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A Novel Aeration Method for the Preparation of Algae (Dunaliella Salina) Biomass for Biofuel Production.

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ABSTRACT: *Preparation of algae (Dunaliela Salina) biomass in ammonia (NH_4^+) and nitrate (NO_3^-) growth media for biofuel production was investigated, with special attention on the elimination of inhibitory oxygen that adversely affects algae growth. A novel aeration method based on high and efficient transfer of carbon dioxide (CO_2) required to stabilize the CO_2 of the algae growth medium in a short time was adopted for the elimination of the inhibitory oxygen. The novel aeration method was found to increase the algae growth rate in the growth media investigated as suggested by increases in pH and decreases in dissolved oxygen concentration. However, algae grown in ammonia medium showed 17% higher growth rate than algae grown in nitrate medium. The high mass transfer of CO_2 and high energy efficiency make the novel aeration method of algae growth in ammonia medium better suited for high yield of algae biomass for biofuel production.*

KEYWORDS: Aeration, algae, biofuel, biomass, growth medium.

I. INTRODUCTION

Over the years, fossil fuels have dominated the energy supply needs of the world market. However, numerous energy issues have led to the search for alternative energy sources. Among them is the dramatic rise in the price of crude oil [1-3] raising global energy fears especially among non crude oil producing nations that depend solely on imported crude as the major source of energy supply. This is in addition to fossil fuel reserves which decrease by the day as they are not renewable energy sources, and would one day, run out. Again, the adverse effects of global warming and climatic change due to high volumes of greenhouse gas emissions from combustion of fossil fuels [3-6] have been on the increase in the past years, raising serious global concern on the environment. These daring energy issues have led to a search for alternative energy sources that are cheap and environmental friendly to reduce over-dependence on fossil fuels. Of all the alternative energy sources investigated, algae biomass proves to be the best potential replacement option. This is largely due to the rapid growth rate and high lipid content of algae, and production of biodegradable fuels unlike other plant biomass. In recent years, algae has attracted global attention as a result of its potential as biofuel feedstock, effluent remediation and lots of valuable natural products they produce [2]. Algae are mostly aquatic and can grow in brackish, fresh and marine water.

They can also grow in different habitats such as snow, banks, deserts, soils, hot springs, rocks, tree trunks ([7,8]. *Dunaliella Salina* is an algae specie and is one of the most important industrial microalgae because of its biofuel feedstock and large β-carotene accumulations. *Dunaliella Salina* is among the most studied green microalgae specie because they can thrive in environments of extreme salinity [1] and can grow in wide range of salt concentrations between 50mM to above 5M NaCL[9] and have large production of β-carotene (about 10%*D.Salina* dry weight in lipid globules located in the chloroplast). *D.Salina* is used in evolution of salt adaptation and attractive β-carotene "cell factory" because of these traits [10]. Biofuels that can be produced with algae biomass feedstock are biodiesel, methane [5,11,12], and biohydrogen [5,12,13]. The major setbacks of algae biofuel production are high cost of harvesting due to low biomass concentration in microalgae culture, drying due to large amount of water in the harvested algae, high intense care and cost when compared to conventional crop production; but all these problems can be overcome by technological developments [12]. Since mass culture of algae for biofuel production requires exploitation of high photo conversion of algae in the presence of carbon dioxide (CO_2) and other growth requirements for the production of biomass that would be used for biofuel production, the most suitable medium and culture conditions for high yield are required to

achieve this purpose, and also to achieve a reduced carbon footprint. For high algae yield and low carbon footprint, this study investigates the use of a novel fluidic oscillator generating micro-bubbles [14] for transfer of CO_2 that is highly required for algae growth into algae culture using NO_3^- and NH_4^+ as different sources of nitrogen in order to determine the most suitable growth medium for high algae growth. Measurement of PH change and dissolved oxygen concentration in the medium before, during and after bubbling CO_2 into the medium was used to evaluate the mass transfer rate of the novel fluidic oscillator micro-bubbles.

II. EXPERIMENTAL

2.1.Materials

Bioreactors, ceramic diffusers, pipes, valves, clips, flow meter, high speed camera, conical flasks, timer, rule, centrifuge, cuvette, 5% CO_2 cylinders, trolley, lights, thermometer, pH meter, clamps, stands, test tubes, Duran bottles, measuring cylinders, distilled water, screws, aluminium foil, masking tape, markers, oxygen probe, measuring tape, magnetic stirrers, weighing balance, sample bottles, oscillator, pipettes, bunsen burner. All the materials were kindly provided by the department of Chemical and Biological Engineering, The University of Sheffield, Sheffield, UK.

2.2. Growth Of Algae

Dunaliella Salina algae strain was used in this study and was obtained from the Biology department of The University of Sheffield, Sheffield, UK and pre-cultured.Two nitrogen sources namely ammonium (NH_4^+) and nitrate (NO_3^-) were used in different growth medium. A modified Dunaliella Salina growth medium recipe was used to supply the elements required for algae growth by dissolving calculated amounts of salts in water (Tables 1 and Table 2). The CO_2required for photosynthesis was supplied from a 5% carbon dioxide bottle.
The microbubbles which transferred CO_2 into the algae growth medium were generated from the patentednovel fluidic oscillator [14].

Table 1.Modified *D. Salina* recipe showing actual chemical composition of NO_3^-growthmedium.

Composition of growth medium (Modified)	Original chemicals	Weight of salt required for 250 litres
*0.5 M/L NaCl	NaCl	14.6kg
10 mM/L KCl	KCl	186g
20 mM/L $MgCl_2$	$MgCl_2.6H_2O$	509g
10 mM/L $CaCl_2$	$CaCl_2.6H_2O$	274g
24 mM/L $MgSO_4$	$MgSO_4.7H_2O$	740g
5 mM/L $NaNO_3$	$NaNO_3$	106g
24 mM/L Na_2SO_4	$Na_2SO_4.10H_2O$	484g
0.1 mM/L NaH_2PO_4	Na_2HPO_4	3.549g
0.0015 mM/L FeEDTA	FeEDTA	0.14g
1 mM/L Trace elements	Trace elements (stock solution)	250ml
20 mM/L HEPES	-	-
1 g/L $NaHCO_3$	$NaHCO_3$	250g

Table 2. Modified *D. Salina* recipe showing actual chemical composition of NH_4^+ growth medium.

Composition of growth medium (Modified)	Original chemicals	Weight of salt required for 250 litres
*0.5 M/L NaCl	NaCl	14.6kg
10 mM/L KCl	KCl	186g
20 mM/L $MgCl_2$	$MgCl_2.6H_2O$	509g
10 mM/L $CaCl_2$	$CaCl_2.6H_2O$	274g
24 mM/L $MgSO_4$	$MgSO_4.7H_2O$	740g
5 mM/L NH_4Cl	NH_4Cl	67g
24 mM/L Na_2SO_4	$Na_2SO_4.10H_2O$	484g
0.1 mM/L NaH_2PO_4	Na_2HPO_4	3.549g
0.0015 mM/L FeEDTA	FeEDTA	0.14g
1 mM/L Trace elements	Trace elements (stock solution)	250ml
20 mM/L HEPES	-	-
1 g/L $NaHCO_3$	$NaHCO_3$	250g

Microbubble Generation : The patented fluidic oscillator [14]was used on a compressed ceramic diffuser to generate microbubbles used in the bioreactor for dissolving CO_2 into algae growth medium.

Algae Pre-Culture : Algae strain of *Dunaliella Salina* was pre-cultured under modified *Dunaliella Salina* recipe in conical flask and kept in a shelve under light and allowed to grow.

Algae Culture : Algae were cultured in a 250L bioreactorcapacity with 5% of CO_2 dissolved daily into the medium for about 30mins at a flow rate of 2L/min. The cultured algae were then used for the determination of algae growth.

Algae Growth Determination : The measurement of chlorophyll content was used to determine the growth rate of the cultured algae. 10ml of culture sample collected over the algae culture period were transferred into a 15ml Falcon tube and centrifuged in a bench centrifuge for 10mins. The supernatant was immediately poured off and the pellets were re-suspended in 1ml of distilled water. Thereafter, 4ml of acetone were added to each of the pellets and allowed to stand for a few minutes in direct sunlight to break open the cells and release the chlorophyll contained therein, and then centrifuged for 5mins until the pellet content was completely white. The optical density of each two supernatants was measured with a spectrophotometer. The chlorophyll content was calculated using the chlorophyll content equation. (Equation 1)

$$Chlorophyll\ (\mu g\ /\ ml) = \frac{OD_{645} \times 202 + OD_{663} \times 80.5}{2 \times 5} \quad (1)$$

Where OD_{645} and $OD_{663}$are absorption wave lengths

Dissolved Oxygen and pH Measurement : Dissolved oxygen probe was used to measure the amount of dissolved oxygen in the growth medium before and during the dissolving of CO_2 in the growth medium while a pH meter was used to measure the PH before and during CO_2 dissolution.

III. RESULTS AND DISCUSSION

Chlorophyll Content Measurement

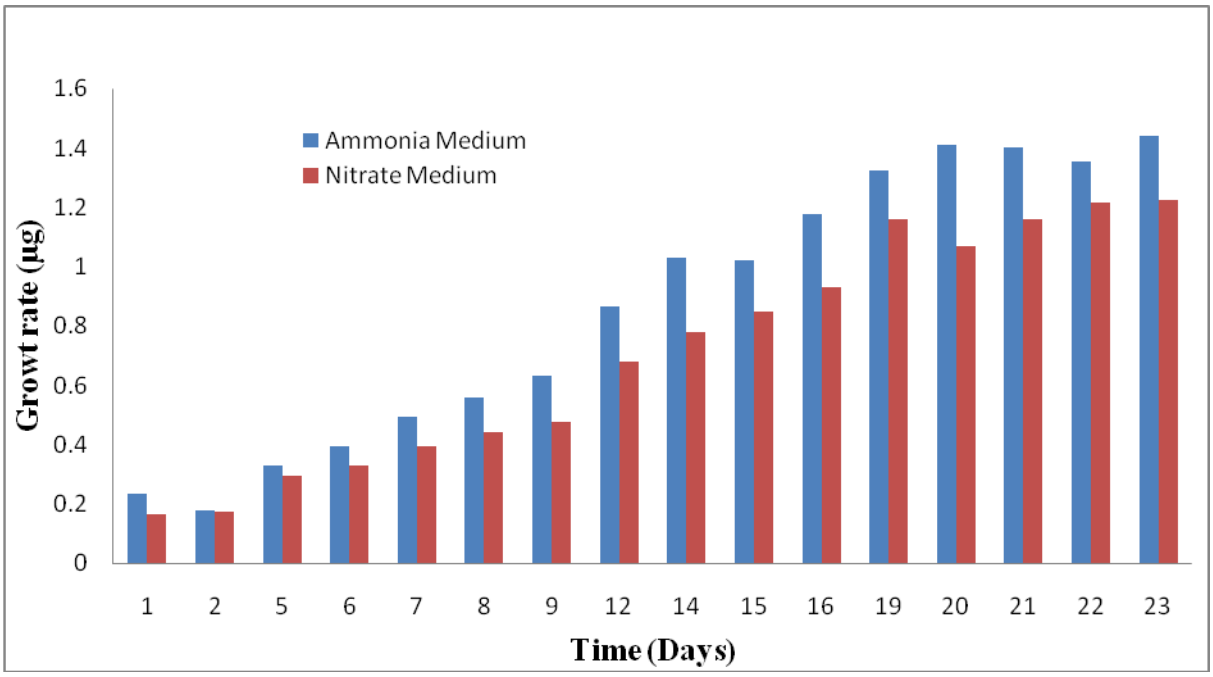

Figure1. Plot of chlorophyl content of Ammonia (NH_4^+) and Nitrate (NO_3^-)growth media versus time.

Figure 1 shows the results of algae growth rate obtained by measuring the chlorophyll content of the algae. The result shows that *D. Salina* specie grows faster in NH_4^+ than in NO_3^- medium. The higher algae growth rate of *D. Salina* in NH_4^+ growth mediumthan in NO_3^- growth medium is in agreement with the result of studies carried out by Giordano, [17],

where algae grown in NH_4^+ medium had higher growth rate than algae cultured in NO_3^- medium. This can be attributed to the fact that algae cells can readily use nitrogen from NH_4^+ directly without any further conversion unlike NO_3^- that requires further conversion to NH_4^+ before nitrogen can be available to algae cells for metabolism [8], Again, algae conserves energy when grown in NH_4^+ medium as a result of readily available nitrogen that does not require energy for conversion. Furthermore, algae grown in NH_4^+ medium saves time that would have been used in the conversion of NO_3^- to NH_4^+ in growth metabolism.

This time saving benefit due to readily available nitrogen in NH_4^+ can also be why fast growing algae like *D. Salina* prefers NH_4^+ growth medium. This is in agreement with the findings of Kumar *et al* [15] who revealed that algae that have high growth rate prefer NH_4^+ growth medium to NO_3^- growth medium as nitrogen source. However, the initial decrease in growth observed in algae grown in NH_4^+ a day after algae inoculation can be related to some undesirable side effects. Richmond [18], pointed out that these undesirable side effects are likely to take place in NH_4^+ growth medium due to sharp drop in pH as shown in the algae grown in NH_4^+ growth medium where the ph dropped from initial inoculation pH of 7.13 to 6.95, even as algae density reduced to about 25% of its initial inoculation density.

This trend was not observed for algae grown in NO_3^- growth medium. This initial side effect due to sharp drop in pH affected the overall algae growth rate because about 25% of algae that were lost to side effects occurred in the algae grown in NH_4^+ growth medium. Without this initial loss, perhaps the overall algae growth observed in NH_4^+ growth medium would have been higher. For this reason, algae cultivation in NH_4^+ growth medium would require higher algae inoculation density to compensate for loses due to side effects to achieve required growth rate when cultivating algae for production of the required algae biomass for production of biofuels and other useful products. Although the algae grown in NO_3^- growth medium did not produce a reduction in algae density after inoculation, they did not start growing very fast and showed 13% loss in growth rate throughout the culture period. At the initial stage, this can be due to adaptation to the new culture environment and the lag phase of growth. However, even without reduction in initial algae growth density in algae grown in NO_3^- growth medium, NH_4^+ growth medium still remains a better and preferred growth medium because of higher overall algae growth. Therefore, to achieve low carbon footprint in algae biofuel production, the use of NH_4^+ growth medium is favoured.

Dissolved Oxygen and pH Measurement : Figure 2 shows the pH change of the algae growth medium on addition of CO_2micro-bubbles. The uptake of CO_2 by algae for growth leads to increased change in pH of the culture medium and can inhibit algae growth at high pH. Because of this, CO_2 has to be added to the growth medium to keep pH at optimum [15]. The rise and fall of pH observed in figure 2 is used to represents the common daily trend of different CO_2 concentrations in the medium. The rise in pH observed from beginning of each day to the end of the previous day, represents algae growth. This is because algae makes use of CO_2 in the medium to grow which leads to increase in pH of the medium. It can be seen from figure 2 that the starting pH of the NH_4^+ growth medium is higher than the starting pH of the NO_3^- growth medium which represents higher photosynthetic rate which is an indication of higher uptake of CO_2 and more algae growth in the NH_4^+ growth medium than in the NO_3^- growth medium. Another reason for higher acidity for NH_4^+ growth medium can be due to the utilization of nitrogen from NH_4^+ source as stated by Goldman and Brewer[16] who revealed that nitrogen uptake from NH_4^+ turns the medium acidic.The rise in pH of both growth medium was controlled by the use of the novel aeration method of Zimmerman et al [14].

This novel aeration system has the capacity to transfer sufficient amount of CO_2 required to stabilize the CO_2 of the medium at optimum level in less than 40mins due to the very high and efficient mass transfer of the microbbubles produced with the novel method. This technique transfers enough CO_2 that can maintain the CO_2 concentration of the medium for a whole day in a short time. The high mass transfer of this novel aeration system has the potential of making microalagae biofuel production a promising option to fossil fuels as well as, a potential climatic change mitigation option.

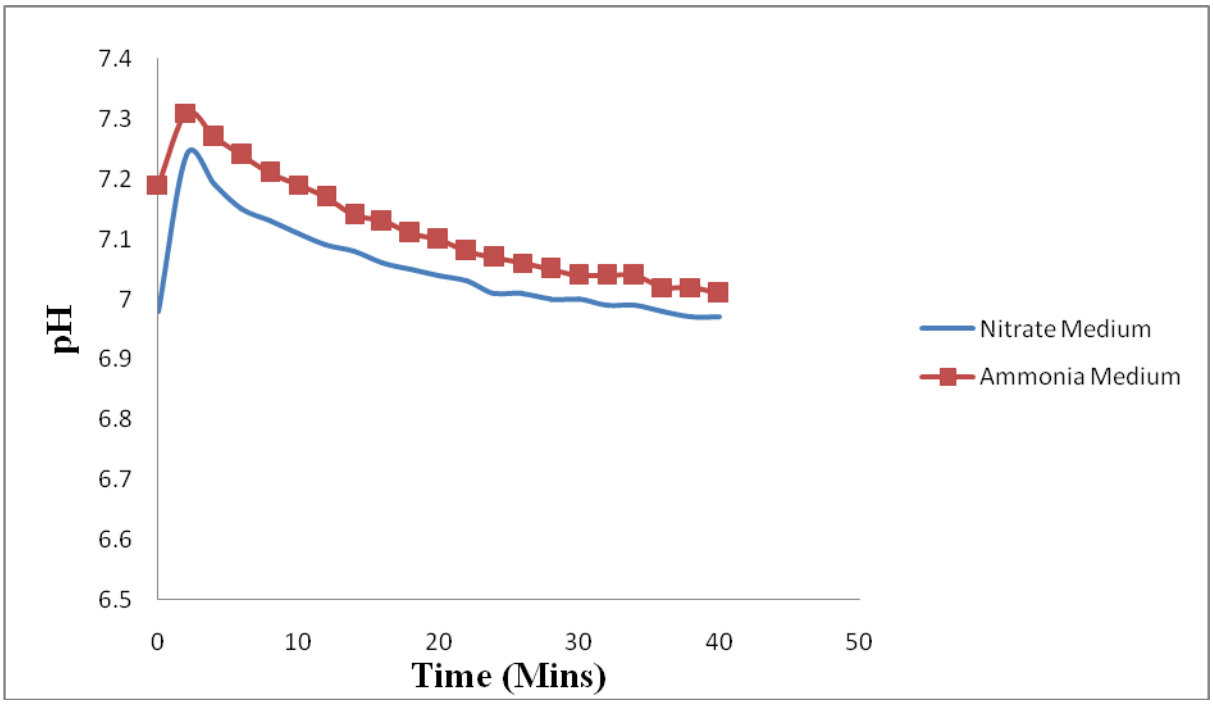

Figure2. Plot ofdaily pH change on addition of CO_2 microbubbles

As Shown in Fig3, the ammonia medium has higher dissolved oxygen which indicates higher growth rate when compared to the nitrate medium. However, the introduction of CO_2 microbubbles into the bioreactor by the novel microbubble aeration method [14] rapidly decreased the concentration of dissolved oxygen. This indicates that the use of the novel aeration method in algae mass culture for biofuel production is more efficient than the conventional methods in eliminating inhibitory oxygen that adversely affects algae growth. This method provides algae mass culture with good growing environment maintaining low oxygen concentrations in algae mass culture. The efficient oxygen striping ability of this novel aeration method is achieved by the oscillatory effect and high momentum of the microbubbles, as well as the low rising velocity of the microbubbles.

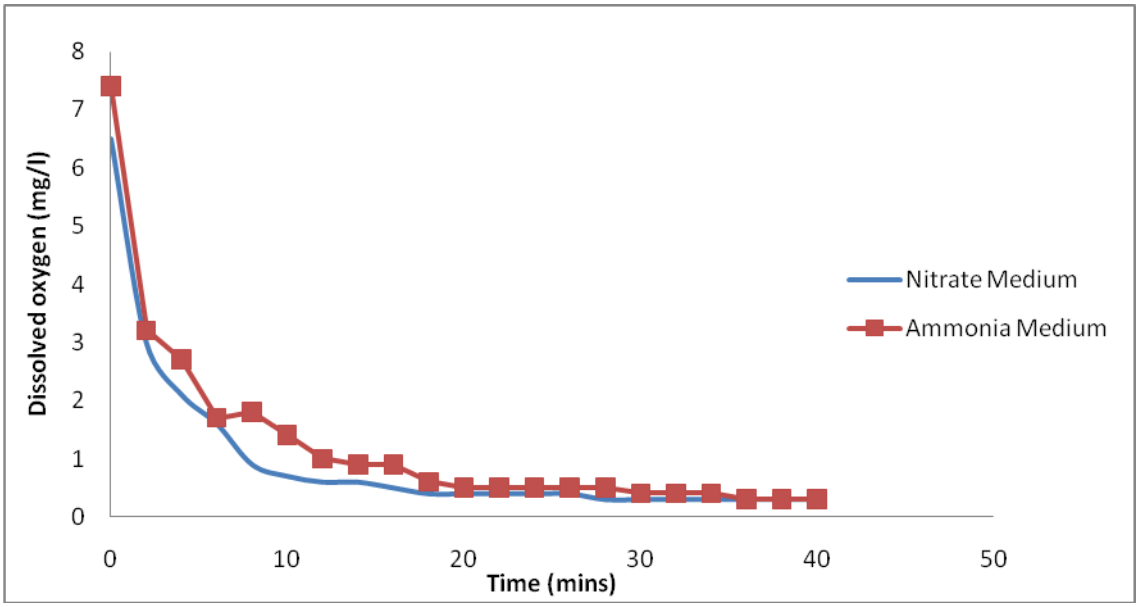

Figure3. Plot of daily dissolved Oxygen in Nitrate and Ammonia growth media versus time

IV. CONCLUSION

The following conclusion can be drawn from this work;

- Algae grown in NH_4^+growth medium had 17% higher growth rate than algae grown in NO_3^- growth medium. This makes NH_4^+ growth medium a better growth medium for commercial cultivation of algae for maximum yield, which would in turn, make algae biofuels viable by supplying enough algae feedstock required for biofuel production.However, Control measures should be taken when using NH_4^+ as nitrogen source due to rapid drop in pH as nitrogen is taken up. On the other hand, if a strain that can withstand the sudden pH change with desired qualities is used, high yields of algae biomass can be achieved because nitrogen in NH_4^+ is readily available to algae for metabolism unlike nitrate that would require further conversion.
- Also, high algae yield withNH_4^+ growth medium would reduce the high cost of harvestingalgae with low biomass concentration. This would in turn increase the potentials of algae biomass as a potential replacement option for fossil fuels.
- The incorporation of the novel fluidic oscillator to large scale algae biofuel production would hasten the replacement of fossil fuels with algae biofuels because of the high mass transfer of the novel method. Again, the energy efficiency associated with the novel method would not only reduce the cost of algae biofuel production, but would also, reduce the carbon footprint of algae biofuel which is of great importance to the environment as it would lead to algae taking up more CO_2.
- The use of algae in biofuel production would provide energy security and global economic benefits like provision of jobs in every part of the globe as algae biofuels can be produced in any part of the globe due to the fact that algae can be grown in all environments even in areas of extreme weather conditions such as in desserts and brackish water unlike economic benefits from oil exploration presently enjoyed by only regions and countries with fossil fuel deposits. The biofuels produced from them would also contribute immensely to the environment as they are biodegradable, can be combined with waste treatment. Even when they release CO_2 back into the atmosphere, it would balance for the CO_2 used during growth.

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