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Screening thermophilic algae for CO\textsubscript{2} sequestration and production of fatty acids

Introduction

Accumulation of anthropogenic carbon dioxide in the atmosphere is a reality and a challenge for science and technology today. At the beginning of industrial era, the CO\textsubscript{2} concentration in the air was around 275ppm and it is considerably increased to 379ppm in first year of this century contributing to the greenhouse effect and consequently causing global warming (Climate Change 2007:Synthesis Report).

United Nations promoted the Kyoto protocol (1997) with the objective of reducing GHG by 5.2%. More than 170 countries have ratified the protocol. There are various methods of CO\textsubscript{2} sequestration including physiological, geological, oceanic, chemical and biological. Chemical reaction based CO\textsubscript{2} sequestration typically consists of three procedures, separation, transportation and sequestration. The cost of CO\textsubscript{2} separation and compression is estimated to be $30-50 per T of CO\textsubscript{2} and transportation and further sequestration cost is estimated to be $1-3 per T. As the method is costly and energy consuming, the mitigation benefits become marginal (Wang et al; 2008).

Biological CO\textsubscript{2} mitigation has attracted much attention as an alternative strategy, which involves fixation of atmospheric CO\textsubscript{2} by photosynthesis leading to biomass energy through plants and microalgae. Terrestrial plants contribute to about 3-6% of CO\textsubscript{2} sequestration and this is due to their slow growth rate (Wang et al loc cit). On the other hand, microalgae and photosynthetic bacteria are fast growing and are reported to have an ability to fix CO\textsubscript{2} 10-50 times more efficiently than terrestrial plants (Li et al; 2008).

Microalgal CO\textsubscript{2} sequestration has several other merits. A very important one is the direct CO\textsubscript{2} utilization from flue gases. This fact is very important as separation of CO\textsubscript{2} from flue gas contributes to 70% of total sequestration cost (Lee and Lee; 2003). Biomass produced can be used in a range of products including animal food, cosmetics, pharmaceuticals and fuels. CO\textsubscript{2} sequestration can be made even more cost effective by
utilising wastewater for algal cultivation (Chinnasamy et al; 2009, Yun et al; 1997, Kumar et al; 2010).

Extensive work has been carried out to find suitable strain of algae tolerant to high concentration of CO₂. The effect of pure CO2 in varying concentration on a number of strains has been examined (Hanagata et al; 1992, Ono and Cuello; 2007). Wantanabe et al; 1992 isolated fresh water green algae Chlorella HA-1 from paddy fields. It showed maximum growth at 5-10% CO₂ but when the concentration of CO₂ was increased higher than 10% the growth rate decreased remarkably. Kodama et al; 1993 reported a new highly CO₂ tolerant marine algae Chlorococcum littorale, the algae has optimum growth rate at 5% and 10% CO₂, with 20% CO₂ growth rate was comparable to optimum and they proposed that the strain will be applied to power plants located near sea shore.

Flue gases emitted from industrial sources contain various concentrations of NOx and SOx and inhibition due to these toxic compounds is inevitable if flue gas is directly fed to the microalgal culture. To overcome the inhibition effect, several studies have been carried out to study the effect of simulated or direct flue gases on algal growth. Sung et al; 1998 isolated highly CO₂ tolerant microalgae Chlorella KR-1. Growth of this strain is not inhibited up to 20% CO₂, 100ppm NOx and 50ppm SOx. Controlling pH of the media increased tolerance of microalgae which allowed them to grow well up to 250ppm SOx and 300ppm NOx. Much of the studies have been conducted on the flue gas originated from power plants. Maeda et al; 1995 cultured Chlorella T1 in 70 L Cylindrical bioreactor with aeration of flue gas (13% CO₂, 5% O₂, 10ppm SOx and 30ppm NOx) from boiler. The culture grew well without any negative impact on growth compared to growth with pure CO₂ source.

Use of microalgae for CO₂ sequestration from flue gases originated from various sources has not been simply restricted to academic level but it has been going on globally in various academic and commercial organizations. Attempts have been made for CO₂ sequestration both in open ponds and closed photobioreactor. Aquatic species program;

Limitations of the open ponds allowed to find the different ways for the large cultivation of the microalgae. Closed photobioreactors prevent direct gas exchange with the atmosphere, as there is less out gassing and loss of the CO₂. They have been reputed for very high productivities. Though the accumulation of O₂ in closed systems inhibits algal growth it can be overcome by having a degassing area. Most importantly closed photobioreactors allows cultivation of algal strains that otherwise could not be cultivated in open ponds.

**A2BE carbon captures Boulder**, Colorado, US made attempts for CO₂ sequestration in photobioreactor, roller film PBR. Similar research carried out at **Ohio State University**, where a pilot scale membrane photobioreactor has been designed for enhanced CO₂ sequestration and characterisation of the growth of thermophilic strain of *Chlorogleopsis* (SC2) isolated by Dr. Keith Cooksey of Montana State University from Yellowstone National Park. **RWE energy, Germany** has successfully developed a, bubble column reactor system connected to series of tubular reactor, for binding CO₂ from power plant in microalgae.

In India, the lead was taken by ACC Cement Company in collaboration with KET's VG Vaze College, Mumbai in 2008. A primary level project was carried out wherein sequestration of upto 23% CO₂ was carried out in a 2 litre air lift photobioreactor using a species of *Chlorella vulgaris* (SAG 211.12). The main constraint in using such strains is that they do not tolerate temperatures beyond 30°C and are not suited for Indian climatic conditions. In tropical countries (like India) ambient temperature is 32-40°C, which does not support its growth. For CO₂ sequestration microalgal species to be used should be able to withstand warm temperatures (since the emitted flue gases would raise the overall temperature of the culture medium), should possess a broad pH optima, ability to withstand accessory gases that accompany flue gases, scalable to industrial size
photobioreactors and most importantly be capable of accumulating high value metabolites under stressing and non stressing conditions (Olaizola, 2003).

Hence attempts were undertaken at KET's V. G. Vaze College to isolate algal strains from hot springs located across the Western Ghats of Maharashtra. Owing to the extreme climatic conditions, these unidentified strains have shown tolerance to higher temperatures as well as high levels of sulphates as they are isolated from thermal sulphur springs.

In the present communication, we dealt with cultivation of isolated algae in tubular airlift photobioreactor for CO₂ sequestration. A lab scale tubular airlift reactor was used to test the feasibility of the gaseous CO₂ reduction, carbon fixation and biomass production. The isolated microalgal strains were screened for their growth at different temperatures, different pH, at increased sulphate concentrations and various CO₂ concentrations to find out suitable strain sustaining at higher temperature (30-35°C) without requirement for pH adjustment for CO₂ sequestration. We also dealt with the screening of the isolated algal species for their ability to produce lipid and fatty acids. Cultures were screened at different temperature to study the effect of temperature on lipid and fatty acid production. Our work comprises of the two chapters;

Chapter I: Isolation, cultivation of algal strains from hot springs located in Western Ghats of Maharashtra and screening their ability to sequester atmospheric CO₂.

Chapter II: Screening of microalgal species isolated from hot springs of Western Ghats of Maharashtra for their ability to produce lipid and fatty acids.
Chapter I: Isolation, cultivation of algal strains from hot springs located in Western Ghats of Maharashtra and screening their ability to sequester atmospheric CO$_2$.

Introduction:

This comprises of the general introduction regarding the biological, plant and algal CO$_2$ sequestration. It explains the advantages of using algae for CO$_2$ sequestration over terrestrial plants. It also explains the work carried out at various institutes regarding the CO$_2$ sequestration using algae and need for screening algae for CO$_2$ sequestration. Different microalgal culture systems and advantages of the use of photobioreactor over open ponds for CO$_2$ sequestration have been explained. Use of simulated and direct flue gas for algal CO$_2$ sequestration is also explained.

Material and methods:

It gives the methods for isolation of algal cultures collected from various hot springs located in the Western Ghats of Maharashtra and cultivation of the algae.

It also discusses the optimization of the culture conditions for the growth of isolated microalgal strains. Strains were cultured at different temperatures and the effect on growth was checked. Requirement of the specific media, pH and SO$_4$ concentration were optimized. It also discusses the assembly of the 2 L airlift photobioreactor for CO$_2$ sequestration. It discusses the methodology for sparging of the CO$_2$ at specified concentrations and its effect on the pH of media and growth of algae.

Results:

Out of the microalgal strains isolate from hot springs the algal cultures A.1.1 (Scenedesmus sp.), A.1.2F (Limnothrix redekei), A.1.5 (Phormidium sp.), A.1.7 (Planktolyngbya crassa), A.2.1 (Chrococcum sp.) and A.3.1 (Geitlerinema sulphureum) were short listed for CO$_2$ sequestration. For these algae various culture conditions like
media combinations, pH, temperature and SO₄ were optimized. Except culture *Scenedesmus* sp. and *Limnothrix redekei* all other cyanophytes showed temperature tolerance upto 42°C. But optimum growth was achieved at 32°C. generally BG-11 medium contains 0.075 mg/ml of SO₄ concentration but since the cultures were isolated from sulphur springs the algae specially *Geitlerinema sulphureum* and *Planktolyngbya crassa* were tolerant to high SO₄ concentration as high as 3.0 mg/ml. Algae *Scenedesmus* sp. and *Limnothrix redekei* showed optimum growth at pH range 6-7. But *Geitlerinema sulphureum* and *Planktolyngbya crassa* were capable of thriving at broader pH range from 6-11. This fact has a great significance in CO₂ sequestration. Because when algae grows and utilizes CO₂ for photosynthesis, the pH of the media become basic increasing pH of the medium as high as 11 but when the algae are fed with artificial flue gases like CO₂, NOₓ or SOₓ these gases brings about decrease in pH.

In the present work for each culture, changes associated with the growth of algae due to CO₂ feeding were elaborately studied. The effect of CO₂ fed in the algal culture on the media pH and growth has been explained.

Effect of CO₂ concentration on the algal growth was studied in detail. Algal species *Scenedesmus* sp., *Limnothrix redekei* and *Geitlerinema sulphureum* were able to sustain concentration of CO₂ up to the 16.67%. Whereas *Planktolyngbya crassa* and *Chrococccum* sp. showed optimum growth at 23.08% of CO₂ concentration.

**Discussion:**

Culture *Geitlerinema sulphureum* has CO₂ tolerance up to 23.08% CO₂ concentration with higher biomass production. The strain is also able to grow at broader pH range 6-11 and tolerate higher temperature (42°C). The culture *Planktolyngbya crassa* showed growth at increased temperature (42°C), increased pH (9-10) and increased tolerance for CO₂ concentration (28.57%) but biomass produced was comparatively less. While culture *Limnothrix redekei* showed higher biomass production at increased CO₂ concentration up to 16.67%. But the pH tolerance of this strain and temperature tolerance was limited.
Chapter II: Screening of microalgal species isolated from hot springs of Western Ghats of Maharashtra for their ability to produce lipid and fatty acids

Introduction:

It discusses the need of biodiesel and various sources of biodiesel available. Advantages of the microalgae over the other sources for biodiesel production are discussed elaborately. Various species utilized earlier for lipid production are reviewed. The lipid productivity and fatty acid produced by various classes of algae are discussed.

Advantages of the cyanobacteria for lipid production are explained. The various environmental factors which stimulate the lipid production are discussed elaborately with supportive examples. Various methods of transesterification and separation of fatty acids are given.

Material and Methods:

This gives the detailed methods regarding the harvesting of algal biomass and lipid extraction. The method for transmethylation of the extracted lipids is explained elaborately for preparation of the fatty acid methyl esters.

HPTLC method was standardized for the separation of the fatty acids depending on their unsaturation level. It also gives the protocol for fatty acid methyl ester analysis both by HPTLC and GC method.

Results:

It discusses the lipid productivity and various cyanophycean strains isolated from hot springs.
Among the cultures screened mesophilic strain Scenedesmus sp. showed maximum lipid content, followed by Limnothrix redekei, Planktolyngbya crassa, Geitlerinema sulphureum and Chroococcum sp. Lowest lipid productivity was found in culture Phormidium sp.

Effect of the temperature on the fatty acid composition was studied for all strains using HPTLC method. It showed higher production of the saturated fatty acids at increased temperature (32°C and 42°C) while unsaturation was increased at lower temperature (22°C).

Detailed study of effect of temperature on the fatty acids was done by GC for shortlisted algal cultures (Limnothrix redekei, Geitlerinema sulphureum and Planktolyngbya crassa). The gas chromatogram supported the results of the HPTLC. Three strains screened showed accumulation of the fatty acids with short chain length (> C18). All these strains showed palmitic acid as major fatty acid. They also showed presence of the stearic (C18:0), oleic (C18:1) and linoleic acid (C18:2).

Discussion:

In all the strains at ambient temperature C16:0 (palmitic acid) was higher. It also showed production of the C18:0 (Stearic acid, oleic acid, linoleic acid and linolenic acid) fatty acids. But at reduced temperature 22°C the amount of the palmitic acid drastically decreased. Lower temperature also facilitates formation of the fatty acids of short chain length (<C16). Slight increase in the unsaturated fatty acids was found.
Bibliography


