel usage are one of the critical issues. However, the global energy demand has been satisfied by fossil fuels. The consumption of fossil fuel could continuously increase for global development, which was predicted in the global energy scenario in all yearly energy reports. Many remedies have been established to reduce fossil fuel emissions by monitoring and controlling emissions from industry and all powered vehicles and encouraging reforestation. This is a temporary solution, and moreover, several studies have reported that renewable energy can permanently overcome all these issues and suppress unsustainable energy usage. Additionally, greenhouse gas (GHG) emissions and global warming are causes of climate change (Hillet al. 2006). Renewable energy is identified as a sustainable energy source because of its greater benefits, because it fulfils the requirements of global energy demands, and because it is eco-friendly in nature and potentially carbon neutral (Demirbas 2009 and Hillet al. 2006).

A statistical review reported that the rate of world energy consumption from fossil fuel could decrease within 45 years (Rühl C 2008). Hence, there is a need to identify an alternative energy source for fossil fuel to reduce the excess shortages of petrol and diesel and minimize the hiking price of transport fuel. Several studies have shown that triacylglycerides (TAGs) are promising alternative energy sources for fossil fuels (Khan,et al., 2009). The production of biodiesel from biomass encourages the rural economy and is also eco-friendly in nature (Barnwal,et al., 2005). Hence, this research focused on the production of biofuel. Thus, roughly four energy generations have been established to harvest biofuel from food crops, nonfood crops, microorganisms, carbon capture and storage to meet those demands. Because it is easier to obtain and more effective than other sources for producing biofuel, extracting fuel from algae has garnered more attention than other types of extraction.

Microalgae are unicellular or multicellular photosynthetic microorganisms

with simple structures and multiple types of primary metabolites. Due to their simple structure, microalgae possess high potential. Generally, microalgae are composed of 6 to 52% proteins, 7 to 23% lipids, and 5 to 23% carbohydrates. Moreover, the protein content is relatively high in algae, which have a 10.2 C/N ratio. Studies have confirmed that certain algal species can stimulate 20−50% of the total TAG (TAG) in terms of cellular dry weight (Williams, et al., 2010). On the basis of algae cultivation, the open-pond microalgae cultivation system involves carbon dioxide (CO_2) utilization, which enriches the yield of biomass; e.g., one kilogram of dry algal biomass utilizes approximately 1.83 kg of CO_2 (Chisti 2007 and 2012).

There are numerous techniques developed in algae technology for biodiesel production. One of the available methodologies is to use a symbiotic bacterial technique for cultivating and producing algae for diesel. In general, natural ecosystems such as fresh and marine water ecosystems are partially occupied by microalgae. Even if these ecosystems are not in a purified condition, algae can still arise in natural ecosystems. An alga tends to cohabit with other microorganisms, and this technique is known as symbiosis culturing (Cole, 1982). Some microorganisms may lead to the suppression of algae growth through different functional parameters, such as physical or chemical factors. On the other hand, few organisms support each other through photosynthetic reactions. In this study, the growth of symbiotic algae–bacteria pairs was studied for biodiesel production. The basic concept encouraged by the symbiotic photosynthetic reaction is that the microalgae exhales oxygen to the environment and it inhales carbon dioxide in their life cycle process, whereas as the bacteria is having the behavior of liberating carbon dioxide into the surroundings and it consumes oxygen for their own cyclic activity (Munoz and Guieysse 2006). Therefore, a system is built to improve the interactions between both biosources, which results in improved cultivation.

In addition, algae produce hormones and organic and antibacterial components that are more useful for bacteria (Pratt et al. 1944). In industry, this method has been implemented in wastewater management and algae cultivation. However, many algae–bacteria interaction studies are theoretically based only. This study aimed to determine how to improve

the use of the symbiotic bacteria-algae technique for diesel fuel production. Generally, nutrients and changes in phosphate or nitrate concentrations promote algae growth. In this work, the symbiotic bacterium *Stenotrophomonas maltophilia*, which was isolated from an algal culture that was older than three months, was introduced. This particular species of bacteria strongly accelerates the growth of algae without requiring a long time. First, stable colonies were separated and subsequently subjected to algal growth; the most productive species was subsequently chosen, and its culture was identified via a molecular technique based on the 16S rRNA sequence.

Stenotrophomonas maltophilia belongs to the family *Xanthomonadaceae*. Formerly called *Pseudomonas maltophilia* or *Xanthomonas maltophilia, Stenotrophomonas maltophilia* is now recognized as the only species in the recently created genus *Stenotrophomonas*. On a range of bacteriological media, the nonfermentative gram-negative bacterium *Stenotrophomonas maltophilia* grows readily and is mostly found in the biosphere layer of the earth (Adegoke et al. 2017; Sherpa MT et al. 2020; Dunne et al. 1997). *Stenotrophomonas maltophilia* mostly infects humans, and other strains of this bacterium promote the growth of plants and the production of different proteins (Crossman et al. 2008; Jakobi et al. 1996; Sherpa et al. 2020). *Stenotrophomonas maltophilia* possesses a number of traits that make it potentially pathogenic, most notably, the capacity to secrete a broad variety of extracellular enzymes, including lipases, fibrolyses, and proteases, which may be crucial for colonization. Understanding the epidemiology of *Stenotrophomonas maltophilia* infection has improved the use of molecular typing systems (Gajdács et al. 2019; Brooke et al. 2021). Thus, *Stenotrophomonas maltophilia*, a kind of symbiotic bacterium, was identified from the algal culture.

An algal–bacteria symbiosis system has been used for natural purification systems in all water systems (Tang et al. 2016; Wang et al. 2008). Bacteria consume oxygen and release carbon dioxide (Munoz et al. 2003). However, in algae, carbon dioxide is utilized by microalgae, and oxygen is released into the environment or from water through photosynthetic activity (Muñoz and Guieysse, 2006). Hence, this alga–bacterium pair works

symbiotically. Therefore, a purified isolated symbiotic bacterial strain was sequentially coupled with different algal species to determine the symbiotic algae–bacteria growth behaviour for the production of biodiesel. Basically, algal cell division occurs through mitosis and meiosis until the end of nutrient starvation in the medium (Lodish et al. 2000, Chellamboli et al. 2014). In symbiotic technology, both algae and bacteria undergo mutualism, commensalism, and parasitism (Saravanan et al. 2021; Chia et al.2023). Numerous studies on the enhancement of algae growth, which may rely on artificial sources directly or indirectly, have been conducted, but the yield efficiency has not yet reached a level that is considered satisfactory. To address this restriction, a unique symbiotic bacterial strain was extracted from the algal culture, and its growth profile was examined in a variety of scenarios to produce biodiesel. Furthermore, the operating factors for algae cultured with symbiotic bacteria should be optimized.

Methodology

Preparation of symbiotic bacteria for the production of algae biodiesel

Initially, this study started with collecting samples/alga culture products after they had matured for three to four months. This algae culture was grown under nonaxenic conditions with good biomass production without additional nutrient supplementation or aeration. Therefore, to determine the culture status, the sample was checked for cross-contamination in the culture system. The microscopic analysis confirmed the presence of *Chlorella pyrenoidosa and Scenedesmus abundans* in the culture system. In addition, bacterial identification techniques such as the streak and pour plate methods were used to identify stable colonies that survived in the culture system. Several rounds of purification of two isolated pure colonies were carried out, and growth curves were plotted for the isolated colonies (white and yellow colonies), which had maximum absorbance values of 0.467 and 0.154, respectively, at 660 nm. Moreover, the isolated bacterial colonies were cocultured with a pure axenic culture of *Chlorella pyrenoidosa* and *Scenedesmus*.

This section focused on identifying the effects of independent parameters such as the bacterial inoculum concentration, the pH of the medium, and the aeration rate of the culture

system. Other known parameters, such as temperature, light intensity, inoculum volume of algae and culture time, were kept constant at 30°C, 13.5 μmoles/m^2/sec, 10% and 20 days for optimization studies. Thus, the effect of the bacterial inoculum concentration was studied by varying the concentration by 0, 2, 4, 6, 8 and 10%. Similarly, the initial pH of the medium varies from 4 to 9, and buffer solutions are used to vary the pH. The aeration rate of the culture system was the foremost important factor for determining the actual outcome of the product without the support of aeration, and it is difficult to establish cell divisions of bacteria as well as algae. Therefore, to determine the influence of the aeration rate in the system, different ranges of volumetric oxygen rates were tested: 90, 80, 70, 60, 50, 40, and 30%. Finally, the present study focused on the symbiotic bacterium *Stenotrophomonas maltophilia,* which was isolated from algae cultivation and subsequently used to increase the growth of algae.

Quantitative analysis

The most often employed techniques in this research are bacterial strain selection and screening, algal biomass quantification, cell disruption or pretreatment techniques, and lipid extraction. In addition, a detailed description of the analytical and quantification methods employed is provided. The obtained parameters were further optimized. Previously, the literature claimed that large-scale algae cultivation in open or closed reactors was carried out in well-equipped, established laboratories or industries; however, for initial investigations, Erlenmeyer flasks are adequate for establishing the necessary parameters, media, and other details.

Microbial isolation technique

Three techniques are available for the isolation of bacteria from culture. These methods include the streak, pour and spread plate methods (Erin R Sanders 2012). These three techniques were used for further study. Samples were withdrawn, and serial dilutions were performed. Several plating techniques have been used to identify stable organisms. Further isolated colonies were subjected to16S rRNA-based molecular analysis to determine the nomenclature of the bacteria**.**

Dry weight method

The gleaned cells were harvested from the culture system, and then, centrifugation was

performed to separate the algal cells from the solution at 5000 RPM for 10 min. The supernatant portion of the centrifuged culture was discarded, the precipitated or deposited algal cells were suspended in ammonium solution to wash out the residues, and the sample was subsequently dried at 104 °C for 24 hr to quantify the acquired biomass following **Eq. (1)**. (Chellamboli et al. 2014 and 2016).

$$\text{Dry weight of biomass} = \frac{[Finalweightofcrucible - Initialweightofcrucible]}{Culturevolume}\ \text{g/L} \quad (1)$$

Optical Density Method

The growth phases of the algal cultures were determined by measuring the absorbance at 660 nm with a UV-2600 Shimadzu, and the corresponding dry weight was calculated from the standard algal growth plot (Chellamboli et al. 2014 and 2016).

Lipid extraction

To determine the total lipid content in algae, several physical disruptions, such as osmotic shock treatment and solvent extraction processes, of intracellular activity were applied to isolate lipids from algae cells. The required amount of algal culture was harvested from the culture system subjected to ultrasonication at 25 kHz for 15 min at 25°C. The appropriate concentration of osmotic shock agent was applied to the aliquot culture by dissolving 0.1 g/ml NaCl in the cell culture mixture, followed by mixing n-hexane and methanol at a ratio of 7:3. The prepared sample was continuously shaken and incubated for 24 h at 100 RPM and 30°C. Furthermore, the sample was transferred to a separating funnel for phase separation. The upper layer was separated and consisted of n-hexane and dissolved lipid cells. The organic phase was removed to a preweighed crucible (W_1) and dried in a vacuum oven at 60°C for 24 h. Quantification of the mass of lipids was performed by calculating the difference between the initial weight (W_1) and the final weight of the dried lipid (W_2) via the formula given in **Eq. (2)** (Chellamboli et al. 2014 and 2016; GursongYoo et al. 2012).

$$\text{Lipid Yield (\%)} = \frac{weightofOilextracted}{weightofBiomass}\ x\ 100 \quad (2)$$

Results and Discussions

A pure bacterium was isolated, and symbiotic bacteria were identified for algae growth. Hence, the isolated symbiotic bacteria

were subjected to maternal culture by culturing pure *Stenotrophomonas maltophilia, Chlorella pyrenoidosa* and *Scenedesmus colonies* in nutrient broth (bacteria) and algae broth medium (algal species) under aseptic conditions. The prepared mother culture was used as a starter culture in the parameter optimization studies. The symbiotic bacteria were cocultured with algae in an enriched nutrient medium to satisfy the cell demand of both algae and bacterial cells, which were 3 N-BBM+V (bold basal medium supplemented with 3-fold nitrogen and vitamins). Therefore, this study also used 3 N-BBM+V media for the study of symbiotic bacteria and algae. Furthermore, optimization has been carried out for maximum growth of biomass production of microalgae in the presence of symbiotic bacteria (Guo and Tong, 2014).

Identification of symbiotic bacteria for algae growth

The growth of the symbiotic bacteria was carried out by measuring the optical density of the growth media at certain intervals (Krishnamurthy et al. 2021). Within 12 hours, the maximum ODs of white and yellow colonies were obtained, i.e., approximately 0.154 and 0.467, respectively. A lack of nutrients in the medium causes bacterial depletion, and the death phase was noted. The growth curves for both pure colonies are displayed in Fig. 1. A yellow colony was later chosen for further experimentation. The activity of symbiotic bacteria is required after twelve hours; this activity begins to support algae growth. In addition, two isolated algal colonies were cocultured. The outcomes of the experiment demonstrated that a yellow colony

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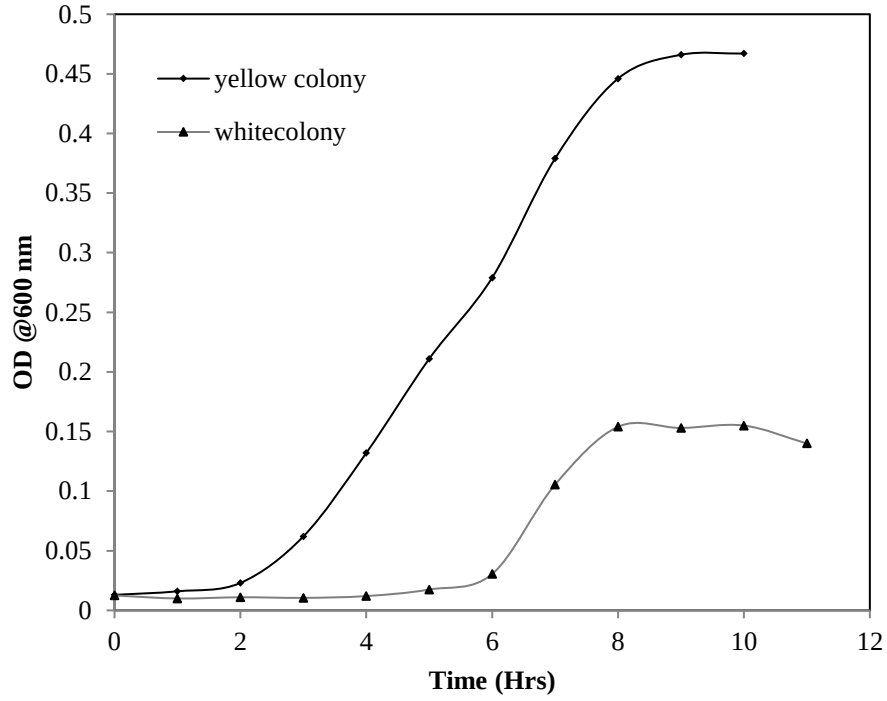

Fig. 1 Bacterial Growth curve for isolated species

bacterium improved the growth of the algal species. Thus, white colonies showed a non-supportive response to the growth of algae, which was neglected in the present study. Furthermore, gene sequence analysis was performed for the yellow bacteria that passed the screening.

Statistical analysis and optimization of the independent factors influencing the biomass and lipid production of symbiotic algae

Every experiment was carried out in triplicate, the data are presented as the average of three runs, and a P value of less than 0.05 indicates a significant effect. This study aimed to determine the operational conditions for the growth of algae supported by symbiotic bacteria. Therefore, symbiotic bacteria and algae coculture studies were initiated with media containing different inoculum (symbiotic bacteria) concentrations, aeration rates and initial pH values for mixed algae culture growth.

Dependence of pH on the production of biomass during algae-bacteria growth

When the initial pH of the nutrient medium was optimized, the growth response improved at pH values of 9, 7, and 5. The gradual progression of algal growth was

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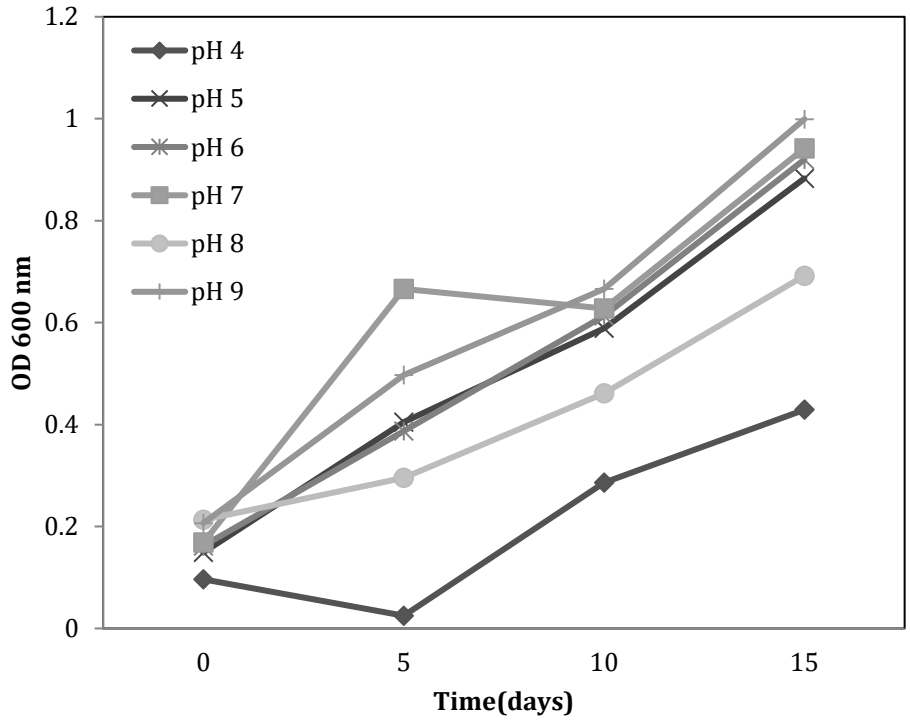

Fig.2 Growth responses of symbiotic bacteria and algae cultured in different pH

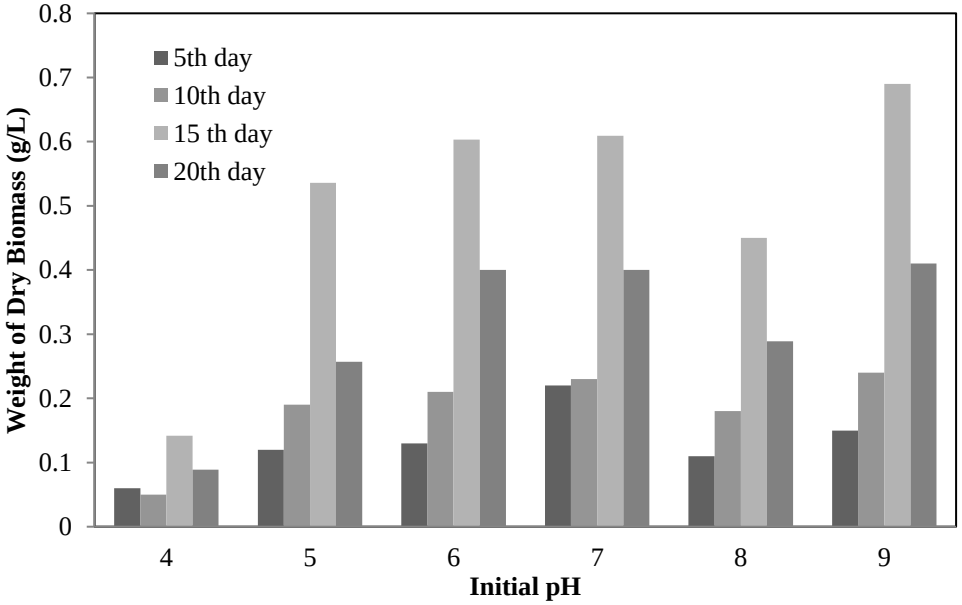

Fig.3 Effect of different initial pH of medium in biomass concentration of algae coculture with symbiotic bacteria

predicted by the optical density at 600 nm. The pH values of 4 and 8 gradually increase in the ranges shown in Fig. 2. The maximum dry biomasses obtained were 0.142, 0.536, 0.603, 0.609, 0.45, and 0.69 g/L for the 4, 5, 6, 7, 8 and 9 pH values, respectively, on the 15th day of the study, as shown in Fig. 3. An increase in pH has a positive effect on dry biomass, but

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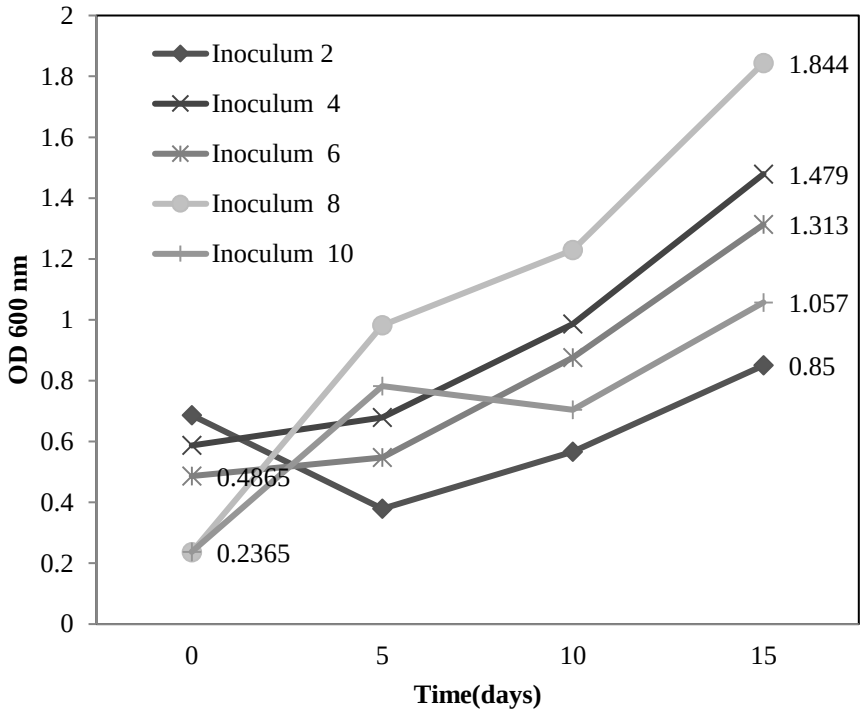

Fig.4 Growth response of mixed algae cultured inoculated in different ranges of bacterial concentration

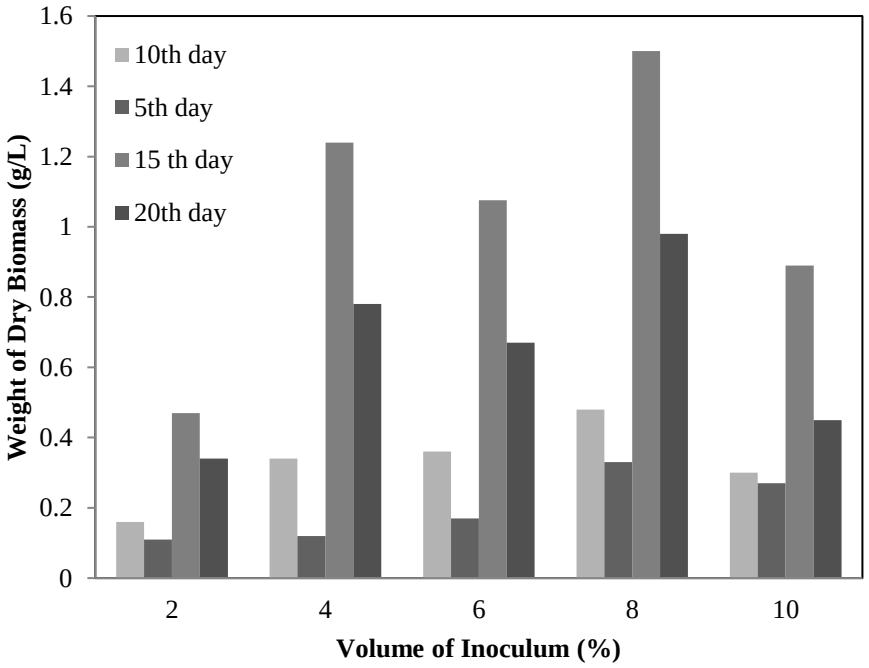

Fig.5 Effect of symbiotic bacteria in different inoculum concentration of algae biomass

a decrease in pH up to 9 results in the agglomeration or destruction of algae. For this reason, nutrient intake decreases, or the blockage of pores present in the cellular membrane acts as a hindering layer. Therefore, algae will be lost if the temperature is greater

than alkaline. In 2006, Travieso et al. reported that the highest algae biomass was 93–98 mg VSS_A/L d (37.2–39.2 g/m^2d) after cultivating algae-bacteria growth by utilizing wastewater. In 2022, Yu et al. reported that the maximum levels of NH^{4+}-N removal and algae growth were observed in cultures maintained at neutral pH 7-8. Conversely, the cultures kept below pH 8 showed better growth in terms of biomass. Therefore, pH plays a crucial role in the development of algae growth

Effect of inoculum concentration on the production of symbiotic bacteria and algae biomass

Fig. 4 shows the effect of different concentrations of bacterial inoculum on the growth of algae. Fig. 5 shows that an increase in the inoculum concentration of symbiotic bacteria did not increase the growth of algae beyond 10%. The inoculum concentrations of 2 and 10% bacteria fluctuated more strongly at the beginning of the experimental runs and later slowly increased the concentration of algae. Compared to the other treatments, 8% of the bacterial inoculum concentrations had a highly influential effect on the growth of algae from the first day onwards. The maximum dry biomass at 2, 4, 6, 8, and 10% inoculum concentrations for the symbiotic bacteria had responses of 0.47, 1.24, 1.076, 1.5, and 0.89 g/L of dry biomass, respectively, on the 15^{th} day of culture. A maximum dry biomass of 1.5 g/L was obtained at an inoculum concentration of 8%. Few bacteria may release algaecides during the growth phase while receiving signals from algae (Seyedsayamdostet al. 2011). Therefore, limiting bacteria was necessary for algae growth.

Effect of aeration rate on the production of biomass by symbiotic bacteria and algae

The effect of aeration rate on the growth of symbiotic bacteria has been studied. The maximum growth response occurred at 90 and 80% aeration, but the overpenetration of aeration in the algae culture led to evaporation or water loss. Therefore, the water loss must be controlled if a 90% aeration rate is used. The aeration rate will definitely improve the growth of algae, but due to the greater growth rate of bacteria, there will be a proliferation of plants utilizing nutrients. Additionally, the inhibition of algae growth may occur due to nutrient

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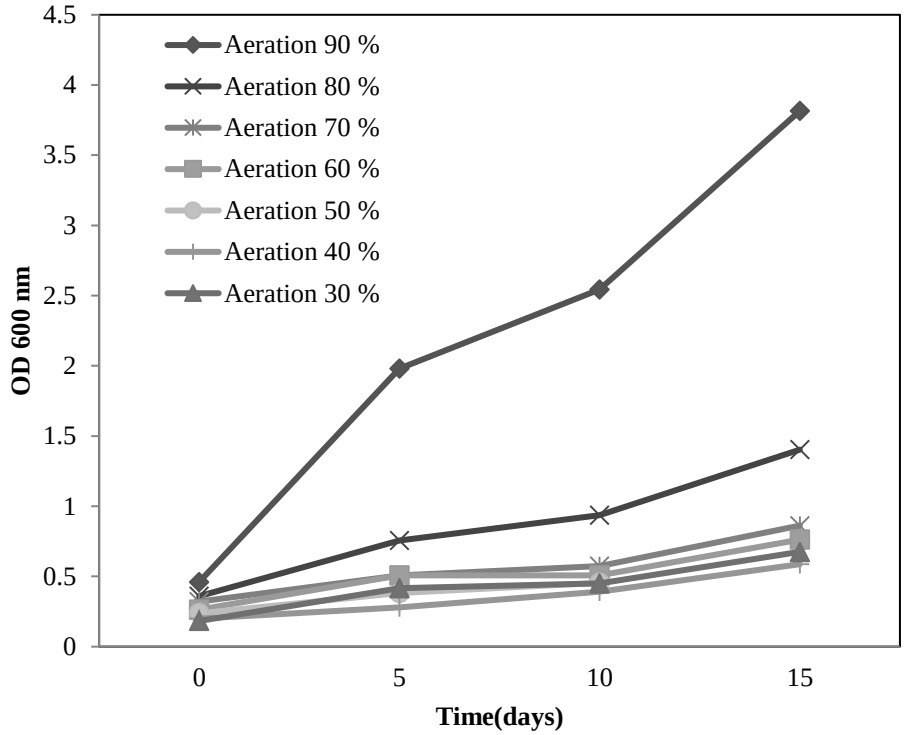

Fig. 6 Growth response of symbiotic bacteria and algae cultured in different aeration rate

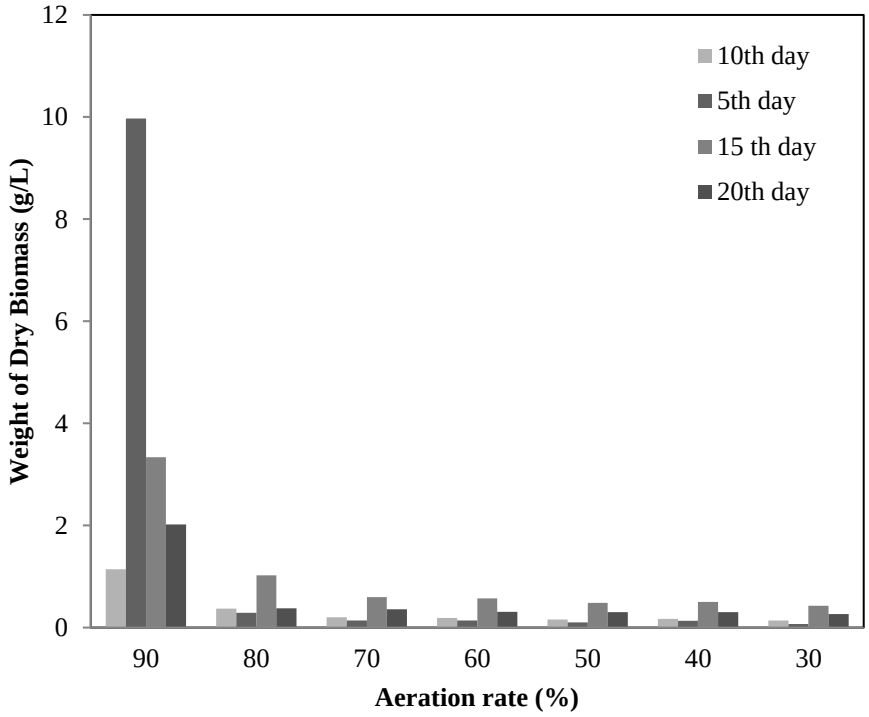

Fig.7 Impact of aeration rate in algae biomass coculture in symbiotic bacteria

shortages (Guo and Tong 2014). Hence, the study showed better growth development at a high aeration rate, as shown in Fig. 6. However, an increase in the aeration rate simultaneously increased the growth of the algae culture. Aeration at a rate < 60% was associated with

decreased growth compared to the other treatments. With different aeration rates, maximum biomass concentrations of 9.97 and 3.34 g/L were obtained for 90% aeration rates on the 5th and 15th days of culture, respectively, as shown in Fig. 7. The results were compared with those of algae culture with a minimum aeration rate of 30%, which produced a biomass of 0.43 g/L on the 15th day. In 2016, Tang reported that the most favorable aeration rate occurred within the reduction range to support the culture system.

Growth response of *Stenotrophomonas maltophilia* cultured with different combinations of algae

Furthermore, algal growth was affected by the culture treatment. The maximum and best growth response was observed for *Chlorella pyrenoidosa* cocultured with *Stenotrophomonas maltophilia*. Similarly, a better growth response was obtained in mixed algae coculture with symbiotic bacteria. Individually growing symbiotic bacteria were considered controls in this investigation. Compared to those of *Stenotrophomonas maltophilia, the growth of Chlorella pyrenoidosa*+ *Stenotrophomonas maltophilia, Scenedesmus abundans*+ *Stenotrophomonas maltophilia* and mixed algae + *Stenotrophomonas maltophilia* was 6.3, 3.54, and 5.04-fold greater, respectively, as shown in Fig. 8. A positive response was detected at biomass concentrations of 0.154, 0.43, 0.8, and 0.637 g/L for *Stenotrophomonas maltophilia, Chlorella pyrenoidosa* cocultured with *Stenotrophomonas maltophilia, Scenedesmus abundans* cocultured with *Stenotrophomonas maltophilia* and mixed algae cocultured with *Stenotrophomonas maltophilia* obtained on the 15th day of culture, respectively. After the 15th day, the growth of the algae decreased, as shown in Fig. 9. The biomass concentrations of *Chlorella pyrenoidosa* + *Stenotrophomonas maltophilia, Scenedesmus abundans*+ *Stenotrophomonas maltophilia* and mixed algae + *Stenotrophomonas maltophilia* were 2.79-, 5.19-, and 4.13-fold greater, respectively. There is evidence that more biomass was produced by *Scenedesmus abundans* cocultured with *Stenotrophomonas maltophilia.*

Effect of the symbiotic bacteria concentration on the lipid yield of the algae

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The effect of inoculum concentration on the lipid yield of the different cultures is shown in Fig. 10. Studies have shown that the maximum lipid concentration occurs within 8% of the inoculum concentration of symbiotic bacteria. Five different concentrations of inoculums taken at concentrations ranging from2, 4, 6, 8, and 10% were assessed for different cultivation combinations maintained in aseptic environments, and the study was carried out for 15 days. The results showed good biomass and lipid yields at an8% inoculum concentration. Increasing the symbiotic bacteria inoculum concentration led to better growth of the algae, but at a10% inoculum concentration, it resulted in a lower percentage of lipids. Moreover, the results showed that the influence of a high range of symbiotic bacteria concentrations leads to the over exhaustion of nutrient media, and a stressful environment will be encountered by bacteria and algae cells. Even a very small amount of incocula will not have lipid production due to insufficient inoculum cells. (Mohamed H et al. 2022)

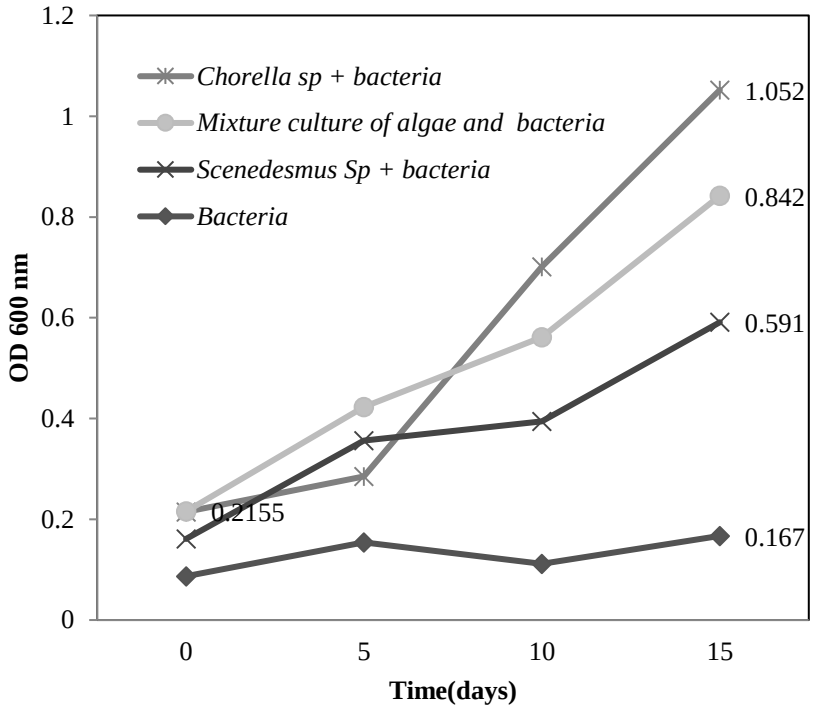

Fig.8 Growth response of different treatments i.e., *S. maltophilia, C. pyrenoidosa+ S. maltophilia, S. abundans+ S. maltophilia* and mixed algae + Symbiotic bacteria

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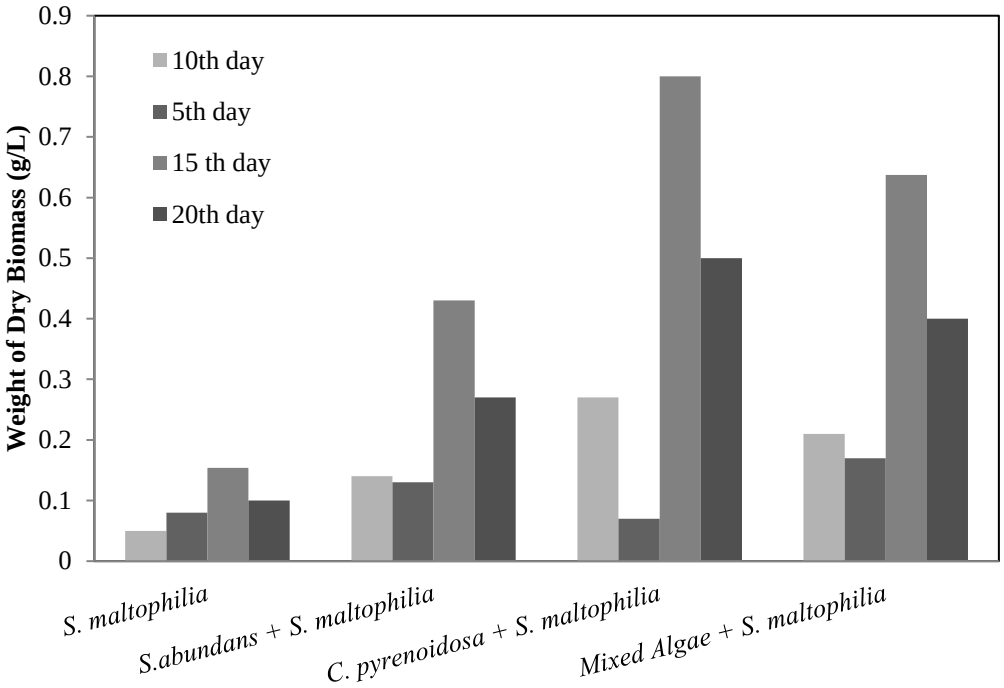

Fig.9 Influence of different treatment conditions such as *S. maltophilia, C. pyrenoidosa+ S. maltophilia, S. abundans+ S. maltophilia* and mixed algae + Symbiotic bacteria in biomass concentration

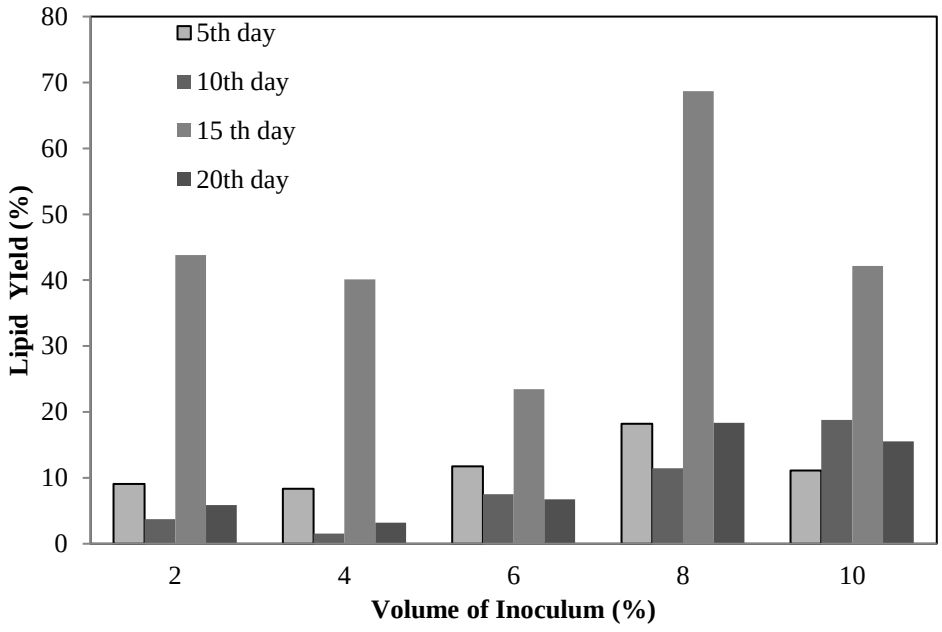

Fig.10 Stimulus of different inoculum (symbiotic bacteria) concentration in Lipid production

Response of lipid production during the growth of algae and symbiotic bacteria to varying pH values

After cultivation, the algae were cocultured with symbiotic bacteria at pH values ranging from 4 to 9 and incubated at 30 °C for 15 days, and the maximum lipid yields obtained

were 5.88±0.5, 47.71±0.9, 31.9±1.5, 48.59±1.0, 61.05±1.1, and 26.9±0.9% for the 4, 5, 6, 7, 8, and 9 pH groups, respectively, on the 15th day of culture, as shown in Fig. 11. The maximum lipid yield was attained at pH 8.

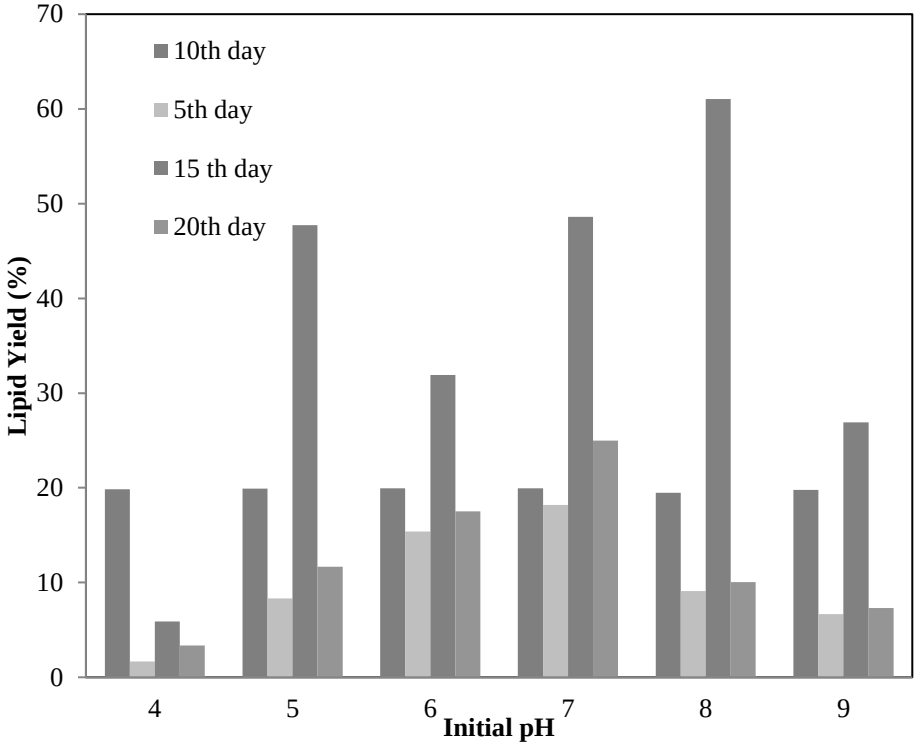

Fig.11 Effect of different initial pH in lipid production occurs in cocultured algae and symbiotic bacterial

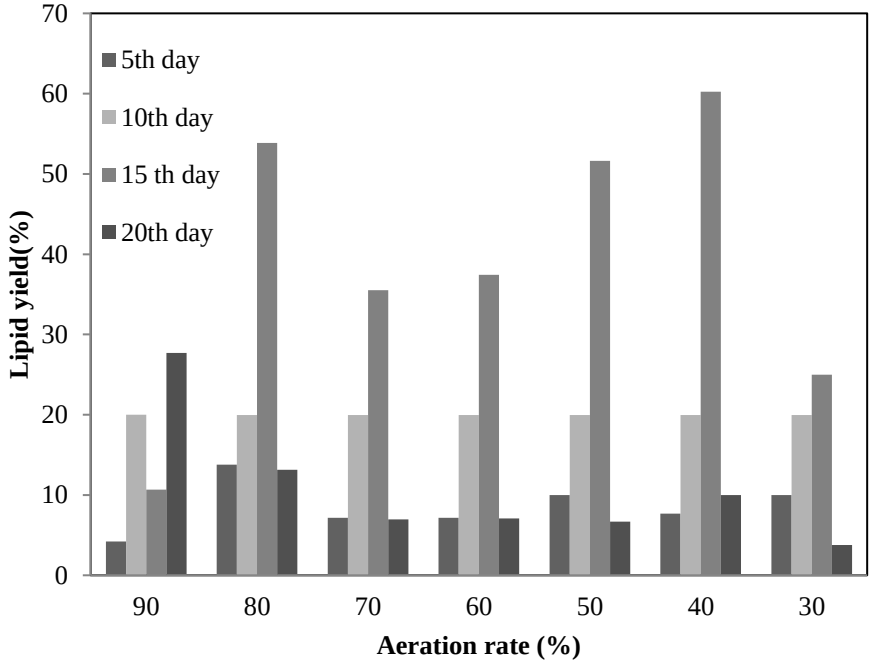

Fig.12 Influence of different aeration rate in lipid production for cocultured algae and symbiotic bacteria

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Influence of aeration rate on lipid production in symbiotic bacteria and algae

The effect of aeration rate on lipid yield in different cultures is shown in Fig. 12. A maximum lipid yield of 60.26% was observed at 40% aeration, i.e., 90, 80, 70, 60, 50, 40 and 30% aeration had 10.65±0.86, 53.85±0.56, 35.51±0.72, 37.41±0.58, 51.61±0.14, 60.26± 1.4 and 25±1.02%, respectively. Mostly, all culture systems require space for cell division and agitation to ensure a proper respiration rate and avoid agglomeration of microorganisms and algae cells. In the culture system, a basic range of 3/4th of the volume of the nutrient will be considered. A 40% decrease in aeration rate results in the maximum production of algal lipids. However, the maximum range of aeration rates results in an increase in the evaporation rate of nutrients from the system. Therefore, an excess aeration rate results in exhaustion of the liquid medium because the cells reach the death phase.

Impact of culture of *Stenotrophomonas maltophilia* on different algae cultures

The maximum lipid yields obtained from *Stenotrophomonas maltophilia, Chlorella pyrenoidosa*+ *Stenotrophomonas maltophilia, Scenedesmus abundans*+ *Stenotrophomonas maltophilia* and mixed algae + *Stenotrophomonas maltophilia* were 19.84±1.9, 40.32±1.13, 35.29±0.89, and 47.69%±1.68, respectively. *Chlorella pyrenoidosa*+ *Stenotrophomonas maltophilia, Scenedesmus abundans*+ *Stenotrophomonas maltophilia* and mixed algae + *Stenotrophomonas maltophilia* had better lipid yields for the different treatments, as shown in Fig. 13. From the study, it was concluded that the optimal concentration of 8% of the bacterial inoculum, pH 7 of the medium, and 90% aeration rate produced the maximum lipid yield.

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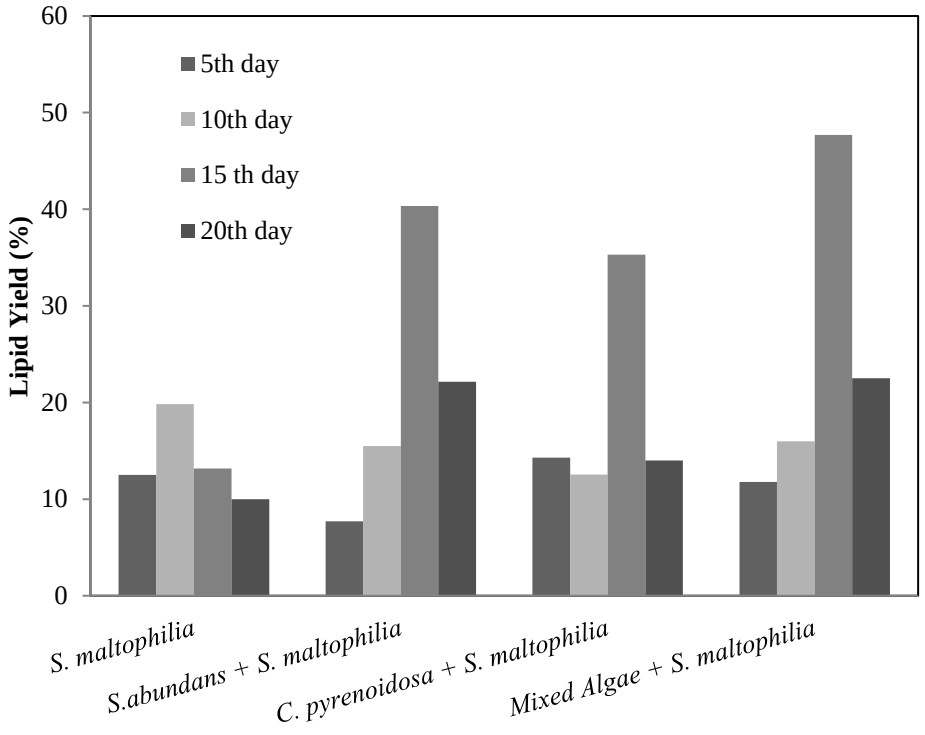

Fig.13 Response of different treatment conditions such as *S. maltophilia, C. pyrenoidosa+ S. maltophilia, S. abundans + S. maltophilia* and mixed algae + *S. maltophilia* in lipid production of biodiesel

Conclusion

Therefore, when *Stenotrophomonas maltophilia* was isolated and coupled with algae, better growth development was shown in the algae cells by symbiotic techniques. Different algal species were treated with symbiotic bacteria for biodiesel production. In this study, the culture conditions were optimized by varying the inoculum concentration of the symbiotic bacteria from 2 to 10%, the pH from 4 to 9 and the 30 to 30% (v/v) aeration rate. The results showed that the maximum lipid yield was obtained at an 8% bacterial inoculum concentration, a medium pH of 7, and a 90% aeration rate. Lipid production was obtained from different treatments, such as cultivation of *Stenotrophomonas maltophilia, Chlorella pyrenoidosa* + symbiotic bacteria, and *Scenedesmus abundans* + symbiotic bacteria, and from mixed cultures of both *Chlorella pyrenoidosa* and *Scenedesmus abundans* with symbiotic bacteria. Lipid yields of 13.16, 40.32, 35.29, and 47.69% were obtained for *Stenotrophomonas maltophilia, Chlorella pyrenoidosa* + symbiotic bacteria, *Scenedesmus abundans* + symbiotic bacteria and mixed cultures of both species with symbiotic bacteria, respectively.

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