#### Selection of optimal microalgae species for CO<sub>2</sub> sequestration

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# INTRODUCTION

 $CO_2$  fixation by photoautotrophic algal cultures has the potential to diminish the release of  $CO_2$  into the atmosphere, helping alleviate the trend toward global warming. To realize workable biological  $CO_2$  fixation systems, selection of optimal microalgae species is vital. The selection of optimal microalgae species depends on specific strategies employed for  $CO_2$  sequestration. In this paper, the criteria used for selecting microalgae species for  $CO_2$  sequestration systems will be discussed, as well as the characteristics of some species which have been tested for use in  $CO_2$  mitigation.

# **COMMERCIAL VALUES**

Some microalgae species, such as *Chlorella*, *Spirulina* and *Dunaliella* have commercial values. It is expected that commercial profit from biomass production will offset overall operational costs for  $CO_2$  sequestration. *Chlorella* sp. has been studied for use in  $CO_2$  sequestration. For example, Hanagata et al. (1992) reported that *Chlorella* sp. can be grown under 20%  $CO_2$  conditions. The species has been used as a health food (Becker, 1994).  $CO_2$  tolerance of *Dunaliella* sp. also has been examined and the species has been used in the industrial production of  $\beta$ -carotene (Graham and Wilcox, 2000).

Further potential applications of microalgal products are the utilization of secondary metabolite, fertilizer and biofuel production. In addition to  $CO_2$  sequestration, another potential strategy to offset operational costs, is to develop multi-functional systems such as waste treatment and aquaculture farms, functions (Pedroni et al., 2001). Since economic feasibility is one of the major issues to realize biological mitigation systems, seeking additional value for the system is an important criterion.

### CULTURAL SYSTEMS

Two distinctive cultural systems have been proposed for  $CO_2$  sequestration with microalgae. One is the open pond system, and the other is the closed photobioreactor system. There is ongoing discussion regarding whether the open pond system or the closed photobioreactor system would be better for  $CO_2$ sequestration (Benemann, 1997; Pedroni et al., 2001). Apparent advantages for utilizing the open pond system are low initial and operational costs. On the other hand, an advantage for the photobioreactor system has a higher potential productivity due to better environmental control and harvesting efficiency.

For an open pond system, the size of the area needed to assimilate significant amounts of  $CO_2$  is being criticized. In fact, the typical size of open pond microalgae production systems range from 0.2 to 0.4 ha (Pedroni et al., 2001). However, there are already existing large-scale open pond systems. For example, the largest single algal production systems, developed by the Sosa Texcoco Co. near Mexico city is 900 ha (Becker, 1994).

The size of some recently constructed wetlands, which have been engineered for waste water treatment harnessing the ability of biological systems, is also suggestive. For example, the wetlands constructed for the Everglades Nutrient Removal Project in Florida occupies 1,406 ha (Arizona Department of Environmental Quality, 1995). The development of such constructed wetlands is increasingly popular, and wetlands have been built in a variety of locations, from the Sonoran desert in Arizona to the Everglades National Park in Florida (Arizona Department of Environmental Quality, 1995).

Another strategy, which has never been explored for  $CO_2$  sequestration use, is to build moderately environmentally controlled systems. In such a system, microalgae is grown under a relatively controlled environment, such as a greenhouse and shade. This is an interesting idea since one can control environment inside a greenhouse while construction costs will not be as high as a photobioreactor with a solar collector system. In fact, a similar concept has been applied for wastewater treatment (Arizona Department of Environmental Quality, 1995; Bennett, 1998; Ono and Koshimizu, 2002).

The choice of the cultural systems is an important factor in selecting microalgae species. In the case of the open pond system, climate conditions over the open pond plays an important role.

In the case of the open raceway pond tested by Tohoku Electric Power CO. in Sendai, Japan, stable cultivation of *Tetraselmis* sp. was possible while other species, *Nannochloropsis salina* and *Phaeodactylum tricornutum* could not be cultivated continuously (Matsumoto et al., 1995). *Tetraselmis suecica* has been used in an outdoor culture experiment conducted at the Natural Energy Laboratory of Hawaii (NELH) in Kona on the island of Hawaii (Laws and Berning, 1991).

#### **HIGH CO2 TOLERANCE**

Direct utilization of power plant flue gas has been considered for  $CO_2$  sequestration systems (Benemann, 1993). The advantage of utilizing flue gas directly is the reduction of the cost of separating  $CO_2$  gas. Since power plant flue gas contains a higher concentration of  $CO_2$ , identifying high  $CO_2$  tolerant species is important. Although  $CO_2$  concentrations vary depending on the flue gas source, 15-20% v/v is typically assumed.

Several species have been tested under  $CO_2$  concentrations of over 15%. For example, *Chlorococcum littorale* could grow under 60%  $CO_2$  using the stepwise adaptation technique (Kodama et al., 1994). Another high  $CO_2$  tolerant species is *Euglena gracilis*. Growth of *Euglena gracilis* was enhanced under 5-45 % concentration of  $CO_2$ . The best growth was observed with 5%  $CO_2$  concentration. However, the species did not grow under greater than 45%  $CO_2$  (Nakano et al., 1996). Hirata et al. (1996a; 1996b) reported that *Chlorella* sp. UK001 could grow successfully under 10%  $CO_2$  conditions. It is also reported that *Chlorella* sp. can be grown under 40%  $CO_2$  conditions (Hanagata et al., 1995) found a strain of *Chlorella* sp. T-1 which could grow under 100%  $CO_2$ , although the maximum growth rate occurred under a 10% concentration. *Scenedesmus* sp. could grow under 80%  $CO_2$  conditions but the maximum cell mass was observed in 10-20%  $CO_2$  concentrations (Hanagata et al., 1992). *Cyanidium caldarium* (Seckbach et al., 1971) and some other species of *Cyanidium* can grow in pure  $CO_2$  (Graham and Wilcox, 2000).

Table 1 summarizes the  $CO_2$  tolerance of various species. Note that some species may tolerate even higher carbon dioxide concentrations than listed in the table.

Overall, a number of high CO<sub>2</sub> tolerant species have been identified.

Table 1. CC	$D_2$ to	lerance	of	various	species.
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Species	Known Maximum CO Concentration	References	
Cyanidium caldarium	100%	Seckbach et al., 1971	
Scenedesmus sp.	80%	Hanagata et al., 1992	
Chlorococcum littorale	60%	Kodama et al., 1993	
Synechococcus elongatus	60%	Miyairi, 1997	
Euglena gracilis	45%	Nakano et al., 1996	
Chlorella sp.	40%	Hanagata et al., 1992	
<i>Eudorina</i> spp.	20%	Hanagata et al., 1992	
Dunaliella tertiolecta	15%	Nagase et al., 1998	
Nannochloris sp.	15%	Yoshihara et al., 1996	
Chlamydomonas sp.	15%	Miura et al., 1993	
Tetraselmis sp.	14%	Matsumoto et al., 1995	

#### TOLERANCE ON TRACE ELEMENTS IN THE FLUE GAS

Some researchers considered the effect of trace acid gases on  $CO_2$  sequestration by microalgae, such as  $NO_x$  and  $SO_2$ . As a source of trace elements, both model flue gas (Maeda et al., 1995; Nagase et

al., 1998; Yoshihara et al., 1996) and actual flue gas (Matsumoto et al., 1995) have been used. It is reported that *Nannochloris* sp. could grow under 100 ppm of nitric oxide (NO) (Yoshihara et al., 1996). Under 1000 ppm of NO and15% CO<sub>2</sub> concentration, *Dunaliella tertiolecta* could remove 51 to 96% of nitric oxide depending on the growth condition (Nagase et al., 1998). *Tetraselmis* sp. could grow with actual flue gas with 185 ppm of SOx and 125 ppm of NOx in addition to 14.1% CO<sub>2</sub> (Matsumoto et al., 1995). Maeda et al (1995) examined the tolerance of a strain of *Chlorella* and found that the strain could grow under various combinations of trace elements and concentrations.

# HIGH TEMPERATURE TOLERANCE

Since the temperature of waste gas from thermal power stations is around  $120^{\circ}$ C, the use of thermophilic, or high temperature tolerant species are also being considered (Bayless et al., 2001). Themophiles can grow in temperature ranging from 42-100°C. An obvious advantage of the use of thermophilies for CO<sub>2</sub> sequestration is reduced cooling costs. In addition, some thermophiles produce unique secondary metabolites (Edwards, 1990), which may reduce overall costs for CO<sub>2</sub> sequestration. A disadvantage is the increased loss of water due to evaporation. *Cyanidium caldarium*, which can grow under pure CO<sub>2</sub> is a thermophilic species (Seckbach et al., 1971). Miyairi (1995) examined the growth characteristics of *Synechococcus elongatus* under high CO<sub>2</sub> concentrations. The upper limit of CO<sub>2</sub>, concentration and growth temperature for the species was 60% CO<sub>2</sub> and 60°C (Miyairi, 1995). Currently, an unidentified thermophilic species isolated from Yellowstone National Park has been examined by the group of researchers supported by the U.S. Department of Energy.

Although less tolerant than thermophiles, some mesophiles can still be productive under relatively high temperature (Edwards, 1990). Such species also can be candidate species for the direct use of flue injection.

#### MARINE MICROALGAE

The use of marine microalgae for biological  $CO_2$  sequestration has been considered. One reason is that seawater could be used directly as a growing media so that maintenance costs of microalgae culture could be reduced. Many  $CO_2$  sources, such as power plants, are located along the coastal area.

A number of marine algae species have been tested for CO<sub>2</sub> sequestration applications. Those marine algae species are, *Tetraselmis* sp. (Laws and Berning, 1991; Matsumoto et al., 1995), *Synechococcus* sp. (Takano et al., 1992), *Chlorococcum littorale* (Pesheva et al., 1994), *Chlamydomonas* sp. (Miura et al., 1993), *Nannochloropsis salina* (Matsumoto et al., 1995; Matsumoto et al., 1996) and *Phaeodactylum tricornutum* (Matsumoto et al., 1995).

### **CO<sub>2</sub> ASSIMILATION ABILITY**

 $CO_2$  assimilation ability is a pivotal criterion in selecting algae species. Since growth conditions vary from experiment to experiment, comparison is not straightforward.

A comparison of bubbling CO<sub>2</sub> gas versus adding carbonated water as a means of introducing CO<sub>2</sub> into the microalgal flumes was conducted. The bubbling CO<sub>2</sub> showed 96 ± 11% utilization efficiencies while adding carbonated water showed  $81 \pm 11\%$  efficiencies. The difference in utilization efficiencies between two methods was statistically significant (Laws and Berning, 1991).

## LIGHT CONDITION

Light condition, especially light intensity, is an important factor because the light energy drives photosynthesis. Typical light intensity requirements of microalgae are relatively low in comparison to higher plants. For example, saturating light intensity of *Chlorella* sp. and *Scenedesmus* sp. is approximately 200  $\mu$ mol/sec/m<sup>2</sup> (Hanagata et al., 1992). Microalgae often exhibits photoinhibition under excess light conditions. Photoinhibition is often suspected as the major cause of reducing algal productivity.

The use of a photobioreactor with a solar collector device for the  $CO_2$  mitigation has been explored. Maximum light intensity of 15.7 Wm<sup>-2</sup> could be attained using the system, and the culture of

*Chlorella* sp. could be maintained. The efficiency of light collection and transmission to the algal cells was 8% (Hirata 1996a). Recently, improvements are being made to the solar collecting devices. For example, Oak Ridge National Laboratory has been developing hybrid lighting systems (Muhs, 2000). The system can utilize infrared heat as well as visible light. In addition, artificial lighting is combined so that lighting is possible when there is no natural sunlight. The use of such novel solar collecting and distributing devices would improve  $CO_2$  sequestration efficiency.

#### SOLID SUPPORT

The application of microalgae on solid support is being considered for  $CO_2$  sequestration projects (Bayless et al., 2001). However, the majority of previous research on microalgae has been conducted under liquid suspension conditions.

The potential advantage of solid support application is an increased surface area and probable improvement on harvesting efficiency. It has been suggested that the development of the efficient harvesting system is crucial for the development of successful CO<sub>2</sub> sequestration systems.

#### DISCUSSION

From previous studies, several high  $CO_2$  tolerant species have been identified, for both freshwater and seawater species. Figure 1. shows habitable temperature- $CO_2$  concentration conditions of microalgae species previously tested. As most of these species are in the mesophilic temperature range, it is apparent that only few studies have been done on thermophilic species.



Figure 1. Habitable temperature- $CO_2$  concentration conditions of microalgae species tested. Publications listed on the table 1 were used to illustrate this figure.

So far, no overwhelmingly useful microalgae species have been found for  $CO_2$  sequestration, even though a number of studies have been conducted. For example, *Chlorella* sp. has commercial value and it can grow under high  $CO_2$  concentration, but it does not grow over 45°C (Hanagata et al., 1992; Hirata et al., 1996b). The use of marine strains is advantageous for biological  $CO_2$  assimilation facilities which are located by the coastline. However, this is less attractive for those facilities which are located inland. Each species has disadvantages to some extent. It is also obvious that only a few studies have been done in certain areas, such as the use of thermophilic species and the behavior of microalgae on the solid support cultivation systems.

# CONCLUSION

For the purpose of  $CO_2$  sequestration, the use of microalgae is a unique technology. For example, microalgae can assimilate  $CO_2$  within various ranges of concentration from ambient (0.04%) to 100% v/v  $CO_2$  by selecting adequate species. The technology also works under a wide range of thermal conditions, ranging from 25 to 100°C. Adapting microalgae for the use of  $CO_2$  sequestration also has the potential to produce useful byproducts, and could function multi-purposely. In addition, it is an environmentally friendly technology.

As discussed, there are a variety of technological solutions possible for microalgae-based  $CO_2$  sequestration systems, and thus optimal microalgae employed would differ from system to system. While efforts to find the "ideal" microalgae species will continue, strategic engineering decisions and engineering modifications will be taken into great consideration to realize effective microalgal  $CO_2$  sequestration systems.

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