JOURNAL OF PURE AND APPLIED MICROBIOLOGY, May 2014. Vol. **8**(Spl. Edn. 1), p. 761-768

Mutagenesis on Cyanobacteria for High CO_2 Uptake: A Review

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(Received: 08 January 2014; accepted: 24 March 2014)

Recently, mutagenesis on cyanobacteria has been performed to enhance carbon dioxide (CO_2) bio-mitigation. The physical and chemical mutagens have been introduced on cyanobacteria to induce genetic mutation. Physical mutagen includes electromagnetic radiation, such as gamma rays, x-rays, and ultraviolet (UV) light, while the chemical mutagen includes are ethyl methanesulphonate (EMS), and N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). Different types of mutagen, concentration, incubation period and temperature affect the rate of mutagenesis. As a result, various kinds of observation were produced. Thus, this review aims at providing an updated account of the research progress in mutagenesis on cyanobacteria and its application in CO_2 mitigation enhancement.

Key words: Cyanobacteria, physical mutagen, Chemical mutagen, Carbon dioxide mitigation.

Cyanobacteria are aquatic photosynthetic bacteria. This prokaryotic microorganism can fix carbon dioxide (CO_2) efficiently from several sources namely industrial exhaust gases, soluble carbonate salts and atmosphere[1]. It has become a favorable microorganism based on its unique characteristics; no food crop's competition as its nature in water[2]; capable of producing lipid and hydrocarbon for energy resource[3]; higher growth rates compared to other plants [4]; rich in genetic resources which is important for metabolic pathways to synthesize valuable bio-based products [2]; more efficient in capturing carbon due to high photosynthetic efficiency[5].

Based on the above characteristics of cyanobacteria, current studies on CO_2 bio-mitigation are worth as it is becoming an alternative way for greenhouse gas mitigation. As reported by World Meteorological Organization[6], the amount of CO_2 in the atmosphere was 390.9 ppm in 2011, increasing on average 2 ppm per year for the past 10 years and reaching 140% of the pre-industrial level (280 ppm). Other studies revealed that the atmospheric concentration of CO_2 has increased from 280 ppm to more than 370 ppm currently[7,8]. The anthropogenic component (atmospheric value reduced by the pre-industrial value of 280 ppm) of atmospheric carbon dioxide has been increasing exponentially with a doubling time of about 30 years since the beginning of the industrial revolution (<1800). Furthermore, carbon emission in atmosphere is one of the cause for global warming (greenhouse effect)[9].

In order to reduce atmospheric concentration of CO_2, biological method of mitigation of CO_2 has been introduced as a sustainable approach for greenhouse effect. To date, few mutagenesis works related to cyanobacteria were conducted to enhance the

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CO_2 bio-mitigation, as mutagenesis clarify metabolic pathways, composition of macromolecular structures, uptake mechanisms for nutrients, and other phenomena in the cell. Consequently, the purpose of this review is to cover some of the recent findings about mutagenesis on cyanobacteria for CO_2 bio mitigation.

Carbon Mechanism in Cyanobacteria

Cyanobacteria have a mechanism which elevatesconcentration of inorganic carbon (C_i) in the cell. The mechanism is called CO_2-concentrating mechanism (CCM). The CCM components include at least four modes of active Ci uptake, including two bicarbonate transporters (HCO_3^-) and two CO_2 uptake systems associated with the operation of specialized NDH-1 complexes[10]. Previous study has shown that the NDH-1 dehydrogenase complex is involved in anabling CO_2 uptake by cyanobacteria[11-14]. Cyanobacteria depend upon a CCM to overcome the poor CO_2 affinity of the major carbon-fixing enzyme, ribulose-bisphosphate carboxylase/ oxygenase (Rubisco). The CO_2 concentration is increased around the active site of Rubisco by active transport of C_i into the cell[15].

Carboxysome is the central to the functioning of the cyanobacterial CCM. It contains the Rubisco of the cell together with a carboxysomal carbonic anhydrase (CA). The CA converts an accumulated cytosolic pool of HCO_3^- into CO_2 within the carboxysome. The CO_2 is generated coupled with a diffusive restriction to the efflux from the carboxysome. This leads the CO_2 to be elevated localized around the active site of Rubisco within the carboxysome. Meanwhile, HCO_3^- is accumulated in the cytosol by the operation of a number of active CO_2 and HCO_3^- transporters[10].

The main difference between wild and mutant type cell lies in the carboxysomes. As mentioned before, carboxysome mainly located in the central nucleoplasmic region in the wild type cell. However, "empty-inclusion" carboxysomes were found in the mutant cells[16]. Furthermore, Wu *et al*. (2000)[17] reported the appearance of various types of abnormal carboxysomes and subsequent higher dependence on external C_i in the mutant cells were observed. It indicate a loss of microenvironment which was organized by CA, RubisCO and its overcoat protein, which caused the CO_2 leakage and subsequent high CO_2 requiring phenotype[17].

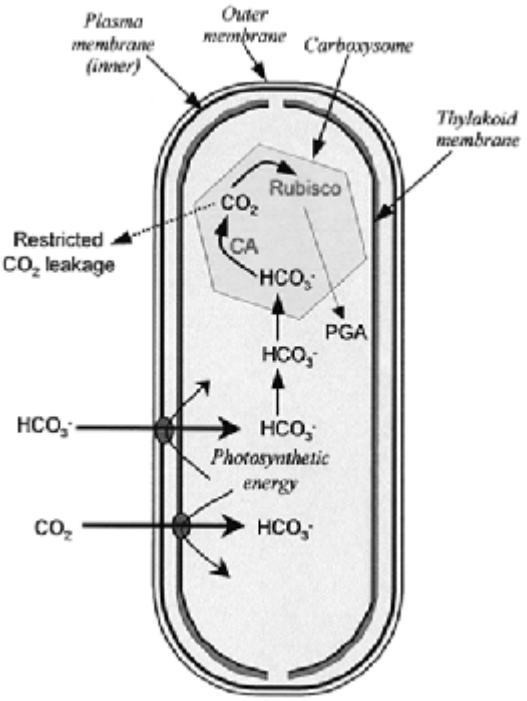

Fig. 1. A model for cyanobacterial CCM. The figure shows the Rubisco-containing carboxysomes with the carboxysomal carbonic anhydrase (CA) and an associated diffusional resistance to CO_2 efflux. The accumulation of HCO_3^- in the cytosol achieved through the action of a number of CO_2 and HCO_3^- uptake system

Figure 1 illustrated a model for the cyanobacterial CCM. This model was developed based on model of cyanobacterial species *Synechococcus* PCC7942, *Synechocystis* PCC6803 and *Synechococcus* PCC7002. It showed the Rubisco-containing carboxysomes with carboxysomal CA and an associated diffusional resistance to CO_2 efflux[10].

CO_2 bio-mitigation by Wild Strain of Cyanobacteria

Several researches[18-21] have conducted studies on cyanobacteria for CO2 bio-mitigation. Table 1 summarized the cultivated conditions and maximum biomass productivity of the studies.

In 2007, Morais and Costa[18] isolated the microalgae *Scenedesmusobliquus* and *Chlorella kessleri* from the waste treatment ponds of coal fired thermoelectric power plant. They investigated their growth characteristic when exposed to

different concentrations of CO_2. The microalgae were cultivated at 30°C in 2 L conical flask photobioreactors and having illumination at 3200 Lux by 40 watts daylight using 12 hours light/dark photoperiod. The cultures were aerated with 0.540 L/min of air supplemented with CO_2 from a cylinder for 15 minutes in every hour. In this study, CO_2 concentration was being supplied at 6%, 12% and 18% (v/v). According to the research, when cultivated with 6% and 12% CO_2, *C. kessleri* showed a high maximum specific growth rate (μ_{max}) of 0.267/day, with a maximum biomass productivity (P_{max}) of 0.087 g $L^{-1}day^{-1}$ at 6% CO_2. For *S. obliquus*, the highest maximum dry weight biomass value was 1.14 g L^{-1} with 12% CO_2. They found that these two microalgae also grew well when the culture medium which contained up to 18% CO_2, indicating that they have potential for biofixation of CO_2 in thermoelectric power plants.

Further research on wild strain of cyanobacteria for CO_2 mitigation has been done by Yoo *et al*. (2010)[19]. They investigated which microalgae are appropriate for cultivation with high levels of CO_2 and the production of biodiesel. The microalgae used in this study were *Botryococcusbraunii*, *Chlorella vulgaris* and *Scenedesmus*sp. The strains were incubated at 25 ± 1°C with continuous illumination of 150 lmol m^{-2} s^{-1} and cultivated for 2 weeks. At first, the microalgae were cultivated with ambient air containing 2% CO_2 for a week before inoculation, to reduce the long lag phase. The flue gas was provided at 0.3 vvm by a heating generator burning liquefied petroleum gas. All species used in this study able to grow with 10% CO_2supplied. The biomass and lipid productivity for *Scenedesmus* sp. with 10% CO_2 were 217.50 and 20.65 mg L^{-1} d^{-1} (9% of biomass), while for *B. braunii* were 26.55 and 5.51 mg L^{-1} d^{-1} (21% of biomass). This study concluded that *Scenedesmu*s sp. is appropriate for mitigating CO_2, due to its high biomass productivity and C-fixation ability.

Tang *et al*. (2011)[20] studied CO_2biofxation of two types of microalgae; *Scenedesmusobliquus* and *Chlorella pyrenoidosa* in response to different CO_2 levels. The strains were cultivated at 25 ± 1°C and 180 µmol $m^{-2}s^{-1}$ in modified BG11 medium under different CO_2 concentrations ranging from 0.03% (air) to 50% (v/v). The cultures were aerated continuously via bubbling from the bottom of modified 1 L Erlenmeyer flask with an aeration rate of 200 mL min^{-1}. Based on the research, both strains could grow at 50% CO_2 (>0.65 g L^{-1}) and grew well (>1.22 g L^{-1}) under CO_2 concentrations ranging from 5% to 20%. These microalgae showed best growth potential at 10% CO_2. Besides, the examined microalgae showed great abilities of CO_2biofixation under the CO_2 concentrations from 5% to 20% and performed best at 10% CO_2. On the other hand, the total lipid content of the strains showed increasing trends with the increase of CO_2 concentration. Based on Gas Chromatography Mass Spectrometer (GC-MS) analysis, it seem that increasing the CO_2 concentration could increase the unsaturated degree in fatty acids, and high levels of CO_2 enhance the production of polyunsaturated fatty acids. In contrast, low CO_2 concentration (0.03%) was good for the accumulation of saturated fatty acids.

Recent study on CO_2 bio-mitigation by wild strain of cyanobacteria was conducted by Anjos et al. (2013) [21]. The rate of CO_2 fixation (R_{CO2}) was maximized by *Chlorella vulgaris* P12. This green microalgae was cultivated photoautotrophically in 110 mL glass bubble column photobioreactors containing 90 mL of medium, during 7 days. The experiment was carried out under different CO_2 concentrations (ranging from 2% to 10%) and aeration rates (ranging from 0.1 to 0.7 vvm). Based on the study, *C. vulgaris* was able to grow under all the evaluated levels of aeration and CO_2; however final biomass concentration (X_{max}) and maximum biomass productivity (P_{max}) were significantly influenced by the cultivation conditions. Regardless the CO_2 level in air, higher values of $X_{max}$and P_{max}were obtained under the aeration rate of 0.4 vvm when compared with those values obtained at 0.1 vvm. Further result,$X_{max}$and P_{max}were almost similar when the aeration rate was increased from 0.4 to 0.7 vvm at 6% and 10% CO_2 concentrations. The value of $X_{max}$and P_{max}at 10% CO_2 is lower than at 6% CO_2. The use of higher CO_2 levels can result in low pH. Decrease of pH will cause the decrease of the activity of carbonic extracellular anhydrase and inhibit cell growth[20]. On the other hand, the highest values of final biomass concentration (10.0 ± 0.5 g L^{-1}), maximum biomass productivity (1.3 ± 0.0 $gL^{-1}d^{-1}$) and maximum specific growth rate (0.95 ± 0.04

d^{-1}) of *C. vulgaris* P12 were all obtained at 6% CO_2 and 0.4 vvm. The maximum R_{CO2} (2.22 $gL^{-1}d^{-1}$) was obtained by using 6.5% CO_2 and 0.5 vvm after 7 days of cultivation at 30°C.

Table 1. Summary for cultivated conditions for cultivated wild microalgae

Types of wild strain	Cultivated lighting	Cultivated temperature (°C)	CO_2 supply (%)	Production system	Maximum biomass productivity	References
Chlorella vulgaris	240 µmol $m^{-2}s^{-1}$	30	6	110 mL photobio reactors	1.3$gL^{-1}day^{-1}$	Anjos *et al.* [21] (2013)
Scenedesmusobliquus	180 µmol $m^{-2}s^{-1}$	25	5	1 L flask	0.158 ± 0.06 g L^{-1} d^{-1}	Tang *et al*.[20] (2011)
Chlorella pyrenoidosa	180 µmol $m^{-2}s^{-1}$	25	10	1 Lflask	0.144±0.011 g L^{-1} d^{-1}	Tang *et al*.[20] (2011)
Scenedesmusobliquus	3200 Lux	30	6	2 L flask	0.085 g L^{-1} d^{-1}	Morais *et al*.[18] (2007)
Chlorella kessleri	3200 Lux	30	6	2 L flask	0.087 g L^{-1} d^{-1}	
Botryococcusbraunii	150 l mol $m^{-2}s^{-1}$	25	10	-	26.55 ± 7.66 mg L^{-1} d^{-1}	Yoo*et al.*[19] (2010)
Chlorella vulgaris	150 l mol $m^{-2}s^{-1}$	25	10	-	104.76 ± 10.73 mg L^{-1} d^{-1}	Yooet al[19] (2010)
*Scenedesmus*sp.	150 l mol $m^{-2}s^{-1}$	25	10	-	217.50 ± 11.24 mg L^{-1} d^{-1}	Yoo *et al.*[19] (2010)

Mutagenesis

At the molecular level, mutation refers to any change to genetic material (DNA) that is heritable[22]. While mutagenesis can be defined as the exposure or treatment of biological material to a mutagen[23]. A mutagen is an agent that induces genetic mutation. Thus, any chemical or physical agent that increase mutagenesis is referred to as a mutagen. Mutagen leads to some chemical change to DNA such as altering bases or breaking the sugar–phosphate backbone. There are two types of mutagen: 1) physical mutagen, 2) chemical mutagen.

Physical mutagens include electromagnetic radiation, such as gamma (γ) irradiation irradiation rays, X rays, and ultraviolet (UV) light, and particle radiation, such as fast and thermal neutrons, beta and alpha particles[24]. Ionizing radiation which is also known as x-rays, ionize atoms and molecules in the cells of the body. The ionizing rays cause the release of electrons from the shell which they are contained. Free radicals are molecules with unpaired electrons that can combine with the bases in DNA, causing errors in DNA replication and producing mutations. A more serious consequence results when the covalent bonds of the sugar-phosphate molecules in DNA are broken, causing breaks in the chromosomes[25].

UV radiation is another type of physical mutagen. It catalyzes the joining of adjacent pyrimidine bases, and these joined bases, or dimers, usually result in point mutations[22]. Ultraviolet radiations are part of the light solar spectrum with intermediate wavelengths between visible light and X-rays. The UV–C part of these radiations, (250–280 nm), is entirely filtered by the ozone layer of the earth's atmosphere. The UV–B, (280–315 nm), is partially filtered, while the UV–A, (315–400 nm), entirely reaches the terrestrial surface[26]. UV light has an effect on DNA by stimulating the formation of detrimental bonds between thymine dimers[25]. The thymine dimers can cause permanent damage because they inhibit the replication of the DNA.

Mutagenesis on Cyanobacteria by Physical Mutagen

Recent study on mutagenesis for CO_2 fixation was conducted by Cheng *et al*. (2013)[27]. *Chlorella pyrenoidosa* were mutated by nuclear irradiation to improve biomass productivity and CO_2 fixation. After mutation of Chlorella by ^{60}Co γ irradiation, the mutants were cultured at 22°C with

12 hours illumination at 1000 Lux and 12 hours of darkness for one month recovery. The culture was then cultivated at 25°C with same 12 hours lighting at 2000 Lux for 15 days. 0.034% CO_2 was continuously flushed during 12 hours photoperiod. The culture was then introduced with air containing mixed gas [3% to 15% (v/v) CO_2] at a rate of 30 mL min^{-1}. In order to increase the tolerance of algae cells to 1% CO_2, the mutants were domesticated with gradually increasing CO_2 concentration in 300 mL column bioreactor. Through this study, the biomass yield of *C. pyrenoidosa* increased by 53.1% (to 1.12 g L^{-1}) under air bubbling. While the biomass of the mutants which were domesticated with gradually increased high concentrations of CO_2 [from 0.038% (v/v) to 15% (v/v)], increased yield to 2.41 g L^{-1}. Based on this study, the mutagenesis by nuclear irradiation improved significantly the biomass productivity of *C. pyrenoidosa*.

Chemical Mutagen

Chemical mutagens are referred as alkylating agents, cross-linking agents, and polycyclic aromatic hydrocarbons (PAHs). The included mutagens are ethyl methanesulphonate (EMS), methyl methanesulphonate (MMS), N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and mitomycin C. EMS; $CH_3SO_2OC_2H_5$ is one of the most frequent used of alkylating agent for chemical mutagenesis in cyanobacteria. The chemical mutagen EMS alkylates guanine leading to the mispairing of guanine (G) with thymidine (T), instead of cytosine (C). The resulting point mutations are mainly lack plastoquinone, demonstrating that plastoquinone GC to AT transitions[28]. The degree of mutagenesis achieved can be altered by changing the reactions conditions, such as concentration of EMS, incubation time and temperature, reaction pH or the length or amount of targeted gene.

Mutagenesis on Cyanobacteria for High CO_2 Uptake by Chemical Mutagen

A few studies[8,17,29,31,32] have been conducted by several researches. Table 2 summarized the parameters study for chemical mutagenesis in cyanobacteria.

In 1989, Price and Badger[29] had isolated two types of high CO_2-requiring-mutants of the *Synechococcus* PCC 7942. After introduced with 0.4 M EMS for 45 minutes at 37°C, a total of 24 isolated mutants appeared and had clearly represented with two extreme phenotype; Type I and Type II mutants. The mutants were incubated under 1% CO_2 conditions (low light; 10 $\mu molm^{-2}s^{-1}$) for 18 hours and then transferred to air for 3 days. After mutagenesis, the mutants were subjected to ampicillin enrichment and then transferred to 1% CO_2 until putative high CO_2-requiring-mutants appeared (8 to 9 days). Measurements were made of the cells ability to take up CO_2 and HCO_3^- both before and after induction of the high affinity transport system at limiting levels of inorganic carbon (C_i). CO_2 and HCO_3^- were supplied at chemical disequilibria and uptake was terminated after 10 second by silicone oil centrifugation. One of the problems of quantifying $C_i$uptake in high CO_2-requiring-mutants is that little or no photosynthesis occurs in these cells at low levels of exogenous C_i. This means that cell photosynthesis cannot be used to deplete $C_i$and in practice it is difficult to produce cell suspensions with less than 30 µM C_i. After the mutants had been incubated under 30 µL L^{-1} CO_2 conditions for 24 hours, both CO_2 and HCO_3-uptake capacity had increased in all six mutants. Through the study, it showed that both types of mutants were able to grow at 1% CO_2 but incapable of growth at air levels of CO_2.

Yu *et al*. (1994)[8] studied similar mutant-type of *Synechococcus* PCC 7942 (TM17) by using 0.2 M EMS. Same incubation method was conducted as mentioned by Price & Badger (1989)[29]. In this study, activity of CO_2 and HCO_3^- uptake during steady-state photosynthesis was measured in a glass cuvette connected to a mass spectrometer. O_2 evolution and CO_2 uptake in the light were measured simultaneously in the closed cuvette at a light intensity of 300µmol of photons $m^{-2}s^{-1}$ and at a temperature of 30°C. The light was turned off after steady-state rates of photosynthetic O_2 evolution and CO_2 uptake were recorded, and the initial CO_2 efflux in the dark was measured. Based on the study, wild strain of *Synechococcus* PCC7942 have the ability to adapt to the growth C_i conditions when grown at various CO_2 concentrations. About 2 mMC_i was required to reach maximal photosynthetic O_2 evolution when the cells were grown at 2% CO_2. As growth C_i decreased to air levels (350 ml L^{-1}) or following induction at 20 µL L^{-1} for 16 hours, much less C_i

was required to saturate photosynthesis. Possibility that CO_2 detection and/or induction signal, or the HCO_3^- transport mechanism have been impaired during the mutation. In summary, the mutant reported grew normally at or above air levels of CO_2 (340 µL L^{-1}) but does not survive at 20 µL L^{-1} CO_2 in air.

In contrast with the above study, Wu *et al*. (2000)[17] had isolated a high-CO_2-requiring mutant of *Synechococcus* sp. PCC7942. A 0.4 M EMS was applied on the wild strain for 40 min at 37°C. The mutants were incubated under 4% CO_2 overnight and transferred to air for 3 days to deplete internal C_i reserves in putative high-CO_2 – requiring mutants. To concentrate the mutants, the ampicillin enrichment has been done. The isolated mutant that appeared under 4% CO_2 were rescreened on duplicate plates under air (nonpermissive) and 4 % CO_2 (permissive) conditions repeatedly, until the true 4% CO_2-requiring mutant cells were obtained. Based on the study, this mutant strain was able to grow at 4% CO_2, but not under ambient CO_2.

Other study on mutagenesis of cyanobacteria was done by Jaiswal&Kashyap (2002)[31]. They had isolated and characterized the mutants of two diazotrophic cyanobacteria; *Nostoccalcicola* and *Anabaena* sp. The wild type strain was supplied by different level of CO_2 (0-50% v/v) which was maintained in gas phase culture by water displacement. The mutant strain was isolated after introduced with 0.1 M of MNNG for 90 minutes. The isolated mutant was incubated for 48 hours in light. The surviving population of *N. calcicola* was exposed to lethal concentration of bicarbonate (500mM $NaHCO_3$).While *Anabaena sp*. were incubated in gas phase containing 40% CO_2 till pin head colonies appeared. Based on this study, maximum growth of *N. calcicola* and *Anabaena sp.*was recorded at 1.5 and 5% CO_2. Besides, *N. calcicola* grew better in $NaHCO_3$ supplemented media, while *Anabaena* preferred CO_2 over $NaHCO_3$. On the other hand, it was indicated that 40% CO_2 was lethal for the cyanobacterium. The HCO_3^{-R} (resistant to bicarbonate) mutant exhibited maximum growth rate at 5% CO_2 and could grow upto 20% CO_2 than the wild type which could tolerate only 10% CO_2 (v/v).

In comparison to all the strains, CO_2^{-R} (resistant to CO_2) mutant had maximum specific growth rate at 20% CO_2 and could grow upto 50% CO_2. The isolated MNNG induced mutants of these cyanobacteria showed growth up to 1M sodium bicarbonate $NaHCO_3$ (*N. calcicola*) or 50% CO_2 (*Anabaena* sp.) in comparison to their wild types. From the finding, bicarbonate and CO_2 resistant mutants isolated from diazotrophic cyanobacteria *N. calcicola* and *Anabaena* sp. grow with low specific growth rate at ambient level CO_2. However, the mutant possesses the capability of growing at fairly high level of 1M $NaHCO_3$ and 50% (v/v) CO_2, respectively. The mutants were altered in multiple properties enabling them to grow at elevated levels of inorganic carbon compounds.

Table 2. Summary of parameters study for chemical mutagenesis on cyanobacteria

Mutant Strain	Types of chemical mutagen	Incubation Temperature (°C)	Incubation period (min)	CO_2 supply (%)	Production on system	References
*Synechococcus*PCC 7942	EMS	37	45	1	Flask	Price & Badger[29] (1989)
Synechococcus PCC 7942	EMS	37	45		Flask	Yu *et al*.[8](1994)
Synechococcus sp. PCC7942	EMS	37	45	4	Flask	Wu *et al*.[17] (2000)
Nostoccalcicola	MNNG	-	90	-	Flask	Jaiswal & Kashyap[31] (2002)
Anabaena sp.	MNNG	-	90	50%	Flask	Jaiswal & Kashyap[31] (2002)
Chlorella sp. MB-9	EMS	-	-	-	PBR	Kao *et al.*[32] (2012)

In the most recent study on mutagenesis of cyanobacteria, a mutant strain of microalgae*Chlorella* sp. MB-9 was isolated by 25-100 mM EMS for an hour. The research was conducted in an outdoor photobioreactor (PBR) to upgrade biogas produced from anaerobic digestion of swine wastewater. Biogas which is produced from anaerobic digestion of biological waste is consisting of mixture of methane (CH_4) and CO_2 with hydrogen sulfide (H_2S) and several minor hydrocarbons. The desulfurized biogas was supplied in 30-min intervals every hour for 8 hour in daytime. The CO_2 capture efficiencies of the *Chrolella* sp. MM-2 captures at10, 20 and 30 min after desulfurized biogas aeration were evaluated at gas flow rate of 0.1 and 0.3 vvm. Through this study, higher CO_2 capture efficiencies from desulfurized biogas (~20% CO_2, ~70% CH_4 and H_2S<100 ppm) were achieved at higher light intensities, approximately 70% on cloudy days and 80% on sunny days. Higher light intensities have deeper light penetration capacities and can cause higher photosynthetic activity in microalgal cultures (Kao *et al*., 2012)[32].

CONCLUSION

In this review, previous mutagenesis studies on cyanobacteria for CO_2 mitigation were discussed. Nuclear irradiation significantly improved the biomass productivity and CO_2 fixation of *C. pyrenoidosa*. The yield of biomass increase in the high CO_2 concentration (15%) after mutation. Besides, it can be seen that EMS had become a common practice treatment on cyanobacteria compared to other chemical mutagen. The concentration, incubation period and temperature was modified and become parameters for mutagenesis treatment. High concentration of EMS improved the CO_2 uptake in mutant type. Based on this review, the isolated mutants by MNNG exhibit high CO_2 uptake compared to isolated mutant by EMS.

ACKNOWLEDGEMENTS

Sincere thanks to Ministry of High Education Malaysia for the research fund (FRGS 11-060-0209) and International Islamic University Malaysia, Edw fund (EDW A12-606-1397).

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