Isolation and screening of microalgae from natural habitats in the midwestern United States of America for biomass and biodiesel sources

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Abstract

Native species of microalgae were isolated from natural water bodies in the Midwestern United States of America and were screened for the ultimate goal of mass cultivation in Missouri and the surrounding states, and for their potential as biomass and biodiesel sources. A number of different nutrient media recipes were utilized to isolate the maximum number of colonies from each field samples. These nutrient recipes were modified in order to optimize the isolation and growth dynamics of specific colonies. All of the isolates were categorized based on the morphological appearance of the culture and the microscopic cellular appearance of the isolated colonies. Isolates included many common green microalgae and cyanobacteria. Lipid content was determined for selected strains that demonstrated relatively quick growth. Scenedesmus sp. that demonstrated the high growth rate, resistance to invasion, and contained sufficient amounts of lipid was investigated for its potential as a sustainable biomass and biodiesel feedstocks.

Key words: Biodiesel, isolation, lipids, microalgal biomass, Midwestern USA

INTRODUCTION

The interest toward biomass as alternative sources of energy and raw material is on the rise due to concerns about depleting petroleum reserves and greenhouse gas problem. Biomass is considered as a renewable energy resource with net zero carbon emissions due to the fact that atmospheric CO₂ is fixed through photosynthesis. Increasing demand for biofuels has exposed a great need for the discovery of more productive, nonfood sources of biomass that can be converted to biodiesel and other transportation fuels. “Aquatic Species Program,” which was supported by the US Department of Energy during 1978-1996, has shown the merits of microalgae as a renewable and sustainable source for biodiesel production. Algae are generally defined as all photosynthetic eukaryotes (with the exception of land plants), although prokaryotic cyanobacteria, also known as blue-green algae, are sometimes included in this broad circumscription. Unicellular microalgae are the fastest growing, photosynthesizing organisms and can complete an entire growing cycle every few days if adequate amounts of sunlight, water, carbon dioxide, and nutrients are available. Some aquatic microalgal species have high oil contents (up to 60% of their biomass dry weight) and the potential to become a significantly more productive source of biodiesel when compared with terrestrial crops such as soybeans. Algae are nonfood resources that are amenable for cultivation on nonproductive land using saline water and wastewater.

Temperature, light intensity, amount and type of nutrients, amount of CO₂, and pH are the key factors influencing algal
Algae represent a very diverse and heterogeneous complex of organisms belonging to many different phyla, and characterized by very different physiological attributes. A direct consequence of this great diversity is that different species of algae have very different growth requirements. Therefore, location is a key determining factor for the selection of microalgal strains that can be used to produce biomass. Of the many algal strains available for the investigation of growth rates and biofuel production potential, the ideal strain will likely be different for each location, particularly if outdoor cultivation is utilized. The environmental conditions of a specific area can greatly influence microalgal populations and their growth dynamics. Therefore, the most logical approach is to screen for highly productive strains with maximum lipid contents at selected sites, and optimize the growth conditions for large-scale cultivation.

Tropical or subtropical regions with an abundant availability of solar radiation throughout the year, and saline water (either from the sea or as groundwater), are generally the best for mass cultivation of algae. In the United States, the southwestern states are characterized by these conditions, which is the reason they are considered the most suitable regions for growing algae. However, in other parts of the country, such as the mid-western states, environmental conditions are affected by much stronger seasonal variation; this region experiences a wide range of temperatures across seasons, which could greatly influence the dominant microalgal strains found there throughout the year. Local algal strains isolated from natural habitats and collected during different seasons are expected to be the best adapted to those specific local conditions; therefore, locally isolated strains would be the best for large-scale cultivation. For this reason, a careful screening and selection process to identify the most suitable local strains is an essential requirement for the successful development of microalgae-based biofuels. However, there has been very little research focused on the identification of microalgal strains suitable for mass cultivation in the Midwest, specifically in Missouri. In general, the microalgal diversity of this region has been studied fragmentarily, and with few exceptions, much of the information available is now outdated. Recent studies on the microalgae of Missouri and other mid-western states have mostly been concerned with ecological aspects and the evaluation of water quality.

The objective of this study was to isolate native microalgal species from a range of natural water bodies in the Midwestern United States and evaluate their potential for the production of lipids, with the ultimate goal of mass cultivation in Missouri and the surrounding states for biodiesel feedstock in the future.

**MATERIALS AND METHODS**

**Field collection**

Water samples with visible microalgal population were collected from ponds, lakes, and rivers that are located in the Midwestern states including Missouri and Nebraska. Sampling of large bodies of fresh water occurred at multiple sites along the waterfront. Collections were made for the top and bottom of the water at each location, with the goal of determining the dominant microalgal species in each area. Additional samples were obtained from the water bodies specifically located adjacent to Missouri coal-fired power plants in the Chamois, Thomas Hill, and New Madrid areas. All field samples were collected in 50 mL tubes and maintained at refrigerated condition while transferring to laboratory.

**Isolation**

In order to isolate single microalgal species from the field water samples, standard plating methods were used to separate algal populations. Multiple media recipes were utilized to isolate the colonies. The field samples were first diluted to aid in the isolation process. Sterilized plastic petri dishes (100 × 15 mm) containing approximately 40 mL of agarized medium were used to plate these diluted samples. One milliliter of the diluted sample was transferred to a media plate and spread evenly across the surface. Inoculated plates were placed in a temperature-controlled greenhouse (20-25°C, approximately 27 μE/m/s) where the algae were allowed to grow for about 14 days. Grown algae cultures were streaked using sterile technique onto additional sets of nutrient media plates and placed back in the greenhouse for isolation. This streaking method was repeated until isolation into axenic unialgal cultures was achieved. The number of colonies that were transferred from each dilution plate onto other nutrient media plates depended on the amount of contamination and the identification of the colonies present based on the colony morphology and the microscopic cellular morphology of each isolate. Following the isolation of individual microalgal colonies, each strain was initially labeled based on the sampling location and special media requirements. Isolated algae were maintained as stock cultures and were stored on a cool, low light shelf. These stock cultures were maintained by re-plating each onto new nutrient media at least once a month, or more frequently depending on the nature of each isolated strain.

**Nutrient media**

A number of different nutrient media recipes were utilized to isolate the maximum number of colonies from each field sample. These nutrient recipes were modified in order to optimize the isolation and growth dynamics of each colony. Common media recipes were used in the first stages of the isolation procedure, including a modified...
Guillard’s f/2 medium. Additional nutrient media were prepared in the laboratory: Jaworski’s medium, Von Stosch’s medium modified following Guiry and Cunningham (1984), and Bold’s Basal medium with vitamins. The general nutrient medium f/2 was purchased as two parts premixed products from the Aquatic Ecosystems (Apopka, Florida), and supplemented with nitrogen and phosphorus by adding Miracle-Gro plant food (Scotts Co, Marysville, Ohio) at a final concentration of 0.1 g/L. Guillard’s f/2 and Von Stosch’s media (meant for marine algae) were prepared using distilled water for the purpose of selecting organisms that are capable of growing in minimal nutrients, the composition contains vital nutrient salts (top off the nutrients of sea water) required for the algal growth.

Cyanobacteria in field samples were also isolated by using nutrient media recipes specifically optimized for the growth of cyanobacteria species. These media recipes were similar in their absence of nitrogen in any form in the actual recipe. The BG-11 medium was prepared from the modified BG-11 medium recipe by removing all forms of nitrogen from the mixture in order to support only heterocystous cyanobacteria. BB-LU containing no nitrogen was derived from the Bold’s Basal medium with vitamins. The final two nutrient media recipes (CyLU-1SM and CyLU-10SM) were composed of the dehydrated seawater (sea minerals) concentrate at 1 or 10 g/L concentrations, respectively, in addition to 0.4 g/L of K₂HPO₄·3H₂O and 15 g/L of agar. All nutrient media recipes were sterilized in an autoclave prior to inoculation, except for the seawater concentrate, which was filter sterilized and added after autoclaving the other portions of the media. Distilled water was used unless otherwise specified in the recipe.

Morphological identification
Microalgal and cyanobacterial cultures were initially separated based on morphological examination of the colonies on an agar nutrient medium. This general classification method was only used to distinguish isolates on the most basic level. Identification of these isolates to the genus level was based on the morphology of the individual cells following microscopic examination. The strains were identified using the methods similar to reported by Wehr and Sheath. Each isolate in the collection was labeled and photographed at three magnifications (20×, 40×, 60×) using the Nikon Eclipse E800 microscope (Nikon Inc., Tokyo, Japan) with the DXM1200 digital camera and the ACT-1 software program. A catalogue of these isolates was established for future reference. Photographic comparison of the original isolates with stock cultures was performed periodically to ensure that contamination had not occurred.

Cultivation and harvesting
Each algal culture sample was monitored every day for cellular growth rates by measuring optical density at 680 nm. A spectrophotometer (Thermo Electron Corp Spectronic 20D+ model) was used after calibrating it with a sample of the F/2 and Miracle Gro mixture media as blank. The cultures were continuously aerated using air pumps with air stones, and the specified media was added to each culture at the end of every week. For the microalgal biodiesel study, the _Scenedesmus_ species isolated from a local (Jefferson City, Missouri) pond was mass-cultivated in two 200-L stainless steel basins (0.6 m dia. × 0.8 m depth), which were placed outdoor next to the greenhouse. Constant mixing of the algal culture in the tank was provided by the aeration. The temperature of the mass culture of algae in the tank remained between 21°C and 32°C. Large volume harvesting of the microalgae was achieved by the flocculation of algae after adding 2 mg chitosan (Federal Laboratory Corp, Alden, NY) per liter of algal culture and subsequent gravimetric settlement.

Lipid content and biodiesel characterization
The algae isolates showing high growth rates were selected for the determination of lipid content. Each algal culture was initially centrifuged at 3700 rpm for 30 min and the water was decanted. The resultant wet algae pellet was lyophilized completely. A modified Bligh and Dyer method was used to determine the total lipid content. First, the dried algae sample was extracted with a chloroform — methanol — water mixture (5:10:4 v/v). The extracts were fractionated by adjusting the final solvent mixture (10:10:9 v/v). The lower chloroform layer containing lipids was evaporated under a stream of nitrogen with gentle heating. The total lipid weight was determined gravimetrically. The microalgal lipids extracted with hexane were converted to biodiesel via a base-catalyzed transesterification reaction. The reaction mixture consisting methanol/oil ratio of 6:1 and 1% (w/w) sodium methoxide was refluxed at 60°C for 6 hours and transferred to a separator funnel. The top layer containing the biodiesel was rotary-evaporated to remove methanol and washed with distilled water to remove remaining residues. The fatty acid methyl ester (FAME) composition of final biodiesel product was analyzed with a gas chromatograph (Model Focus GC, Thermo Electron Co., Waltham, MA, USA) equipped with a flame ionization detector (FID). A fused silica capillary column (DB-225, 30 m × 0.25 mm i.d., 0.25 μm film, Agilent JandW Scientific Co., Folsom, CA, USA) coated with 50% cyanopropylphenyl-dimethylpolysiloxane stationary phase was used. The retention time and peak size were compared with the FAME standard mixture (Supelco Analytical, St. Louis, Missouri) and the soybean biodiesel obtained from the Missouri Soybean Merchandizing Council.
(Jefferson City, Missouri). Helium was used as a carrier gas at constant flow of 1.2 mL/min. The temperature of GC oven was initially held at 50°C for 1 min and increased to 230°C at 10°C/min rate. The temperature of inlet and FID was maintained at 250°C and 250°C, respectively.

RESULTS

More than 300 algal strains from approximately 30 field sampling locations in the Midwestern U.S., which included the states of Missouri and Nebraska, were collected and evaluated as potential sources of biomass and biodiesel. All of the isolates were categorized based on the morphological appearance of the culture and the microscopic cellular appearance of the isolated colonies. Isolates included many common green microalgae and cyanobacteria. The isolated strains of microalgae ranged from unicellular to filamentous in form. A number of the isolated cyanobacteria specifically possessed heterocysts, giving them the potential to fix nitrogen. The color of the cultures ranged from dark green to brown for the eukaryotic microalgae, while most of the cyanobacteria demonstrated the characteristic blue-green coloration, with the exception of a few brown strains as well.

The majority of our isolated strains were identified at the genus level based on microscopic morphological examination. Many different strains of microalgae were identified based on the cellular morphology of each strain isolated [Table 1]. Specifically, five different cyanobacteria were isolated. [Figure 1] shows microscopic pictures of a few selected microalgae. The strains isolated ranged in morphology from microalgae with unicellular organization to colonial and filamentous forms with either branched or unbranched organization. *Chlorella*, *Chlorococcum*, *Cosmarium*, *Monoraphidium*, and *Dictyochloropsis* were the most common unicellular algae, whereas forms with colonial organization included *Scenedesmus*, *Coelastrum*, and *Ankistrodesmus*. Filamentous strains included *Cladophora* (branched), *Hydrodictyon* (branched), *Klebsormidium* (unbranched), *Anabaena* (unbranched), *Nostoc* (unbranched), *Leptolyngbya* (unbranched), *Lyngbya* (unbranched), *Oscillatoria* (unbranched), *Phormidium* (unbranched), and *Ulothrix* (unbranched). A few of these identified strains are capable of being either unicellular or colonial, including *Oocystis* and *Kirchneriella*. *Anabaena* and *Nostoc* are cyanobacteria that contain heterocysts as part of the filamentous strand that comprises their structures, giving them the ability to readily fix nitrogen.

Lipid contents were determined for selected algae strains and are compared in [Table 2]. These strains demonstrated relatively rapid growth, and provided an adequate amount of biomass for the lipid analysis to be performed. *Scenedesmus* strains produced consistently high levels, 19.9-43.6% of their cellular mass, of lipids. These lipid contents are comparable to the previously reported

![Figure 1: Photomicrographs of selected microalgal isolates from natural waters in Midwestern US. Each division of the scale represents 10 μm.](image)

| Location          | Sampling month | Genera identified          |
|-------------------|----------------|---------------------------|
| Jefferson City, MO| December       | Ankistrodesmus            |
| 38°33'53.64" N 2|                | Coelastrum                |
| 92°50'00.89" W   |                | Scenedesmus               |
| Central Park, NE  | November       | Chlorella                 |
| 42°01'31.89" N 2|                | Coelastrum                |
| 97°24'52.04" W   |                |                           |
| Adams Park, NE    | November       | Chlorella                 |
| 41°17'09.47" N 2|                | Dictyochloropsis          |
| 95°57'53.17" W   |                | Phormidium                |
| Fontenelle Park, NE| November     | Chlorella                 |
| 41°17'50.02" N 2|                | Lyngbya                   |
| 95°58'54.83" W   |                | Phormidium                |
| New Madrid, MO    | May            | Chlorella                 |
| 36°31'01.28" N 2|                | Oscillatoria              |
| 89°36'47.11" W   |                |                           |
| Chamois, MO       | June           | Chlorella                 |
| 38°40'56.43" N 2|                | Leptolyngbya              |
| 91°45'30.05" W   |                | Phormidium                |
| 12°00'14.26" W   |                |                           |
| Westphalia, MO    | September      | Anabaena                  |
| 38°27'08.16" N 2|                |                           |
| 92°00'14.26" W   |                |                           |
| Thomas Hill, MO   | May            | Anabaena                  |
| 39°35'02.06" N 2|                | Chlorella                 |
| 92°38'06.34" W   |                | Scenedesmus               |

Denotes cyanobacteria
productivity; 12-14% and 16-40% for *Scenedesmus obliquus* and *Scenedesmus dimorphus* strains, respectively.[26]

The FAME composition of the biodiesel derived from the mass-produced *Scenedesmus* lipids is shown in Table 3. Two major fatty acid components were palmitic acid (C16:0) and oleic acid (C18:1) at 27.3% and 32.7% of the total lipids, respectively. This proportion correlates closely with the previous studies. Makulla[27] reported the lipid contents of *Scenedesmus obliquus* to be 35.9-43.1% for C16:0 and 11.5-28.6% for C18:1. *Scenedesmus* sp. cultivated with high level (10%) of carbon dioxide resulted in the 36.3% and 25.9% levels for C16:0 and C18:1, respectively.[28] Contrast to the conventional soybean-derived biodiesel, the biodiesel derived from *Scenedesmus* lipids contained significantly lower level of oxidation-prone methyl linolenate [Figure 2]. The high oleic acid composition suggests the improved properties of the resulting algal biodiesel fuel.[29]

**DISCUSSION**

In general, the culture medium that proved to be the most successful in cultivating a wide range of isolates was f/2 mixture supplemented with Miracle-Gro plant food, which offered increased nitrogen and phosphorus amounts. The majority of microalgal isolates received adequate concentrations of their required nutrients from this general medium, and they seemed to respond positively. However, for heterocystous cyanobacteria isolation purposes nitrogen-free media recipes that were more selective for nitrogen-fixing cyanobacteria were utilized. The CyLU<sub>0</sub>-1SM media was successful for the isolation of several cyanobacteria, as well as BBLU<sub>0</sub> and BG-11. Cyanobacteria isolates grown on these three media recipes demonstrated very similar growth patterns, with no

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**Table 2: Lipid content of selected algae isolates**

| Genus   | Origin               | Location                     | Media<sup>a</sup> | Lipid content<sup>b</sup> (% dry wt.) |
|---------|----------------------|------------------------------|-------------------|---------------------------------------|
| Chlorella | Jefferson City, MO   | 38°33'53.64" N 92°50'00.89" W | BB                | 9.0                                   |
| Chlorella | Jefferson City, MO   |                             | VS                | 18.5±7.4                              |
| Chlorella | Central Park, NE     | 42°01'31.89" N 97°24'52.04" W | J                 | 29.1±3.4                              |
| Chlorella | Fontenelle Park, NE  | 41°17'50.02" N 95°58'54.83" W | f/2               | 31.7                                  |
| Chlorella | Fontenelle Park, NE  |                             | J                 | 28.3                                  |
| Chlorella | Fontenelle Park, NE  |                             | VS                | 20.2±6.3                              |
| Cladophora | Thomas Hill, MO     | 39°35'02.06" N 92°38'06.34" W | f/2               | 10.9                                  |
| Coelastrum | Jefferson City, MO  | 38°33'53.64" N 92°50'00.89" W | VS                | 26.0±22.3                             |
| Dictyochlorosis | Adams Park, NE | 41°17'09.47" N 95°57'53.17" W | VS                | 17.4                                  |
| Scenedesmus | Jefferson City, MO | 38°33'53.64" N 92°50'00.89" W | f/2               | 21.7                                  |
| Scenedesmus | Jefferson City, MO |                             | J                 | 19.9±7.2                              |
| Scenedesmus | Adams Park, NE     | 41°17'09.47" N 95°57'53.17" W | J                 | 43.6±6.8                              |
| Scenedesmus | Fontenelle Park, NE | 41°17'50.02" N 95°58'54.83" W | VS                | 28.0±0.7                              |
| Scenedesmus | Fontenelle Park, NE |                             | VS                | 34.3                                  |
| Ulothrix | Chamois, MO          | 38°40'56.43" N 91°45'30.05" W | f/2               | 10.4                                  |
| Anabaena<sup>c</sup> | Jefferson City, MO | 38°33'53.64" N 92°50'00.89" W | f/2               | 7.8                                   |
| Lyngbya<sup>c</sup> | Adams Park, NE | 41°17'09.47" N 95°57'53.17" W | J                 | 9.1±2.2                               |
| Lyngbya<sup>c</sup> | Fontenelle Park, NE | 41°17'50.02" N 95°58'54.83" W | VS                | 18.4                                  |
| Phormidium<sup>c</sup> | Adams Park, NE | 41°17'09.47" N 95°57'53.17" W | J                 | 22.5±8.7                              |
| Phormidium<sup>c</sup> | Fontenelle Park, NE | 41°17'50.02" N 95°58'54.83" W | BB                | 11.6                                  |

<sup>a</sup>BB: Bold’s basal, VS: Von stosch’s, J: Jaworski’s, f/2, <sup>b</sup>Mean ± Standard deviation, Results from 1-4 cultures, <sup>c</sup>indicates cyanobacteria

**Table 3: Fatty acid profile of lipids extracted from microalga Scenedesmus sp. cultivated outdoor as 400 L cultures in open-top tanks**

| Fatty acid | % Composition<sup>a</sup> |
|-----------|----------------------------|
| 14:00     | 1.1±0.2                    |
| 16:00     | 27.3±0.3                   |
| 16:01     | 1.6±0.2                    |
| 16:02     | 1.1±0.3                    |
| 18:00     | 3.8±0.2                    |
| 18:01     | 32.7±0.3                   |
| 18:02     | 14.5±0.3                   |
| 18:03     | 17.4±0.5                   |
| 20:00     | 0.5±0.4                    |

<sup>a</sup>Mean ± Standard deviation from three replicates

Figure 2: Comparison of major fatty acids content of biodiesel derived from microalga *Scenedesmus* sp. cultivated in large-scale and soybean. (n = 3 replicates)
particular medium resulting in an observable difference in growth rate. The other cyanobacteria-selective media recipes did not show the same growing potential for our cyanobacteria isolates as the three listed above, or resulted in no growth of any cultures. Multiple green algae isolates were also inoculated on these cyanobacteria-selective media recipes, along with the general f/2 and Miracle-Gro mixture. The results indicated reduced, or completely inhibited, growth as well as reduced chlorophyll concentrations of the green algae grown on any of the cyanobacteria media recipes in comparison to our general f/2 and Miracle-Gro mixture. The isolation of algae from natural water samples required the use of multiple media types containing various concentrations of many key nutrients in order to ensure the recovery of all algal species present in the samples. For example, green algae do not thrive on media lacking a nitrogen source. Yet, cyanobacteria that are able to fix nitrogen using nitrogenase confined inside heterocysts can thrive without the addition of nitrogen to the medium recipe.\[30\] Green algae are thought to consume intracellular nitrogen pools, such as chlorophyll, when extracellular nitrogen is not available, resulting in hindered growth.\[8,9\] However, other nutrient requirements including iron and phosphorus can limit the growth of these cyanobacteria, demonstrating the importance of meeting the nutrient requirements of specific species for isolation.\[31\]

Microalgal species require various concentrations of the major nutrients composing most media recipes, but the ratio of these components is also very important in lipid production. The types of nutrient media used during the cultivation of these samples could have multiple effects on the composition of the algal cell, which may also be influenced by the particular species of each isolate and the environment from which the isolates were collected. There are many general characteristics that make certain microalgal strains more desirable than others for biodiesel production. The first is the fast growth rate of the selected strains. The second characteristic is a high content of lipids, which are used to produce biodiesel through a transesterification process. It has been demonstrated that limitation of certain nutrients, especially of nitrogen, can result in higher lipid storage in microalgal cells.\[32,33\] Since a high content of nutrients is necessary for a high growth rate, this implies that fast growth and accumulation of large amounts of lipids normally cannot take place at the same time. Therefore, the focus is geared toward the fast growing species because those strains with high lipid contents tend to grow slower. Even though fast-growing species tend to have lower lipid contents on average, the accumulated biomass may be utilized for various other energy-producing avenues (methane or ethanol production, etc.) aside from biodiesel production.\[8,9\]

Although a number of algae isolates adapted well to the environmental conditions in our central Missouri study location, one strain demonstrated the greatest dominance in our testing region. *Scenedesmus* sp. collected from the immediate vicinity of our culturing facility showed the best growth dynamics. This *Scenedesmus* isolate had an average lipid content of 20.8% by weight, and demonstrated the greatest potential as a source of biomass and lipids because of the shear body mass amount that could be accumulated in a short amount of time compared with other isolates. Invasion and contamination by other species was not observed, even when grown outdoors in a 200-liters open basin as long as a dense culture had been established. The isolate sustained a pure culture in the presence of other isolates growing in adjacent vessels. Therefore, this strain was used in the majority of the growth experiments. Upon harvesting the *Scenedesmus* strain yielded approximately 1 g dry weight biomass per liter of culture.

Another strain of particular interest was identified as a member of the genus *Ulothrix*. This isolate showed a relatively quick growth rate compared with the other filamentous isolates. With a relatively low lipid content of 10.4%, the *Ulothrix* isolate may not be a high lipid producer compared with *Scenedesmus*, but it appeared to be a suitable candidate for the power plant site from which it was isolated due to its adaptation to the environmental conditions of the specific location. Cyanobacteria isolates also have the potential for successful cultivation because they are photosynthetic organisms with the capability of nitrogen fixation. Exclusion of nitrogen allows *Anabaena* to grow uncontaminated by healthier green algae that require nitrogen to survive. *Anabaena* cultures were successfully grown in a media mixture containing only concentrated seawater, which is low in both nitrogen and phosphorus.

**CONCLUSION**

Local weather is one of the major limiting factors when determining which microalgal strains can be grown quickly in an established area. Despite having relatively high lipid content, the microalgal species cannot be used for biodiesel production if it does not grow adequately in that predetermined location.\[4\] Local species have long been adapted to the prevailing regional abiotic and biotic factors, and thus are evolutionarily primed for local bioresource production. Through this work certain species like *Scenedesmus* had found to have scope for the successful production of biomass for biodiesel feedstock in massive scale.

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REFERENCES

1. Usui N, Ikenouchi M, The biological CO2 fixation and utilization project by RITE (1): Highly effective photobioreactor system. Energy Conver Manage 1997;38:5487-92.
2. Stepn D, Shockey RE, Moe TA, Dorn R. Carbon dioxide sequestering using microalgal systems, EERC, publication-EERC-02-03. Grand Forks, ND: Energy and Environmental Research Center; 2002.
3. Sheehan J, Dunahay T, Benemann J, Roessler P. A look back at the U.S. Department of Energy’s Aquatic Species Program: Biodiesel from algae. Close-Out report. National Renewable Energy Lab, Department of Energy, Golden, Colorado, Report number NREL/TP-1998. p. 580-24190.
4. Delwiche CF. Algae in the warp and weft of life: Bound by plastids. In: Brodie J, Lewis J, editors. Unravelling the algae: The past, present and future of algal systematics, The Systematics Association Special Volume Series 75. Boca Raton, London and New York: CRC Press; 2007. p. 7-20.
5. Lee RE. Phycolgy. 2nd ed. Cambridge: Cambridge University Press; 1989. p. 645.
6. Li Y, Horsman M, Wang B, Wu N, Lan CQ. Effects of nitrogen sources on cell growth and lipid accumulation in green alga Neochloris oleoabundans. Appl Microbiol Biotechnol 2008;81:629-36.
7. Liu ZY, Wang GC, Zhou BC. Effect of iron on growth and lipid accumulation in Chlorella vulgaris. Bioreosour Technol 2008;99:4717-22.
8. Chisti Y. Biodiesel from Microalgae. Biotechnol Adv 2007;25:294-306.
9. Hossain AB, Saleh A, Boyce AN, Chowdhury P, Naquddin M. Biodiesel Fuel Production from algae as renewable energy. Am J Biochem Biotechnol 2008;4:250-4.
10. Sakai N, Sakamoto Y, Kishimoto N, Chihara M, Karube I. Chlorella strains from hot springs tolerant to high temperature and high CO2. Energy Conver Manage 1995;36:693-6.
11. Kurano N, Ikemoto H, Miyashita H, Hasegawa T, Hata H, Miyachi S. Fixation and utilization of carbon dioxide by microagal photosynthesis. Energy Conver Manage 1996;36:689-92.
12. De Morais MG, Costa JA. Isolation and selection of microalgae from coal fired thermoelectric power plant for biofixation of carbon dioxide. Energy Conver Manage 2007;48:2169-73.
13. Borowitzka MA. Commercial production of microalgae: Ponds, tanks, tubes and fermenters. J Biotechnol 1999;70:313-21.
14. Grobelaar JU. Algal biotechnology: Real opportunities for Africa. South Afr J Bot 2004;70:140-4.
15. Dempster TA, Sommerfeld MR. Effects of environmental conditions on growth and lipid accumulation in Nitzchia communis (Bacillariophyceae). J Phycol 1998;34:712-21.
16. Andersen RA, Berges JA, Harrison JP, Watanabe MM. Appendix A: Recipes for freshwater and seawater media. In: Andersen RA, editors. Algal culturing techniques. Burlington, Elsevier, San Diego and London: Academic Press; 2005. p. 429-532.
17. Tompkins J, Deville MM, Day JG, Turner MF. Culture collection of algae and protozoa. Catalogue of strains. The culture collection of algae and protozoa. Ambleside: Institute of Freshwater Ecology; 1995. p. 204.
18. Guiry MD, Cunningham EM. Photoperiodic and temperature responses in the reproduction of north-eastern Atlantic Gigartina acicularis (Rhodophyta: Gigartinales). Phycologia 1984;23:357-67.
19. Bischoff HW, Bold HC. Phycological Studies IV. Some soil algae from Enchanted Rock and related algal species. Austin: Texas: University of Texas; 1963. . Publication No. 6318.
20. Starr RC, Zeikus JA. UTEX-The culture collection of algae at the University of Texas, Austin. J Phycol 1993;29:1-106.
21. Allen MM. Simple conditions for growth of unicellular blue-green algae on plates. J Phycol 1986;4:1-4.
22. Allen MM, Stanier RY. Growth and division of some unicellular blue-green algae. J Gen Microbiol 1968;51:199-202.
23. Rippka R, Deruelles J, Waterbury JB, Herdman M, Stanier R. Generic assignments, strain histories and properties of pure culture of cyanobacteria. J Gen Microbiol 1979;111:1-61.
24. Wehr JD, Sheath RG. Freshwater algae of North America. Ecology and Classification. Amsterdam: Elsevier Inc; 2003. p. 918.
25. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physiol 1959;37:911-7.
26. Becker EW. In: Microalgae: Biotechnology and microbiology. Ed Baddiley J, et al., New York: Cambridge University Press; 1994. p. 178.
27. Makulla A. Fatty acid composition of Scenedesmus obliquus: Correlation to dilution rates. Limnologica 2000;30:162-8.
28. Yao C, Jun SY, Lee JY, Ahn CY, Oh HM. Selection of microalgae for lipid production under high levels of carbon dioxide. Bioreosour Technol 2010;101:71-4.
29. Knothe G. Designer biodiesel; optimizing fatty acid ester composition to improve fuel properties. Energy Fuels 2008;22:1358-64.
30. Adams DG. Cyanobacterial phylogeny and development: Questions and challenges. In: Brun YV, Shimkets L, editors. Prokaryotic development, Washington DC: ASM press; 2000. p. 51-81.
31. Berman-Frank I, Quigg A, Finkel Z, Irwin A, Haramaty L. Nitrogen-fixation strategies and iron requirements in cyanobacteria. Limnol Oceanograph 2007;52:2260-9.
32. Negoro M, Shioji N, Miyamoto K, Micira Y. Growth of microalgae in high CO2 gas and effects of SOx and NOx. Appl Biochem Biotechnol 1991;28:877-86.
33. Shen Y, Pei Z, Yuan W, Mao E. Effect of nitrogen and extraction method on algae lipid yield. Int J Agric Biol Eng 2009;2:51-7.

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