



An Integrated Methodology towards Mitigation of Global Warming and Biomass Production for Biodiesel using *Chlorella sorokiniana* BTA 9031

Anoar Ali Khan, Madhumanti Mondal, Gopinath Halder

Abstract: The rise of atmospheric carbon dioxide (CO_2) concentration as well as depletion of fossil fuel reserves calls for the development of clean and ecofriendly alternative fuel source. Recently, lipid rich microalgal biomass is being extensively studied for generation of biodiesel however, the expenses incurred on production of microalgal biomass is a significant hurdle. Almost 80 % of the production cost is generated from the cultivation medium which majorly comprise of carbon, nitrogen and phosphate. If the microalgal cultivation could be linked to a CO_2 capturing unit than the cost of production could be reduced to a large extent. CO_2 absorption by means of aqueous amine solvents is known to be a mature technology and could be integrated with microalgal cultivation unit for efficient utilization of the captured CO_2 . In this present research work, blended solution of piperazine (PZ) and 2-amino-2-methyl-1-propanol (AMP) (5/25 wt. %) was used to capture CO_2 and then the captured CO_2 was utilized as an inorganic carbon stream for growing *Chlorella sorokiniana* BTA 9031 for biodiesel production. The CO_2 rate absorption was governed by series of process variables viz. solvent flow rate ranges (1.5 to 3) $\times 10^{-4}$ $\text{m}^3 \text{min}^{-1}$, absorption temperature (298 to 313) K, concentration of CO_2 (10 to 15) kPa and gas flow rate (5 to 8) $\times 10^{-3}$ $\text{m}^3 \text{min}^{-1}$. The detected final biomass strength of *Chlorella sorokiniana* BTA 9031 was 0.955g L^{-1} . The fatty acid methyl esters (FAME) determined subsequently acid transesterification was observed to contain fatty acids suitable for biodiesel production.

Keywords: Carbon dioxide; Absorption; 2-amino-2-methyl-1-propanol; Piperazine; *Chlorella sorokiniana* BTA 9031; Biodiesel.

I. INTRODUCTION

The progressive rise in the amount of greenhouse gases (GHGs) in the ambience caused by numerous anthropogenic intrusions directing to worldwide climate change has been a subject of global consideration and significant research over the past decades [1].

Among the other GHGs, 60% of the global warming is caused only due to the carbon dioxide (CO_2) because of its huge emission rate [2].

The CO_2 concentration in atmosphere increased from 300 ppm in the pre-industrial period to 400 ppm in the present decade. This gradual escalation of CO_2 in the present day's atmosphere stresses on dedicated studies and research towards the improvement of suitable capturing methods towards lessening the influence of global warming effect due to CO_2 emission.

Extensive methods of carbon capture pathways are available with conventional routes of chemical and physical sorption, membrane based capture, gas liquefaction means and CO_2 capture by biological fixation. Physical adsorption and chemical absorption techniques are considered to be the extremely encouraging process towards post combustion CO_2 capture among all other separation processes since, membrane separation is still at their nascent stage and capture of CO_2 via cryogenic routes is a greatly energy demanding method and is not cost effective [3], [4]. Biological CO_2 fixation has attracted many researchers but it requires longer time and energy. Chemical solvent (as an absorbent) attracted interest of researchers since it possess suitable potential towards CO_2 removal through absorption process and is also considered to be the most technologically advanced capture technique. Alkanolamine solvent develops a greater affinity towards CO_2 capture which is acidic in nature through carbamate formation.

In this research work, solution blend constitute of piperazine (PZ) as 5 wt% and 2-amino-2-methyl-1-propanol (AMP) as 25 wt% was prepared to absorb CO_2 from a lab scale flue gas generator unit. AMP is a primary amine having special hindered characteristics. It has been suggested to be an encouraging CO_2 absorbent having high CO_2 loading capacity and modest rate of CO_2 absorption. Stoichiometrically, the maximum possible loading capability of AMP is 1 along with a significant sorption rate. However, addition of PZ further boosts rate of absorption by escalating the rate of reaction and thereby the reaction kinetics [5-10]. The CO_2 captured from exhaust gas by the aqueous amine blend was transformed into clean fuel source-biodiesel through microalgae. Several studies have been reported on the growth of microalgae using flue gases rich in CO_2 or pure CO_2 but, microalgal cultivation using the CO_2 obtained after desorption from amine system is rarely reported. The CO_2 recovered after desorption was fed into photobioreactors for growing microalgae.

Manuscript published on November 30, 2019.

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Microalgae are single celled photosynthetic microorganisms which can use CO₂ by means of a carbon supplying agent for growing photoautotrophically [11]. They have been recognized as new biofuel feedstock however, the cost of production of microalgal biomass is a serious obstacle since, the cost incurred on cultivation medium (mainly carbon) is significantly higher than other requisites. Therefore, if the CO₂ capture unit is integrated with the microalgal cultivation unit then the cost of production of microalgal biomass could also be reduced to a large extent. The CO₂ utilized by microalgae for growth is incorporated in them as lipids, which can be extracted and transesterified into biodiesel which could be considered as an unconventional fuel source. Therefore, in this integrated approach (as described in Figure 1), along with mitigation of CO₂ and energy crisis the cost of production of microalgal biomass was also reduced.

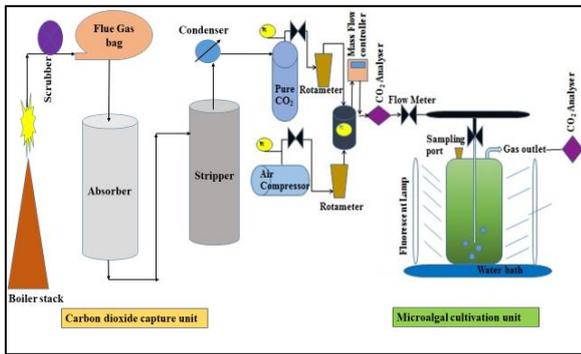


Figure 1. Schematic representation of the integrated carbon dioxide capture unit and microalgal cultivation unit

II. METHODS AND METHODOLOGY

A. Absorption and Desorption of Flue gas

In this current research, flue gas was produced from a miniature coal-fired boiler unit. The working gas or the element of concern in the flue gas was CO₂. The gas generated from the boiler was analyzed by means of flue gas analyzer (TESTO 350-S, Germany). The exhaust gas which liberated from the boiler stack was sucked through a water scrubber unit and finally collected in a flue gas storage bag. The absorption of CO₂ from flue gas was carried out by means of amine blend (PZ 5 wt. % + AMP 25 wt. %) as an absorbent in a packed absorber considering pre-determined variables like liquid absorbent flow rate ranges (1.5 to 3) × 10⁻⁴ m³ min⁻¹, absorption temperature (298 to 313) K, concentration of CO₂ (10 to 15) kPa and absorbate (gas) flow rate (5 to 8) × 10⁻³ m³ min⁻¹. Stripping operation was performed after absorption process in a stripping unit using CO₂-enriched blends of amine. Detailed experimental procedure was reported in author's earlier research (Khan et al., 2016). After regeneration, pure CO₂ (99%) was stored in a CO₂ storage cylinder from the top section of the stripper unit (i.e. condenser section). The pure CO₂ obtained via the above mentioned process was utilized as a source of microalgal growth towards biodiesel (clean fuel) production.

B. Microalgal Strain and Cultivation Condition

In the current investigation *Chlorella sorokiniana* BTA 9031 was used as microalgal species. It was isolated from a coal mine named Mahavir (23°37'44''N 87°06'54''E) placed in Raniganj, West Bengal, India.

Chlorella sorokiniana BTA 9031 was cultured and maintained in a broth named Blue Green-11 (BG-11) medium retaining pH- 7.4. The components of the media were according to Rippka et al., 1979 [12]. The culture was maintained at the light concentration under 80 μmol m⁻² s⁻¹ with photoperiod of 12h: 12h (light: dark cycle) at 25 °C. At a specific time period of 2-3 days cultures were stirred manually.

C. Biomass Productivity and Growth Determination

Spectrophotometric analysis was carried out towards the determination of microalgal growth. The absorbance of the microalgal cells was recorded at 540 nm every day through a spectrophotometer. By means of weighing the microalgal cells after drying them at 60 °C in an air oven, the dry cell weight (DCW) of the microalgae (g L⁻¹) was estimated. Linear regression equations were used to establish relationship between DCW and optical density.

The yield of biomass, P (g L⁻¹ d⁻¹) for a time interval (cultivation time) was determined by calculating the variation in the amount of total biomass observed. The equation used to measure biomass productivity is:-

$$P = (X_1 - X_0) / (t_1 - t_0) \quad (1)$$

Where, X₁ and X₀ signifies the total biomass amount (g L⁻¹) on t₁ day and t₀ day respectively [13].

D. Microalgal Cultivation Using the CO₂ Obtained After Desorption Process

Chlorella sorokiniana BTA 9031 was cultivated in a photobioreactor (capacity 1 L) fabricated with Perspex sheet which filled with 0.5 L of sterile BG-11 (pH 7.4) medium without sodium carbonate since, 15 % CO₂ was fed into the photobioreactor which served as the carbon source for the microalgae. The pure CO₂ (from the CO₂ storage cylinder after stripping operation) was connected to a mixer vessel. In the mixer vessel, air was mixed along with pure CO₂ for achieving the exact CO₂ concentration required for cultivation. A CO₂ analyzer was interconnected with the mixer vessel for verifying the CO₂ percentage. The CO₂ analyzer accurately states the exact CO₂ concentration in the mixture gas. The pre-determined mixer gas (15 % CO₂) was provided and flowing at 250 mL min⁻¹ continually for 15 days from the lower part of photobioreactor. As an experimental control, a similar photobioreactor with same species was also maintained and fed with only air (containing 0.04 % CO₂). The photobioreactors were illuminated with 70 μmol m⁻² s⁻¹ light intensity and photoperiod of 12h: 12h (light: dark cycle) at 25 °C. For all the experimental runs, 0.05 (g L⁻¹) cell concentrations were used as the initial biomass. The axenic environments of cultures were conserved by frequent monitoring of the samples of microalgal culture under light microscope.

E. Total Lipid Content Determination

The improved procedure of Folch was employed towards the estimation of total lipid content of microalgal cells. The detailed procedure has been elucidated in our earlier published research [1].

F. Preparation and Profiling of FAME

The lipids extracted by the above mentioned Folch protocol from the microalgal cells are not appropriate through gas chromatography (direct injection) analysis since,

structurally they are extremelpolar. Consequently, the conversion of it towards the methyl ester derivatives is an essential step for analysis.The procedure for FAME preparation and its analysis via Gas chromatography has been described in author’s priorresearch article [14].

III. RESULT AND DISCUSSIONS

A. Absorption and Desorption of CO₂

The sorption phenomenaof CO₂ using blended solution of (PZ 5 wt.% + AMP 25wt.%)was determined under consideration of specific rate of absorption, absorbed CO₂ percentage and regeneration efficiency of solvent blend after stripping operation. Figure 2 revealed that the rate of absorption steadily rises with growing solvent flow rate and the value ranges from (10.6-26.7) ×10⁻⁶kmol m⁻² s⁻¹.It canbe suggested that the absorption of CO₂ using amine blend is distinctly linkedwith contact time of amine blend and flue gas (absorbate). The increased rate of absorption resultsasenhancement of interfacial area which directlyrelated with solvent flow rate.Conversely, elevated mass transfer coefficient was attained since larger accessible are of mass transfer infers the enhanced absorption rate. The effect of CO₂ concentration associated withabsorption rate as displayed in Figure 2states that the rate steadily escalates from 10-15 kPa with a highest rate of 26.7×10⁻⁶kmol m⁻² s⁻¹. The faster sorption rate ofCO₂ took place because of more CO₂ molecular transformation from bulk gas stream to solvent-gas interface since of steady increase of CO₂ intensity in inlet gas stream. Figure 3 represents the percentage absorption CO₂ and it gradually rises with rise in CO₂ concentration and solvent flow rate.

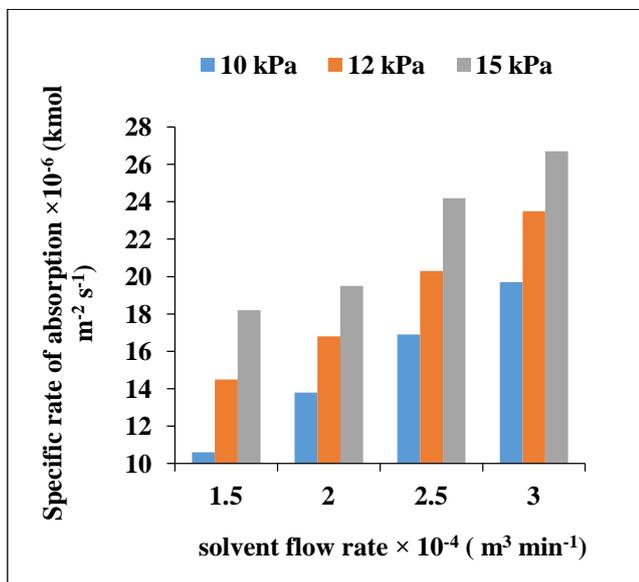


Figure 2: Specific absorption rate of CO₂ in aqueous (AMP 25 wt. % + PZ 5 wt. %) blend

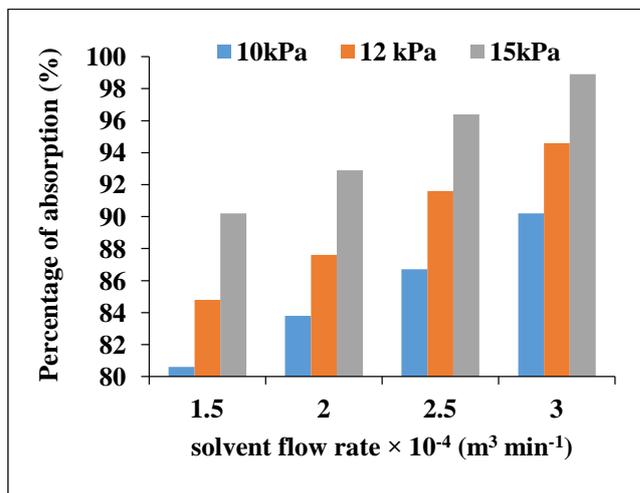


Figure 3: Percentage absorption of CO₂ in (AMP 25 wt. % + PZ 5 wt. %) blend

Regeneration temperature and requisite time for regeneration of CO₂ enriched amine blends are the important parametric conditions to be considered towards regeneration performance. Figure 4 depicts that the regeneration efficiency of considered amine. It displays that the efficiency steadily increases with rise in temperature from 368 to 383 K and regeneration time from 30 to 120 minutes with the resultant value ranges of 85.45 to 97.51 %. The requisite energy of separation for CO₂ gas liberated from the CO₂ enriched blended solutionsupplied by regeneration temperature and it is subject to intensity of carbamate formed. After completion of regeneration of CO₂ rich (AMP 25 wt. % + PZ 5 wt. %) blend, the stripped off CO₂ gas (99% pure) was stored in a CO₂ cylinder. The exact concentration of the stripped off CO₂ was confirmed through CO₂ analyzer and desorption cell study.

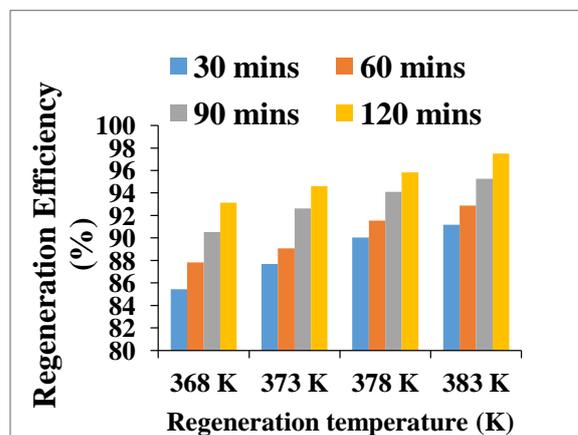


Figure 4: Regeneration efficiency of aqueous (AMP 25 wt. % + PZ 5 wt. %) blend

The CO₂ obtained after desorption process is continuously being stored in the storage tank of the CO₂ capture unit. This CO₂ is connected to the microalgae cultivation unit, where the CO₂ serves as an inorganic carbon resource for phototrophic microalgae growth.

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The pure CO₂ is mixed with air and 15 % CO₂ is fed into the photobioreactor continuously throughout the cultivation period.

B. Effect of 15 % CO₂ on the Production of Biomass in *Chlorella sorokiniana* BTA 9031

The basic requirements of microalgal growth are light, carbon source and water. The carbon source can be inorganic or organic according to the availability. The microalgal isolate used in the study *Chlorella sorokiniana* BTA 9031 is capable of utilizing both organic and inorganic carbon. Depending upon the availability of carbon it is decided whether it will grow photoautotrophically, heterotrophically or mixotrophically. In the present study, the microalgal culture is fed with 15 % CO₂ which serves as an inorganic carbon source and directs the microalgae to grow photoautotrophically. The CO₂ obtained after desorption process is continuously stored in the storage tank of the CO₂ capture unit and from there it is fed into the bottom of the photobioreactor after passing through a mixer vessel where the appropriate percentage of CO₂ is obtained by mixing the pure CO₂ with air.

The microalgal isolate was observed to grow well utilizing CO₂ provided to it. The final amount of biomass in *Chlorella sorokiniana* BTA 9031 was detected to be 0.955 g L⁻¹ while the final amount of biomass in the control photobioreactor fed with air was observed to be 0.547 g L⁻¹. The biomass concentration enhanced from 0.061 g L⁻¹ to 0.955 g L⁻¹ over a period of 15 days with air enriched with 15 % CO₂ as depicted in Figure 5. The Biomass yield was noticed to be 0.059 g L⁻¹ d⁻¹. The high biomass concentration can be attributed that *Chlorella sorokiniana* BTA 9031 was isolated from a coalmine in West Bengal. The microalgal species isolated from coalmines and regions around the coalmines or thermoelectric power plants tends to possess the capability of growing under circumstances dominant in such areas like, occurrence of excess carbon quantity in soil and combustible gas-air mixture produced by the power stations. Sankar et al., 2014 [15] conveyed a final biomass concentration 0.8824 g L⁻¹ of *Chlorella minutissima* grown in 15 % CO₂ in a stirred tank reactor. Basu et al, 2014 [16] also reported that *Scenedesmus obliquus* SA1 generated a maximum amount of biomass of 1.1 g L⁻¹ at 15 % CO₂.

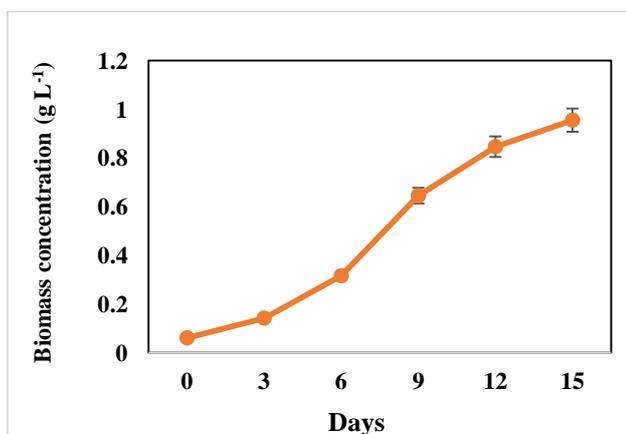


Figure 5: Biomass concentration of *Chlorella sorokiniana* BTA 9031 observed with 15 % CO₂ over the cultivation period

Several studies have been performed and reported which have explored the possibility of growing *Chlorella* sp. in

higher percentages of CO₂ but, very few studies are there where a decent amount of biomass concentration and productivity has been observed. The species might be tolerant to higher percentages of CO₂ but it is also important to know whether it is able to grow and reproduce in that percentage of CO₂, only than it will be useful in CO₂ capture studies. Certain researchers like, Maeda et al., 1995 [17] informed that *Chlorella* sp. T-1 was able to cultivate successfully in 100 % CO₂ but the highest growing rate and biomass production was observed only at 10 % CO₂.

C. Effect of 15 % CO₂ on Production of Lipid in *Chlorella sorokiniana* BTA 9031

The total lipid content in the microalgal cells grown in 15 % CO₂ was observed to follow the similar trend as the concentration of biomass. The total lipid content increased from 8 % to 23 % over the cultivation period as represented in Figure 6. The total lipid content in the microalgal cells was found to be 14.5 % therefore, it can be suggested that the air enriched with 15 % CO₂ helped enhance the total lipid content in the microalgal cells from 14.5 to 23 %.

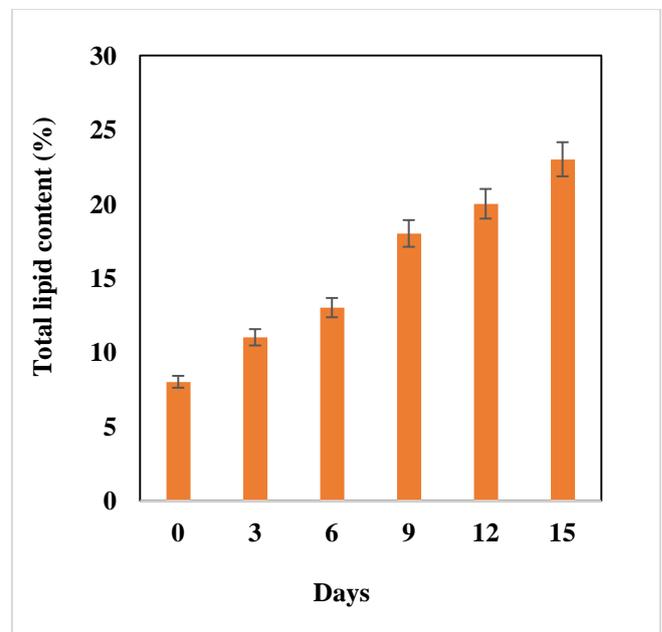


Figure 6: Total lipid content of *Chlorella sorokiniana* BTA 9031 observed with 15 % CO₂ over the cultivation period

The composition of fatty acid in the microalgae grown in 15 % CO₂ has been conveyed in Table 1. Fatty acids from C-10:0 to C-24:0 was found in the FAME solution. Along with nine saturated fatty acids (SFAs) and nine mono unsaturated fatty acids (MUFAs), eight poly unsaturated fatty acids (PUFAs) were also found. SFAs like Pentadecanoic acid (C_{15:0}), Arachidic acid (C_{20:0}) and Palmitic acid (C_{16:0}) were observed to be in abundance while Elaidic acid (C_{18:1n9t}) and Cis-11-Eicosenoic acid (C_{20:1}) were observed to be the leading MUFAs. SFAs, MUFAs and PUFAs represented 77.80 %, 15.32 % and 5.93 % respectively of the total FAME esters. Since, the fatty acid profile contains fatty acids required for biodiesel production, it could be suggested that *Chlorella sorokiniana* BTA 9031 might be a stated as a potential biodiesel feedstock.

Table 1: Fatty acid composition of *Chlorellasorokiniana* BTA 9031 whilst grown up in 15 % CO₂

Type	Lipid number	% of fatty acid
Saturated fatty acid (SFAs) content	C _{10:0}	0.019
	C _{12:0}	0.357
	C _{13:0}	0.661
	C _{14:0}	1.230
	C _{15:0}	52.11
	C _{16:0}	10.30
	C _{17:0}	4.20
	C _{18:0}	0.72
	C _{20:0}	8.21
% of total SFAs		77.80
Monounsaturated fatty acid (MUFAs) content	C _{14:1}	0.306
	C _{15:1}	2.08
	C _{16:1}	0.292
	C _{17:1}	0.712
	C _{18:1n9t}	2.13
	C _{18:1n9c}	0.25
	C _{20:1}	9.17
	C _{22:1}	0.08
	C _{24:1}	0.30
% of total MUFAs		15.32
Polyunsaturated fatty acid (PUFAs) content	C _{18:2n6t}	0.524
	C _{18:2n6c}	0.350
	C _{18:3n6}	1.36
	C _{18:3n3}	0.138
	C _{20:3n6}	0.129
	C _{20:3n3}	2.15
	C _{20:4n6}	1.096
C _{20:5n3}	0.182	
% of total MUFAs		5.929
Grand Total		99.05

IV. CONCLUSION

In this research work, aqueous solution of AMP and PZ (25/5 wt. %) was used to capture CO₂. The CO₂ captured was recovered through desorption process and utilized as a supply of carbon resource for growing microalgae towards biodiesel production. It could be summarized that aqueous solution of AMP and PZ proved to be an efficient method for capturing CO₂ through absorption. *Chlorella sorokiniana* BTA 9031 also was found to have capability to acclimate to higher CO₂ levels and considered as a CO₂ resource for biomass production. In this way, along with CO₂ mitigation the microalgal biomass generation cost was also curtailed. Therefore, the integrated approach of CO₂ capture through AMP-PZ and its utilization for cultivating microalgae for biodiesel production was found effective.

ACKNOWLEDGEMENT

The authors might want to thankfully recognize Vignan's Foundation for Science, Technology & Research, Vadlamudi and National Institute of Technology Durgapur for providing the facilities to perform the research experiment.

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