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REVIEW ARTICLE

Fluorochemical as an oxygen carrier for the growth of microalgae for biodiesel production

Shelendra Kumar Manglavat, Manjeet Singh, Pushpendra Singh, Mahavir Yadav, Archana Tiwari

School of Biotechnology, Rajiv Gandhi Proudyogiki Vishwavidyalaya, Airport Bypass Road, Bhopal-462033, Madhya Pradesh, India

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Corresponding Author

Shelendra Kumar Manglavat

Abstract

The microalgae cultured with the aid of PFCs for algal cell culture presented here, PFCs were used to supply carbon dioxide for cell growth and sweep out oxygen, which is considered as an inhibitor for algal growth. The results indicate that cultivation of the microalgae on the inter phase between liquid growth medium and PFC liquids increases cell growth rates and biomass in a variety of carbon dioxide concentrations ranging from 2 to 20%. The use of per fluorocarbon also caused remarkable increases in both glucose and oxygen consumption rates. The implication of this novel culture method can be harnessed to develop environmentally friendly technology for sequestering carbon dioxide by massive biomass growth of microalgae. By utilizing a fluorochemical, the free oxygen will be removed by degradation; it also helps in increasing photosynthesis which will provide a greater amount of biomass.

Introduction

Perfluorocarbons (PFCs) are petroleum-based compounds synthesized by substituting fluorine for the hydrogen molecules of hydrocarbons. They are both stable and chemically inert due to the presence of very strong carbon fluorine Bonds. Oxygen solubility in PFCs is 10-20 times higher than that in pure water (J.G. Riess 1982).

Perfluorochemicals (PFCs), also known as fluorocarbons or perfluoroalkanes are synthetic fluorine-substituted derivatives of hydrocarbons, i.e. they are similar to hydrocarbons, but all hydrogen ions are replaced by fluorine. Due to the strength of the carbon-fluoride bond, they are stable and inert compounds with a high resistance to heat (Lowe, 2002; Mattiasson & Adlercreutz, 1987; Riess, 2006).

The effect of O_2 was measured as free parameter at prohibited conditions of temperature, pH, light intensity and biomass concentration (Raso et al., 2012).

The present invention provides a method to deliver carbon dioxide in increased concentrations using perfluorodecalin for growth of algae in a photo bioreactor to use in the production of biofuel. The method also increases fatty acids with in algae when perfluorodecalin is used to increase the concentration of carbon dioxide. In addition, the present method uses perfluorodecalin to carry oxygen away from algae after photosynthesis.

In this study the main focus is kept on resolving the problem created by the free oxygen present within the media, which hinders the biomass productivity in culture. Due to the presence of oxygen within the system, the microalgae was not able to flourish well. With the help of a fluorochemicals this difficulty will be defeated. As this fluorochemical is able to mixed the CO_2 present within the environment. That will result in increased growth rate will lead to increased level of lipid will add on to biodiesel production.

To overcome this limited solubility of carbon dioxide in biological growth media, emulsion Perfluorocarbons (PFC) are introduced into the biological growth media. PFCs allow for increased gas solubility, are chemically inert, and have not been demonstrated to be biodegradable or been shown to be toxic to microorganisms. Gas solubility in PFCs follows Henry's law. Gas laden PFCs contacting microorganisms in the growth media increase the transfer of gases to the microorganisms and thereby increase the metabolic rates of the microorganisms. By emulsifying the PFCs, the surface area is increased allowing for increased contact with microorganisms and an even greater rate improvement. Examples of perfluorocarbons used for increasing the solubility of biological growth media are perfluorodecalin, perfluorohexane, perfluorobentane, and perfluorobenzene (Dressler & Chirkov, 2008).

Microalgae

The microalgae are unicellular photosynthetic organisms that use photon energy, CO_2 and they are responsible for photosynthesis in plants for the production of biodiesel. (Converti et al., 2009). The algal culture can capitulate 30–50% oil. The fatty acids were found in the form of algal oil in these proportions: 36% oleic (18:1), 15% palmitic (16:0), 11% stearic (18:0), 8.4% iso-17:0, and 7.4% linoleic (18:2) (Wang et al.,2008).

As one of the oldest life-forms, algae are identified. They are old plants (thallophytes), i.e. absence of roots, leaves and stems, have no sterile covering of cells around the reproductive cells but algae have chlorophylls as their primary photosynthetic pigments. (Brennan & Owende, 2010). Biodiesel from microalgae has the potential as a sustainable fuel, since some species show exceptionally high lipid accumulation potential under various stresses. (Gorain, Bagchi, & Mallick, 2013). Fatty acids are constituents of lipid molecules, a fatty acid (FA) molecule consists of a hydrophilic carboxylate group attached to one end of a hydrophobic hydrocarbon chain (Halim et al., 2012).

The microalgae are extremely responsible for production of Biofuels. A large variety of microalgae that produce algal oils can be considered for biodiesel production. The cost of biodiesel and demand of vegetable oils can be reduced by algal oil instead of vegetable oils, because it has good potential as an alternative diesel fuel. Algal oils have been found to be promising lipids for the production of biodiesel. Algal oils are very important for developing and petroleum-poor countries.

Fluorochemicals

Perfluorocarbons (PFCs), also recognized as fluorochemicals are imitation fluorine-substitutes which are comparable to hydrocarbons, however all hydrogens are replaced by fluorine molecules. The perfluorinated oxygen transporter(PFCs) are used in a miniaturized batch system to improve the yield of cultured cells (Pilarek, Glazyrina, & Neubauer, 2011). Normally fluorochemicals are used for deoxygenation in culture of microalgae, it removed the oxygen (O_2) in micro algal culture (Wasanasathian & Peng, 2001).

Perfluorocamicals (PFCs) are fuel-based compounds synthesized by fluorine substitutes in hydrocarbons. They are established and chemically inert because of the incidence of extremely strong bonds of carbon-fluorine. In PFCs solubility of O_2 has 10-20 times high than in chaste water (Elibol & Mavituna, 1999).In the previous studies four fluorochemicals are used for oxygen removal in micro algal culture, such as perfluorooctyl bromide, methoxynonafluorobutane, perfluorodecalin and ethoxynonafluorobutane (Lee et al., 2012).

These fluorochemicals also helps in carbon dioxide emulsification which in addition facilitate the growth of microalgae. Beside the problem created with the sparging and bubbling also overcome as it does not require use of sparger. Accordingly improved growth rate would result in enhanced lipid productivity through which more quantity of biodiesel will be produced.

Property

PFCs has found properties such as inertness & higher gas solubility create gorgeous for utilization in culture biotechnology. Perfluorochemicals and their emulsion based liquids facilitated release to eucaryotic & procaryotic cells. Perfluorocarbon liquids liquefy larger volumes of non-polar and respiratory- gases. The solubility of PFCs are related to molecular volumes of the dissolved gas and the decresing order are $CO_2 > O_2 > CO > N_2$. For example in PFC liquids the oxygen solubility is 35-44 mmol l^{-1} in biomedical applications, as compare to 2.2 mmol l^{-1} for water, CO_2 solubility in PFCs can go above 200 mmol l^{-1}. The oxygen solubility in perfluorocarbon is directly

proportional to the number of fluorine molecules and inversely proportional to the molecular weight of fluorine atom (Lowe, 2002).

Liquid fluorochemicals are characterized by higher solubility of carbon dioxide, oxygen and other gases. The physical property of Perfluorocarbons are such to assist carbon dioxide would be released in excess of a comprehensive time (Wardrop et al., 1997).

Mechanism

Fluorochemical (PFOB) are used enhanced growth rate of algae for biodiesel production.

The fluorochemical are emulsion based hydrocarbon, which contain high gas solubility, which are principle based

Henry law.

$$P = k. C$$

P = pressure

k = constant

C = concentration

The present invention generally relates to a method of increasing the productivity and growth of algae in a photobioreactor system. More specifically, the present invention is a method used to deliver carbon dioxide in increased concentrations using a perfluorodecalin solution for production and growth of algae in a photobioreactor system. The same perfluorodecalin solution is also effective for binding free oxygen, created during photosynthesis, which can inhibit algae growth. The algae grown by the present invention is suited for usage in the production of Biofuels, biomass and hydroponics (Dressler & Chirkov, 2008).

The perfluorochemical (PFC) liquid, perfluorodecalin (PFD) has also been evaluate as an O_2 carrier in cultures of organism (Naeimpoor & Mavituna, 2000), it has been also used to enhance the carbon dioxide provide to culturing shoots of Rosa chinensis. The oxygenated perfluorocarbons (PFC) liquids enhanced plating effectiveness in cell suspension culture of protoplasts (Wardrop et al., 1997). PFCs exhibit exceptional respiratory gas dissolve capacities associated to the low consistent forces that. The uptake and discharge of O_2 (and N_2, CO_2 and other non-polar gases) by a PFC depend fundamentally on the partial pressure of the gas (Audran et al., 2000).

PFCs present more suitable approach for facilitated exchange of respiratory-gas in plant cell cultures than straight sparging or gassing . It is particularly true for breakable plant protoplasts that are extremely vulnerable to osmotic or mechanical damage (Lowe, 2002).

Oxygen increase in the culture media has been extensive considered as a drawback for enclosed microalgae culture. through this concern is not commonly reported in nearly all types of small-scale culture strategy that is most likely because the gas convey apparatuses used are able to supply adequate carbon dioxide and rapidly remove the dissolved oxygen concurrently, it does critically hamper the growth effect in many profitable systems. In fact, the photosynthesis reaction in many micro algal species will be considerably inhibited when the concentration of O_2 are over air saturation (i.e., 0.225 mM of dissolved oxygen at 20^0C) even although the concentration of CO_2 are maintained at prominent levels (Lee et al., 2012).

Hydrofluoropolyethers (HFPEs) can be moderately used as replacements for halogens and PFCs because it has numerous of their similar physical and thermo chemical properties (Tsai,2007). The microalgae cultured in photobioreactor with 60% oxygen atmosphere, It can grow up in all the photobioreactors which consist the fluorochemicals moreover, the Perfluorooctyl bromide system exhibited the most tough efficacy of oxygen removal in the culture media (methoxynonafluorobutane perfluorooctyl bromide, ethoxynonafluorobutane, perfluorodecalin) and yielded a 3-fold increase of biomass production (Lee et al., 2012).

Table 1. Characteristics of PFC liquids compared to those of water at standard pressure and temperature (Ntwampe et al., 2010).

Liquid	Oxygen	Carbon dioxide	Density c	Boiling point d	Molecular weight e
Water	2.2a	57 a	1.0	100	18
FC-40	37b	142b	1.87	155	650
FC-77	56b	214b	1.78	97	415
FC-84	59b	224b	1.73	80	388
Bis-(Perfluorobutyl) ethane	44.0a	203a	1.41	60	464
Perfluorobutyl tetrahydrofur an	51b	209b	1.77	102	416
Perfluorodecalin	35.5a	125a	1.92	142	462
Perfluoro-n-hexane	65b	248b	1.68	59	340
Perfluorooctyl bromide	44.0a	185a	1.93	142	499
Perfluorotributylamine	35.2a	123a	1.85	155	671
Perfluorotripentylamine	31b	117b	1.94	215	821

aGas dissolving capacity in mM at 25°C; bGas dissolving capacity in ml gas/100 ml PFC at 37°C; cDensity in g.cm-3; dBoiling point in °C; eMolecular weight in g/mol.
FC 40/43/77/84-are Fluor inert electronic liquids, products of 3M Company

Toxicity

The fluorochemical has found high density approx 1,400 kg m^{-3} and they immiscible in culture media. PFCs are resolve in bottom of the container and its phase separated. They are not mixed in culture media, because it only mixed on non polar & respiratory gases. The toxicity of each fluorochemical was not found in favor of media and microalgae (Lee et al., 2012).

Perfluorooctane sulfonate (PFOS) was evaluated unaided and in dual mixtures among atrazine, diuron and pentachlorophenol correspondingly to examine the effects of communications between PFOS and other compounds on the growth rate in *Scenedesmus obliquus*. solo application of PFOS showed no inhibition on the growth of *S. obliquus* under 40 mg L^{-1}, while PFOS acting with pentachlorophenol resulted in higher inhibition of algae culture in contrast with pentachlorophenol (Liu et al., 2009).

Conclusion

Biodiesel has become further gorgeous in recent times because of its ecological reimbursement and the fact that it is prepared from renewable resources. Therefore, biodiesel may be well thought-out as diesel fuel substitutes. The use of Biofuels as engine fuel can play a critical role in serving the developed and developing countries to diminish the environmental impact of fossil fuels. In micro algal lipids produced that can be changed to biodiesel fuel, effects of concentration of carbon dioxide aeration on the production of biomass and accumulation of lipids on *Nannochloropsis oculata.*

The fluorochemicals are used as oxygen carrier to surmount the challenge where four liquid fluorochemicals namely Perfluorooctyl bromide, methoxynonafluorobutane, perfluorodecalin and methoxynonafluorobutane By utilizing a fluorochemical, the free oxygen will be removed by degradation. It mixed the environmental CO_2 in culture media so helps in increasing photosynthesis which will provide a greater amount of biomass. Accordingly improved biomass & growth rate would result in enhanced lipid productivity through which more quantity of biodiesel will be produced.

As increases oxygen concentration in culture media then the results was observed decline the growth rate in cell culture. Future increasing the O_2 concentration for 20 to 75% aeration the effect of specific growth was negligible but as the air saturation was increased from 75 to 250% then the specific growth rate was decreased .so in the large scale culture oxygen concentration will be controlled in favor of microalgae for biodiesel production.

References

Agarwal, A. K. (2007). "Biofuels (alcohols and biodiesel) applications as fuels for internal combustion engines." Progress in energy and combustion science 33(3): 233-271.

Audran, M., M. P. Krafft, J. De Ceaurriz, J.-C. Maturin, M.-T. Sicart, B. Marion, G. Bougard and F. Bressolle (2000). "Determination of perfluorodecalin and perfluoro-< i> N</i>-methylcyclohexylpiperidine in rat blood by gas chromatography–mass spectrometry." Journal of Chromatography B: Biomedical Sciences and Applications 745(2): 333-343.

Brennan, L. and P. Owende (2010). "Biofuels from microalgae—a review of technologies for production, processing, and extractions of biofuels and co-products." Renewable and Sustainable Energy Reviews 14(2): 557-577.

Converti, A., et al. (2009). "Effect of temperature and nitrogen concentration on the growth and lipid content of< i> *Nannochloropsis oculata*</i> and< i> Chlorella vulgaris</i> for biodiesel production." Chemical Engineering and Processing: Process Intensification 48(6): 1146-1151.

Chisti, Y. (2007). "Biodiesel from microalgae." Biotechnology advances 25(3): 294-306.

Chiu, S.-Y., et al. (2009). "Lipid accumulation and CO< sub> 2</sub> utilization of *Nannochloropsis oculata* in response to CO< sub> 2</sub> aeration." Bioresource technology 100(2): 833-838.

Demirbas, A. (2009). "Political, economic and environmental impacts of biofuels: A review." Applied Energy 86: S108-S117.

Elibol, M. and F. Mavituna (1999). "A remedy to oxygen limitation problem in antibiotic production: addition of perfluorocarbon." Biochemical engineering journal 3(1): 1-7.

Gorain, P. C., et al. (2013). "Effects of calcium, magnesium and sodium chloride in enhancing lipid accumulation in two green microalgae." Environmental Technology(just-accepted): 1-17.

Halim, R., et al. (2012). "Extraction of oil from microalgae for biodiesel production: A review." Biotechnology advances 30(3): 709-732.

J.G. Riess, M. Le Blanc, Pure Appl. Chem. 54 (1982) 2383±2406.

Korman, T. P., B. Sahachartsiri, D. M. Charbonneau, G. L. Huang, M. Beauregard and J. U. Bowie (2013). "Dieselzymes: development of a stable and methanol tolerant lipase for biodiesel production by directed evolution." Biotechnology for biofuels 6(1): 70.

Lardon, L., et al. (2009). "Life-cycle assessment of biodiesel production from microalgae." Environmental science & technology 43(17): 6475-6481.

Lawton, R. J., R. de Nys and N. A. Paul (2013). "Selecting Reliable and Robust Freshwater Macroalgae for Biomass Applications." PloS one 8(5): e64168

Lee, Y.-H., et al. (2012). "Using fluorochemical as oxygen carrier to enhance the growth of marine microalga *Nannochloropsis oculata*." Bioprocess and biosystems engineering: 1-8.

Liu, B. and Z. K. Zhao (2007). "Biodiesel production by direct methanolysis of oleaginous microbial biomass." Journal of Chemical Technology and Biotechnology 82(8): 775-780.

Liu, W., Y.-B. Zhang, X. Quan, Y.-H. Jin and S. Chen (2009). "Effect of perfluorooctane sulfonate on toxicity and cell uptake of other compounds with different hydrophobicity in green alga." Chemosphere 75(3): 405-409.

Lowe, K. C. (2002). "Perfluorochemical respiratory gas carriers: benefits to cell culture systems." Journal of fluorine chemistry 118(1): 19-26.

Ma, F. and M. A. Hanna (1999). "Biodiesel production: a review." Bioresource technology 70(1): 1-15.

Mandal, S. and N. Mallick (2012). "Biodiesel production by the green microalga *Scenedesmus obliquus* in a recirculatory aquaculture system." Applied and environmental microbiology 78(16): 5929-5934.

Naeimpoor, F. and F. Mavituna (2000). "Metabolic Flux Analysis in *Streptomyces coelicolor* under Various Nutrient Limitations." Metabolic engineering 2(2): 140-148.

Pilarek, M., J. Glazyrina and P. Neubauer (2011). "Enhanced growth and recombinant protein production of *Escherichia coli* by a perfluorinated oxygen carrier in miniaturized fed-batch cultures." Microb Cell Fact 10: 50.

Raso, S., et al. (2012). "Effect of oxygen concentration on the growth of *Nannochloropsis sp*. at low light intensity." Journal of applied phycology 24(4): 863-871.

Ren, J., A. Manzardo, A. Mazzi, A. Fedele and A. Scipioni (2013). "Emergy Analysis and Sustainability Efficiency Analysis of Different Crop-Based Biodiesel in Life Cycle Perspective." The Scientific World Journal 2013.

Rukminasari, N. "Effect of nutrient depletion and temperature stressed on growth and lipid accumulation in marine-green algae *Nannochloropsis sp*."

Sazdanoff, N. (2006). "Modeling and simulation of the algae to biodiesel fuel cycle."

Tran, L. S., B. Sirjean, P.-A. Glaude, R. Fournet and F. Battin-Leclerc (2012). "Progress in detailed kinetic modeling of the combustion of oxygenated components of biofuels." Energy 43(1): 4-18.

Tsai, W.-T. (2007). "Environmental risk assessment of hydrofluoropolyethers (HFPEs)." Journal of hazardous materials 139(2): 185-192.

Wang, B., et al. (2008). "CO2 bio-mitigation using microalgae." Applied Microbiology and Biotechnology 79(5): 707-718.

Wardrop, J., K. Lowe, M. Davey, R. Marchant and J. Power (1997). "Carbon dioxide-gassed fluorocarbon enhances micropropagation of rose (Rosa chinesis Jacq.)." Plant Cell Reports 17(1): 17-21.

Yang, F., M. A. Hanna and R. Sun (2012). "Value-added uses for crude glycerol-a byproduct of biodiesel production." Biotechnol Biofuels 5: 13.

Zaimes, G. G. and V. Khanna (2013). "Microalgal biomass production pathways: evaluation of life cycle environmental impacts." Biotechnology for Biofuels 6(1): 88.