A Green Approach to Mitigate CO2 Using Mixed Microbial Culture

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1. Introduction
In 19th century, science community came to know about the gases that cause “green house effect” which contributes to the global warming. Carbon dioxide (CO2) is found to be one of the green house gases (GHGs) causing “green house effect” and it is found that its level has continuously increased and has raised global temperature. The emissions of GHGs have increased significantly due to human activities and CO2 is considered as one of the most important anthropogenic (Human impact) GHG [1, 2]. As of July 2011, CO2 in the earth’s atmosphere is at a concentration of 392 ppm by volume which is 35% higher as compared to the mid-1800s [3].

The increased levels of CO2 concentration in atmosphere is mainly due to the use of fossil fuels either for the energy production or for the consumption of the energy. In order to meet the energy demand for substantial growth, there is a great challenge to develop the mitigation and sequestration method to reduce CO2 concentration in the earth’s atmosphere [3-5].

1.1 Techniques for CO2 mitigation and sequestration
CO2 capture and sequestration or CCS involves separation of CO2 from the point source, transportation to the storage site and storing it in such a way that it does not enter into the atmosphere. Storage of the CO2 is envisaged either in deep geological formations, in deep ocean masses, or in the form of mineral carbonates. A major concern with CCS is the leakage of stored CO2. For ocean storage, the retention of CO2 would depend on the depth. Presently, most carbon capture and sequestration discussions are about geological storage of CO2. Even if this is proven safe, the biggest difficulty with this approach is the added cost of separation and transportation of CO2 from the emission streams. The CCS requires three basic steps: separation, transportation and storage. The cost of CO2 separation is projected around $30 – 50 per ton of CO2. The other cost such as transportation and sequestration is around $1 – 3 per ton per 100 km and $1 – 3 per ton of CO2, respectively [6, 7, and 8].
1.2 Biological route

In the recent years, biological route has been identified as one of the most viable options available for the mitigation of CO\textsubscript{2}. There are six pathways through which CO\textsubscript{2} known to be fixed in atmosphere out of which the Calvin cycle (Calvin- Benson – Bassham) used by photoautroph’s to fix CO\textsubscript{2}. All the green plants, micro-alga, \textit{Cyanobacteria} comes under the category of Photoautotroph’s. It is found that CO\textsubscript{2} fixing ability of micro-alga is 1.8 times greater than terrestrial plant. These micro-algae, \textit{cyanobacteria} can also be used to produce bio-diesel, lipids, methane, hydrogen, amino acids etc. Thus, CO\textsubscript{2} fixation using micro-alga and \textit{cyanobacteria} has been reported as an alternate solution. The major problems of this route are (i) the continuous mitigation of CO\textsubscript{2} from waste streams, (ii) low efficiency of open pond cultivation, (iii) resistance of micro-alga towards NO\textsubscript{x} and SO\textsubscript{x} and (iv) the feasibility at industrial level is not very certain (v) Photo-bioreactors are expensive (9, 10, 11, 12, 13, 14, and 15). The remaining CO\textsubscript{2} fixing pathways are found to be operated in different aerobic and anaerobic microbes. These microorganisms are mainly from bacterial, archaeal domain and are called as extremeophiles. These microorganisms are omnipresent in nature and having capabilities to flourish in severe environmental condition. The ability of different microbes to remove pollutants from air has been already established. They can utilize CO\textsubscript{2} in one or another way in the absence of solar radiation and convert it into useful product (16, 17, 18, 19, and 20).

Mixed culture can be naturally selected or can be engineered on the basis of behavior of different microbial species towards each other. Naturally selected mixed culture helps in identification and characterization of microbes capable of CO\textsubscript{2} fixation. Engineered mixed culture helps to enhance the performance of CO\textsubscript{2} fixing ability in harsh industrial environment along with simultaneous waste water treatment, production of bio-methane and bio-hydrogen. The major advantage of mixed culture is that species together develop a symbiotic relationship in that environment in which they can’t survive individually (21, 22, and 23).

The need of industries in present scenario is the continuous mitigation of CO\textsubscript{2} from gaseous effluent streams at large flow rate and having high concentration of CO\textsubscript{2} along with other gases. It has been observed in several studies, that most of the industrial based applications (engineering based applications), mixed cultures are preferred over pure strain during long term operations (24, 25, 26, and 27). The reason is the adaptability of mixed culture in any environment unlike pure strain where certain specified conditions are to be maintained throughout.

Kumari, R., et al., (16) carried out an exhaustive review for the use of various microbial species to utilize inorganic compound CO\textsubscript{2} as a food source and its conversion into other organic compounds. Six pathways were discussed for fixing CO\textsubscript{2} using different microorganisms from archaeal and bacterial domain.

Megharaj et al., (22) carried out an exhaustive review on the behavior of different combinations of microbial species for the mitigation of CO\textsubscript{2}. This paper emphasized on the production of microorganisms metabolites along with simultaneous removal of environmental pollutants and waste water treatment. The combination of cyanobacteria, microalga and bacteria found to be more proficient than mono culture in detoxification of organic and inorganic pollutants, and removal of nutrients from wastewaters. The combination of different species and the construction of combination of preferred partners serve the dual purpose of CO\textsubscript{2} fixation along with removal of environmental pollutants.

Shu et al., (28) used the mixed culture of \textit{Chlorella} sp. and \textit{Saccharomyces cerevisiae} for CO\textsubscript{2} fixation and compared the result with mono-algal culture. The result showed a symbiotic
relationship of the mixed culture. The maximum CO₂ mitigation rate of a mixed culture was obtained as 64.76 mg L⁻¹h⁻¹, which was 195% improvement as compared to that of the monoculture of *Chlorella* sp. aerated with air. The biodiesel produced from the mixed culture showed better quality in terms of oxidative stability as compared to those from the monoculture. As seen from the earlier studies, there has been lot of work carried out in the field of CO₂ mitigation through biological route. Most of the studies are limited to the use of micro algal species for CO₂ mitigation. The major problems associated with the use of micro algal species in the industrial conditions are availability of light, tolerance to NOₓ & SOₓ, performance at high CO₂ concentration, slow rate of CO₂ mitigation, high capital and operating costs, contamination etc.

The present work deals with the possibility of mixed microbial culture collected from the waste stream of effluent treatment plant for CO₂ mitigation. The culture was acclimatized in the laboratory environment. Then batch study was carried out for different CO₂ concentration ranging from 4 to 25%. CO₂ concentration range was chosen on the basis of percentage of CO₂ from different industrial gaseous effluent data. Mitigation rate for different concentration of CO₂ and bio-mass growth rate has been analyzed on the per hour basis and per day basis. Mixed culture found to be superior candidate than photoautotrophic organisms for CO₂ mitigation. They show a promising future to develop a low cost, compact size closed system for *in-situ* CO₂ mitigation.

2. Experimental

2.1 Microbial culture

The mixed culture was obtained from the secondary treatment of the sewage treatment plant at Pilani, Rajasthan, India. It was kept in a beaker with equal amount of distilled water for 12 h for settling. The 50 mL of lower portion of this mixture was taken and mixed with equal amount of distilled water. It was kept for 2 minutes. The upper portion of this mixture was used for mitigation experiment.

2.2 Preparation of media

The media, Minimal Salt Medium (MSM), prepared has the following composition (in g l⁻¹): K₂HPO₄—0.8, KH₂PO₄—0.2, CaSO₄.2H₂O—0.05, MgSO₄.7H₂O—0.5, (NH₄)₂SO₄—1.0, FeSO₄—0.01 in distilled water. 100 mL of MSM was taken in 250 mL Erlenmeyer flask and was autoclaved. The pH of MSM obtained after autoclaving was 6.7. Stock glucose solution was prepared by dissolving 10 g of glucose in 100 mL distilled water.

2.3 Cultivation

The culture was cultivated in a MSM medium having the following composition of K₂HPO₄ 0.8g, K₃H₂PO₄ 0.2g, CaSO₄.2H₂O 0.05 g, MgSO₄.7H₂O 0.5g, (NH₄)₂SO₄ 1g, FeSO₄ 0.01g. 10 mL. 1000 ppm of glucose solution is added in a 2.5 L conical flask along with 300 mL of MSM and 5 mL of mixed culture. Conical flask was sealed at top using rubber stopper and 100 mL of carbon dioxide is added to it using 50 mL air tight syringe. It was left for 24 h to grow on a shaking incubator with 80 rpm of speed.

Two 2.5 liters of conical flask was taken with fresh 300 mL of MSM solution. 5 mL of cultured growth from the previous flask was added to it. The conical flask was sealed at the top
by rubber stopper. 100 mL, 150 mL of CO\textsubscript{2} was added using air tight syringe. These two flasks were kept for 4 days on a shaking incubator with 80 rpm for study.

2.4 CO\textsubscript{2} determination
The initial concentration and per day concentration of CO\textsubscript{2} was done by using CO\textsubscript{2} analyzer model number 906 provided by Quantek Instruments, USA.

2.5 Optical density measurement
The Optical Density per day measurement was done by using UV Spectrophotometer. The OD values were then converted to dry cell weight concentration via proper calibration. The microbial culture which was found absorbs the wavelength of 298.40 nm in UV spectrophotometer.

3. Result and discussion

3.1 CO\textsubscript{2} mitigation per day basis
Fig. 1 shows degradation of CO\textsubscript{2} at different initial concentration using assimilated mixed culture on per day basis. The initial concentration range is 25.6%, 22.3%, 16.5%, and 3.4%. The consumption of CO\textsubscript{2} by assimilated mixed culture show fast degradation rate on day 1 & 2 but becomes steady afterwards. The batch was continued for four days and no change in concentration was observed after fourth day.

The CO\textsubscript{2} removal efficiency of different micro-algal culture and cyanobacteria was upto 40% and was reported by many researchers in their work. The assimilated mixed culture shows the removal efficiency of 50% in 24 h. Thus assimilated mixed found to superior in degrading CO\textsubscript{2} as compared to different micro-algal culture and cyanobacteria reported in literature (15, 29, 30, 31, 32, and 33). The tolerance of assimilated mixed culture towards NO\textsubscript{x} and SO\textsubscript{x} need to be investigated further. The assimilated mixed culture shows a promising fast CO\textsubscript{2} removal efficiency.

![Graph showing CO\textsubscript{2} concentration (\% V/V) vs. Time (24 hrs)]

**Fig.1 CO\textsubscript{2} Fixation Rate \% V/V per day basis**
3.2 Bio-mass growth rate

Fig. 2 shows the bio-mass concentration profile of assimilated mixed culture on per day (24 h) basis. The report is observed for 25.6 %, 22.3 %, 16.5 %, and 3.4 %. The bacterial growth rate is characterized by four phases, these are lag phase, exponential phase, and stationary phase and dead phase. Turbidity is one of the methods of its measurement. The concentration is found to increase or the growth rate increases up to second day. It also shows a steep downfall in growth rate after second day and become steady means no growth after 3rd day. The observation was done for the period of four day batch experiment. The culture taken was a natural mixed culture. It’s having combination of different microbial species. The bio-mass growth rate shown below is a growth of all the microbial species together. Though there is a need of kinetic modeling to predict the actual kinetic model for the growth of our mixed culture.

The bio-mass growth rate and carbon dioxide fixation rate are important parameters used to evaluate the potential of mixed culture towards carbon dioxide fixation. In the table 1 below reported the biomass growth per day basis with pure strain of micro-algae is presented.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Operating Conditions Of Bioreactor</th>
<th>Microbial Species</th>
<th>Biomass Production (g L⁻¹ d⁻¹)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15 Inlet CO₂ % 50 Temp. 0°C</td>
<td>Hot spring algae</td>
<td>0.2667</td>
<td>[15]</td>
</tr>
<tr>
<td>5</td>
<td>15 Inlet CO₂ % 25 Temp. 0°C</td>
<td>Nannochl- oris</td>
<td>0.350</td>
<td>[29]</td>
</tr>
<tr>
<td>6</td>
<td>50 Inlet CO₂ % 22 Temp. 0°C</td>
<td>Chloroccum littorale</td>
<td>0.044</td>
<td>[30]</td>
</tr>
<tr>
<td>7</td>
<td>15 Inlet CO₂ % 25 Temp. 0°C</td>
<td>Nannochl-oropsis</td>
<td>0.300</td>
<td>[34]</td>
</tr>
<tr>
<td>8</td>
<td>20 Inlet CO₂ % 35 Temp. 0°C</td>
<td>Chlorella sp</td>
<td>0.700</td>
<td>[35]</td>
</tr>
<tr>
<td>15</td>
<td>25 Inlet CO₂ % 26 Temp. 0°C</td>
<td>Chlorella sp. MTF-7</td>
<td>2.870</td>
<td>[33]</td>
</tr>
</tbody>
</table>
Table 2. Maximum biomass observed for mixed culture for different CO$_2$ concentration

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Operating BATCH Conditions</th>
<th>Max. Biomass Observed (g L$^{-1}$ d$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inlet CO$_2$ %</td>
<td>Temp. $^0$C</td>
</tr>
<tr>
<td>1</td>
<td>3.4</td>
<td>37</td>
</tr>
<tr>
<td>2</td>
<td>16.5</td>
<td>37</td>
</tr>
<tr>
<td>3</td>
<td>22.3</td>
<td>37</td>
</tr>
<tr>
<td>4</td>
<td>25.6</td>
<td>37</td>
</tr>
</tbody>
</table>

Table 2 shown above is for the maximum biomass observed for mixed culture for different CO$_2$ concentration. It suggests the potential of our natural mixed culture is superior to pure strain for capturing CO$_2$ at faster rate and produced biomass can be utilized for the waste water treatment. However, the continuous carbon dioxide fixation from industrial gaseous waste stream would vary with operational factors, such as, air flow, pH, working volume of the column which has to be investigated further. The mixed culture not only shows its ability to fixed CO$_2$ faster but it shows greater sustainability. The inhibition in growth after two days was mainly because of reduction in concentration of CO$_2$ and production of different gases. This has to be further investigated for defining the growth kinetics. Also, the analysis of gaseous part is required to investigate the production of other gases.
4. Conclusions

As seen from the earlier studies, there has been lot of work carried out in the field of CO₂ mitigation through biological route. Most of the studies are limited to the use of micro algal species for CO₂ mitigation. The major problems associated with the use of micro algal species in the industrial conditions are availability of light, tolerance to NOₓ & SOₓ, performance at high CO₂ concentration, slow rate of CO₂ mitigation, high capital and operating costs, contamination etc.

Mixed culture shows CO₂ fixation rates and productivities better than the reported by other species of microalgae used for CO₂ removal. This mixed culture may have the potential to be grown in gas exhaust from thermal power industry since it shows sustainability also at 25% CO₂. The maximum biomass growth was found at 25.6 % which is 231 g L⁻¹ d⁻¹. Thus, assimilated mixed culture shows a promising future for efficient solution of CO₂ mitigation via biological route.

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