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Influence of media composition on the growth rate of *Chlorella vulgaris* and *Scenedesmus acutus* utilized for CO_2 mitigation

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Abstract

Atmospheric carbon dioxide levels have increased since the industrial revolution due to increasing combustion of fossil fuels. One possible CO_2 mitigation strategy is the use of microalgae for mitigation of CO_2. This paper focuses on the influence of media composition on the growth rate of two microalgae strains, *Chlorella vulgaris* and *Scenedesmus actus*. A KNO_3 based medium was found to work better for *Chlorella*, while a urea based equivalent worked better for *Scenedesmus*. The urea based media investigated here resulted in growth similar to that found with previously reported KNO_3 based media. This should result in an economic benefit in large scale algae cultivation for CO_2 mitigation, as urea is typically less expensive than KNO_3. Additional media components were tested and it was found that EDTA, vitamin B, and the addition of B, V, and Mo do not result in a significant increase in algae growth rate under process conditions used.

Key words: CO_2 mitigation, *Chlorella*, *Scenedesmus*, growth media, urea.

Introduction

The current CO_2 concentration in the atmosphere is believed to be the highest it has been in the last 650,000 years (Canadell ea al. 2007). The carbon dioxide from energy-related activities represented 82% of total U.S. greenhouse gas emissions in 2008 (EIA 2008). Coal-fired power plants alone emit about 1.9 billion metric tons of CO_2 annually, representing 33% of these energy-related CO_2 emissions. One possible strategy for CO_2 mitigation is the use of microalgae (Sayre 2010; Wang et al. 2008; Ho et al. 2011).

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The fundamental concepts to develop a microalgal CO_2 mitigation system include strain selection, media development, cultivation system design, and downstream processing. Optimizing medium is to balance the minimal amounts of nutrients to provide and the maximum amounts of nutrients required by cells. Nutrient deficiency generally induces the decrease of CO_2 fixation and cell growth (Healey 1973). However it has been reported that nitrogen deficiency lead to accumulation of long chain fatty acids and benefit biofuel production which is another application of microalgae, but not the main focus of CO_2 bio-mitigation (Ho et al. 2010). On the other hand, over dosing is a waste of resources and adds operation cost.

This paper focuses on the influence of media composition on the growth rate of two microalgae strains proposed as suitable for CO_2 mitigation, *Chlorella vulgaris* and *Scenedesmus actus*. After first considering the literature data underpinning the selection of these strains and the required nutrients, the results of an experimental study aimed at optimizing the culture medium for these strains is presented. Nitrogen source dependence was studied comparing KNO_3 (M-8 media) and urea. Various urea loadings were testing to determine optimal levels of urea addition. Different EDTA sources were tested and compared to the growth rate without EDTA. Vitamin B and micronutrients (B, V, and Mo) were added to determine if these additions could enhance growth. Finally, the growth rate at various initial pH values was determined. Other factors were held constant (CO_2 levels, temperature, light intensity, and photobioreactor design).

Microalgae exist and thrive everywhere on Earth, however not just any microalga is suitable for the capture of CO_2 from flue gas. Flue gas is emitted from coal power plants at a relatively high temperature (even after gas clean up), and usually contains 10-15% CO_2. If closed photobioreactors are chosen for the bio-mitigation, the operating temperature often exceeds ambient level. Therefore two primary properties of the ideal strains should be tolerance to high CO_2 concentrations and high temperatures. With these goals in mind, the search for the ideal strains has been ongoing for decades. Scientists have isolated and selected various species from high temperature (30-70 ˚C) and high CO_2 locations, like warm natural carbonate springs (Sakai et al. 1995; Westerhoff et al. 2010), wastewater treatment facilities (de-Bashan et al. 2008), or artificial extreme conditions (Morais and Costa 2007). On the other hand,

instead of searching for wild candidates, researchers have tried to acclimate some strains to the working environment (Yun et al. 1996). For example, after ten days' cultivation, the dry cell weight (g/L) of *Chlorella vulgaris* UTEX259 is approximately 0.5 or 3 g/L under 2 or 20% CO_2, respectively. When gradually increasing CO_2 concentration from 5 to 30% over 10 days incubation, the dry cell weight reaches 3.5 g/L at the end. Apparently, this species of *Chlorella* is tolerant to high CO_2 concentration after acclimation (Yun et al. 1996).

From a historical perspective, chlorophyta is one of most studied groups of microalgae in terms of CO_2 bio-mitigation and biofuel production. Among the most promising species, *Chlorella* and *Scenedesmus* have been the most studied to date (Morias and Costa 2007). *Chlorella* occurs as 2~12 μm spherical or ellipsoidal unicells thriving in fresh and marine waters and soils (Graham and Wilcox 2000). Molecular phylogenetic analyses indicate that *Chlorella* is polyphyletic and descended from multiple ancestors. *Scenedesmus* typically appears as a flat colony of 4, 8, or 16 linearly arranged cells growing in fresh and occasionally brackish water (Graham and Wilcox 2000). Note that they rarely form dense populations in natural environments.

A number of fresh water based media have been applied in the cultivation of *Chlorella* and *Scenedesmus* (Table 1). Nitrogen is often added to media in the form of potassium nitrate, sodium nitrate or calcium nitrate. Phosphorus can be in the form of monopotassium phosphate, dipotassium phosphate or disodium phosphate, which also function as buffers to maintain the neutral pH of media. Iron and magnesium play critical roles in photosynthesis, so a sufficient supply of iron and magnesium directly favors CO_2 fixation and biomass production. Iron can be provided as ferrous sulfate, Fe EDTA or ferric ammonium citrate ($C_6H_{5+4y}Fe_xN_yO_7$). Magnesium mainly comes from its sulfate form. Calcium is added as calcium chloride. *Chlorella* cells also contain trace amounts of zinc, copper and manganese, which are linked to cell division (Healey 1973). Therefore, manganese (II) chloride, copper (II) sulfate, and zinc sulfate are employed as micronutrient supplements. Moreover, based on different observations regarding microalgae composition and metabolism, several other minerals are provided to the cells, for example, in Fitzgerald medium (Table 1), a total of eleven trace minerals are supplied.

Optimal media provide the minimum quantity of nutrients to support maximum growth of algae. Nutrient deficiency generally induces decreased cell growth rates and as a result, lower CO_2 fixation rates (Healey 1973). However, it has been reported that nitrogen deficiency leads to accumulation of long chain fatty acids that improves biofuel production (which is another benefit of microalgae), but is not the main focus of CO_2 bio-mitigation (Ho et al. 2010). On the other hand, oversupplying the medium with nutrients requires additional energy (embodied in the nutrients), adds operational costs, potentially results in additional N_2O off gassing (a major greenhouse gas), and additional water treatment costs. Different media have varying nutrient quantities that can significantly change the quantity of cell biomass produced during cultivation (Mandalam and Palsson 1998). *Chlorella vulgaris* has been cultivated in N-8 and M-8 media for over 500 hours. Due to lower amounts of nitrogen, iron, magnesium and sulfate compared with M-8 (Table 1), N-8 resulted in biomass accumulation during the first 250 hours, but not a steady accumulation of chlorophyll that is responsible for photosynthesis and continued cell growth. The extremely low synthesis rate of chlorophyll ultimately inhibits the cell growth. Because the consumption of nutrients synchronizes with cell growth, it is necessary to continuously monitor the composition of the medium and continuously replace nutrients (Chen et al. 2011).

Most marine and fresh water microalgae can utilize nitrate or urea as nitrogen sources. Both the nitrogen concentration and source in the medium can be responsible for important changes in the growth and biochemical composition of microalgal cells, chlorophylls, proteins, carbohydrates, and lipids (Fabregas et al. 1989). For practical applications at the pilot plant and industrial scale the choice of N-source of the medium should be based on algae metabolism.

The nitrogen source for the medium (sans the one introduced in this paper) shown in Table 1 is KNO_3. However, there has been some work with *Spirulina platensis* that showed that replacing KNO_3 with urea achieved a biomass gain of 37% and consequently larger total amounts of chlorophyll at a lower cost as compared to cultures grown on KNO_3 (Danesi et al. 2002). Utilization of mixed N-sources, KNO_3 and urea, may involve inhibition or repression since NO_3^- and NH_4^+ (hydrolysis of urea gives ammonia) are metabolized via a common pathway. Evolutionary optimal growth of bacteria, algae, and yeast on a mixture of C- or N-sources is achieved via control of uptake of a so called "faster" substrate which acts as a repressor to the uptake of the "slower" substrate. The use of a single compound with two different N-sources, such as NH_4NO_3, could also lead to inhibition.

Materials and methods

Chlorella vulgaris (#152075) was obtained from the algae collection at Carolina Biological Supply Company (Burlington, NC). *Scenedesmus acutus* (UTEX 72) was purchased from the algae culture collection at the University of Texas at Austin. Seed cultures of *Chlorella vulgaris* and *Scenedesmus* were grown and maintained in M-8 or urea media (Table 1). Media were prepared with city water (dechlorinated with 0.06 g/L sodium thiosulfate) and filtered through a 0.2 μm Nalgene nylon membrane filter (47 mm diameter). Once a week, microalgal cells were transferred into new media for subculture or used as inoculums for an experiment. Working in a laminar flow hood, 2 mL liquid stock culture was transferred to 400 mL fresh media in a 500 mL Erlenmeyer flask. Newly transferred flasks were incubated under warm (Philips F32T8/TL741 Alto, 32 Watts) and cool white (Philips F32T8/TL735 Alto, 32 Watts) fluorescent lights (70 μmol/m^2 per second) in a 16:8 hours light:dark illumination period. Flasks were bubbled with 3% anaerobic grade cylinder CO_2 (approximately 0.14 L/min CO_2 and 4.4 L/min air for the total system of flasks), this percentage of CO_2 being sufficient to ensure saturation of the flask with CO_2. Flasks were placed on a shaking table (100 rpm) and kept at room temperature (22°C).

Dry weight and growth rate

Chlorella and *Scenedesmus* biomass samples in suspension were filtered using Whatman binder-free glass microfiber filters (type 934-AH, 24 mm diameter). The dry weights (DW) of samples were measured by drying at 105°C for 24 hours. Biomass content was calculated from microalgal dry weight produced per liter (g/L). Growth curves of cultivation time versus DW were linear, hence the slope of the growth curve was used as the growth rate (mg/L/hr; Shuler and Kargi 1992).

Media development

M-8 medium was considered the best starting point for optimization of the media for the flask experiments. Initial studies utilized the M-8 media recipe containing 3.0 g/L KNO_3 (Mandalam and Palsson 1998), which was developed for a higher final biomass density

Table 1: Composition of fresh water based media

	Medium							
	N-8	M-8	BG-11	Fitzgerald	MC	ASM-1*	Detmer	Urea**
Macronutrients (mg/L)								
KNO_3 or $NaNO_3$	1000	3000	1500	496	1250	1000	-	-
KH_2PO_4	740	740	-	-	1250	740	260	118.5
$Na_2HPO_4 \cdot 2H_2O$ or K_2HPO_4	260	260	30.5	39	-	260	-	-
$CaCl_2 \cdot 2H_2O$	13	13	36	36	-	-	-	55
Fe-EDTA	10	10	-	-	-	10	-	-
$FeSO_4 \cdot 7H_2O$	-	130	-	-	2	-	20	-
$MgSO_4 \cdot 7H_2O$	50	400	75	75	1250	50	550	109
Na_2CO_3	-	-	20	20	-	-	-	-
Citric acid	-	-	6	6	-	-	-	-
Na-EDTA	-	-	1	1	-	-	200	-
Ferric ammonium citrate	-	-	6	6	-	-	-	-
$NaSiO_3 \cdot 9H_2O$	-	-	-	25	-	-	-	-
$Ca(NO_3)_2 \cdot 4H_2O$	-	-	-	-	-	-	1000	-
KCl	-	-	-	-	-	-	250	-
$CO(NH_2)_2$	-	-	-	-	-	-	-	550
Reference	1	1	2	3	3	4	5	*
Micronutrients (mg/L)								
$Al_2(SO_4)_3 \cdot 18H_2O$	3.58	3.58	-	-	-		-	-
$MnCl_2 \cdot 4H_2O$ or $MnSO_4 \cdot 4H_2O$	12.98	12.98	181	2.23	2.5		1.81	-
$CuSO_4 \cdot 5H_2O$	1.83	1.83	7.9	-	0.079		0.08	-
$ZnSO_4 \cdot 7H_2O$ or $ZnCl_2$	3.2	3.2	22	0.287	0.22		0.11	-
H_3BO_3	-	-	286	3.1	2.86		2.9	-
$Na_2MoO_4 \cdot 2H_2O$	-	-	39	-	0.02		-	-
$Co(NO_3)_2 \cdot 2H_2O$	-	-	4.9	0.146	-		-	-
$(NH_4)_6MoO_{24} \cdot 4H_2O$	-	-	-	0.088	-		0.018	-
$Na_2WO_4 \cdot 2H_2O$				0.033				
KBr				0.119				
KI				0.083				
$NiSO_4(NH_4)_2SO_4 \cdot 6H_2O$				0.198				
$VOSO_4 \cdot 2H_2O$				0.02				
$Al_2(SO_4)_3K_2SO_4 \cdot 24H_2O$				0.474				
Reference	1	1	2	3	3	4	5	6

* Micronutrients of ASM-1 are not provided. ** Urea formula is the only one using tap water as the water source.
1. Mandalam and Palsson 1998; 2. Tang et al. 2011; 3. Sakai et al. 1995; 4. Jeong and et al. 2003; 5. Ho et al. 2010; 6 This paper

than possible in our set-up (due to light and reactor limitations). Based on the final biomass density achieved in our set-up (typically 1 g/L) the KNO_3 level was scaled down to 0.75 g/L
and the remaining ingredient levels were scaled down by the same factor. By using the elemental composition of *Chlorella vulgaris* biomass (Table 2) and a linear programming procedure (LPP), M-8 was evaluated to see to what extent it is stoichiometrically balanced.

The assumptions for performing LPP procedure were as follows: 1) CO_2 in the liquid phase, light availability, and mixing are not limiting factors; 2) N-source is a reference for calculation of biomass concentration; and 3) micronutrients and sodium ions are supplied by the city water and during pH control. The LPP was coded in MAPLE 12. The LPP results (Table 3) showed that the expected biomass concentration for M-8 medium is 1.35 g/L.

Phosphate concentration in M-8 is higher than the stoichiometric requirement based on elemental analysis. Calcium concentration differences are not crucial for large scale cultivation of fresh algae because both tap (42 ppm) and industrial waters are a source of Ca^{2+}. On the other hand, Ca^{2+} ions and phosphates are responsible for precipitates and algal agglomerates at high pH level (Oh-Hama and Miyachi 1988). Hence, Ca^{2+} ions must be controlled at the lowest possible level or avoided depending on the tap water calcium content. Sulfates are also not a problem, which is in agreement with the water chemistry of flue gases SO_2 and SO_3. Magnesium and iron concentrations in M-8 medium are not optimal, which was also shown by Mandalam and Palsson (Mandalam and Palsson 1998). In this case, micro-elements of both media are not a concern because flue gas and technical grade chemical components are good sources of them. Analytical urea contains Cu^{2+} which is an important micro-element for control of contaminants in industrial applications. The developed algorithm worked well and identified an optimized media formula based on the original M-8 formula from the literature, with urea instead of KNO_3 as the nitrogen source, to be tested experimentally, simply referred to as urea media.

Table 2: Elemental composition of *Chlorella sp.* (by weight)[a]

Element	% by weight	Elemental composition % by weight	
		Min[a]	Max[a]
Macro-elements			
N	7.7	6.2	7.7
P	2.0	1.0	2.0
K	1.62	0.85	1.62
Mg	0.8	0.36	0.8
S	0.39	0.28	0.39
Fe	0.55	0.04	0.55
Micro-elements			
Ca	0.080	0.005	0.080
Zn	0.005	0.0006	0.005
Cu	0.004	0.001	0.004
Mn	0.01	0.002	0.01
B	0.0026	-	0.0026
Mo	0.001	-	0.001
Co	0.001	-	0.001

[a] Elemental composition values (Min, Max) for N, P, K, Mg, S, Fe, Zn, Cu, Mn obtained from (Mandalam and Palsson 1998).

This urea medium did not contain EDTA, since EDTA has the potential to be one of the most expensive components on a large scale. EDTA acts as a chelator in the media and is intended to ensure the iron ions remain dissolved and thus available for algal metabolism. It has been hypothesized that in the absence of EDTA, *Chlorella* would synthesize and extracellularly excrete siderophores to increase the bioavailability of iron (Sukenik and Shelef 1984). A summary of the two formulas tested are shown in Table 4.

Table 3: Evaluation of M-8 media composition on the basis of elemental composition of the biomass and by applying linear programming procedure (LPP).

Ingredient (g/L)	M-8 75%, Literature[a]	LPP results[b]
KNO_3	0.75	0.75
KH_2PO_4	0.185	0.1185
$NaHPO_4$	0.065	not included
$CaCl_2.2H_2O$	0.00325	0.0004
$FeSO_4.7H_2O$	0.0325	0.037
$MgSO_4.7H_2O$	0.1	0.10925
Micronutrients		
$MnCl_2.4H_2O$	0.003245	0.000486
$CuSO_4.5H_2O$	0.000458	0.000212
$ZnSO_4.7H_2O$	0.0008	0.000298

[a] Concentrations are 75% of the recipe for M-8 found in the literature (Mandalam and Palsson 1998).
[b] Concentrations are intended to support the same final concentration as the M-8 based on 75% of the values in the literature, but are adjusted using the Linear Programming Procedure (LPP).

Table 4: Summary of media recipes based on M-8 and urea used to culture *Scenedesmus* and *Chlorella vulgaris*.

Chemical compound	M-8 (literature)	Urea - EDTA
KNO_3	0.75	-
$(NH_2)_2CO$	-	0.55
KH_2PO_4	0.185	0.1185
$NaHPO_4$	0.065	-
$CaCl_2.2H_2O$	0.00325	0.055
$FeSO_4.7H_2O$	0.0325	0.015
$MgSO_4.7H_2O$	0.1	0.109
Na.EDTA.Fe	0.0025	-

Culture Preparation

Prior to experiment, algae stock culture (cultivated in 400 mL urea media with 3% CO_2 for 96 hours) was centrifuged at 1800 rpm for 30 min and the supernatant (media) was discarded. The dewatered algae were re-suspended in deionized water to ensure a uniform algal concentration. Unless otherwise noted, the same preparation procedure was used in all the experiments listed in the paper.

Experimental Procedure

Chlorella and *Scenedesmus* were cultured on a shaking table (100 rpm) in triplicate with M-8 and urea medium (Table 4) at 22°C for 96 hours. Media were prepared with city water (dechlorinated with 0.06 g/L sodium thiosulfate) and filtered through a 0.2 µm Nalgene nylon membrane filter (47 mm diameter). For both cultures, 15 mL of re-suspended algae was added to 400 mL media in a 500 mL flasks bubbled with 3% CO_2. The gas flow rates of 0.14 L/min CO_2 and 4.40 L/min air were regulated with a mass-flow controller (model 5850E, Brooks Instrument, Hatfield, PA) and a flow meter (model VA20439, Dwyer Instrument, Inc., Michigan City, IN), respectively. The culture was illuminated with warm and cool white fluorescent light in 16:8 hours light:dark period (70 µmol/m^2 per second). Dry weight (30 mL samples) and pH measurements were taken at time 0, 24, 48, 72, and 96 hours (±1 h). A schematic drawing of the experimental set up is shown in fig. 1.

Additional studies were done with *Scenedesmus* to better understand which ingredients were necessary for increased growth. All other aspects of the experiment were kept constant, only the media ingredients were changed. The urea dosages were varied at three levels (0.07, 0.14, and 0.275 g/L) and the other components were held constant. Triplicate treatments of growth media containing different levels of iron chelating agent (EDTA) were tested. Urea media was prepared by adding various amounts of EDTA (no EDTA, 0.02 g/L Na.EDTA.Fe, and 0.2 g/L Sprint 330 Na.DTPA.Fe(III)) but keeping the other components constant ($FeSO_4$ was added to make up for the missing Fe in the no ETDA treatment). The effect of vitamin on algae growth was investigated by adding two levels of vitamin B complex (25 and 50 µg/L) to the urea media (control). Micronutrients of boron (0.415 mg/L H_3BO_3), vanadium (0.07 mg/L NH_4VO_3), and molybdenum (0.06 mg/L $Na_2MoO_4.2H_2O$) were added to 400 mL urea media individually or together into triplicate 500 mL flasks (a control was also included). *Scenedesmus* was cultured in urea media at various pH levels (5, 5.5, 6, 6.5, 7, 7.5, and 8). The urea media was adjusted to the desired the pH level with Good's buffers (MES, ADA, and HEPES).

Results and Discussion

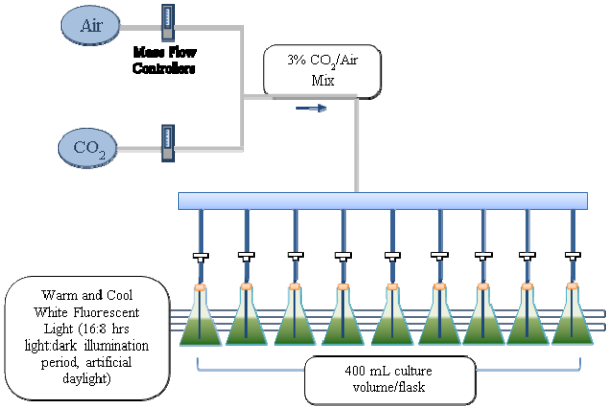

Figure 1: Schematic of the experimental culture system.

Typical growth curve for Chlorella and Scenedesmus growth in urea media are shown in fig. 2, illustrating that the growth curve is best represented as the slope of the linear regression resulting in a production rate in g/L/h.

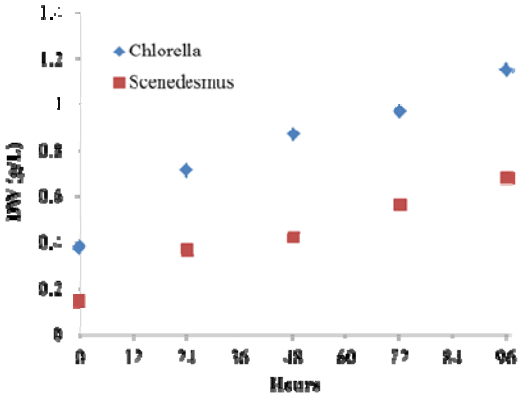

Figure 2: Typical growth curve for *Chlorella* and *Scenedesmus*.

Chlorella and *Scenedesmus* were cultured with M-8 media (based on the literature) and urea media (based on the LPP), the media compositions are summarized in Table 4. For both strains, the growth rates are higher with the urea media (Table 5), although there was not a significant difference (p_{value} equal to 0.17 and 0.74, respectively).

These results illustrate that urea is an adequate nitrogen source for *Scenedesmus* and *Chlorella*. Cell biochemical evidences show that microalgae can effectively use NO_3^-, NO_2^-, N_2 or NH_4^+ as nitrogen sources, but via absolutely different pathways, since only the most reduced form is used to synthesize biomolecules (Glass et al. 2009). To assimilate NO_3^-, nitrate reductase and nitrite reductase are secreted by algae cells to catalyze the two-step reduction reaction shown in Eq. 1 and 2.

$$NO_3^- + 2e^- + 2H^+ \xrightarrow{\text{nitrate reductase}} NO_2^- + H_2O \quad \text{Eq. 1}$$

$$NO_2^- + 6e^- + 8H^+ \xrightarrow{\text{nitrite reductase}} NH_4^+ + 2H_2O \quad \text{Eq. 2}$$

Urea, as dissolved organic nitrogen, can also be assimilated by algae cells. The pathway begins with a reduction reaction facilitated by urease (Eq. 3).

$$CO(NH_2)_2 + H_2O + 2H^+ \xrightarrow{\text{urease}} 2NH_4^+ + CO_2 \quad \text{Eq. 3}$$

After foregoing conversions, ammonium is assimilated through the glutamine synthetase-glutamate synthase (GS-GltS) pathway, and enters cell metabolism eventually (Glass et al. 2009).

Considering urea should be more cost effective, it makes sense to use the urea media in place of M-8. Subsequent experiments were done based on the urea recipe with various changes to see if the changes would make a significant enhancement in the growth rate.

Table 5: Growth rates ± standard error (n=3) for *Chlorella* and *Scenedesmus* grown with M-8 and urea media recipes.

	Growth rate (mg/L/hr)	
Media	*Chlorella*	*Scenedesmus*
M-8	7.50 ± 0.32	2.13 ± 0.11
urea	8.02 ± 0.67	3.90 ± 0.19

According to the LPP simulation, the amount of urea required for a >1 g/L final culture density is 0.55 g/L. To potentially reduce media costs, experiments were done to test three different levels of urea (Table 6). The resulting growth rates were not statistically different (α = 0.05). It even appeared that the lower urea level supported better growth rates overall. Several inorganic forms of nitrogen, including nitrate, nitrite, ammonia ion, as well as urea, have been found to serve as N sources for algae growth (Graham and Wilcox 2000; Glass et al. 2009; Syrett 1988). Urea can serve as a nitrogen source for many algae, including dinoflagellates, *Chlorella* and *Chlamydomonas* (Healey 1973; Syrett 1988; Solomon and Glibert 2008).

Table 6: Growth rate ± standard error for *Scenedesmus* grown with different varying urea levels.

Urea Level	Growth rate (mg/L/hr)
0.55 g/L	6.84 ± 0.36
0.275 g/L	7.03 ± 0.14
0.14 g/L	6.96 ± 0.074
0.07 g/L	7.21 ± 0.18

EDTA was one of the components that was left out of the original urea recipe tested. An additional study was done to specifically test whether the presence and type of EDTA would enhance the growth rate (Table 7). Na.EDTA.Fe is the laboratory grade EDTA reagent (purchased in small quantities from Sigma-Aldrich), while Sprint 330 EDTA is an industrial grade of EDTA (purchased in bulk from a fertilizer supplier), being more cost effective for large scale applications. There was no significant difference between both of the EDTA types and the absence of EDTA. There was a significant difference between the lab and commercial grade (p_{value} = 0.031). While these results show that the presence of EDTA doesn't necessarily improve the growth rate, it was kept in the final version of the urea media because it did help keep iron in solution and made various laboratory tasks easier.

Table 7 Growth rate ± standard error of *Scenedesmus* in the absence of EDTA and with a lab grade (Na.EDTA.Fe) and commercial grade EDTA (Sprint 330 EDTA).

EDTA Type	Growth rate (mg/L/hr)
Na.EDTA.Fe	5.64 ± 0.28
Sprint 330 EDTA	6.83 ± 0.23
No EDTA	6.84 ± 0.36

The inclusion of B vitamins was tested at two different levels (25 and 50 µg/L) and compared to a control (Table 8). The addition of vitamins did not have an effect on the growth rate of *Scenedesmus* in this system. This does not totally agree with Krichnavaruk's results on *Chaetoceros calcitrans* (Krichnavaruk et al. 2005). In that study, it was proved that 1 µg/L of Vitamin B_{12} gave a significant boost on growth rate. It is possible with this small volume; batch cultivation vitamins are not necessary or advantageous for growth. However, the addition of vitamins in an industrial, continuous system could prove to be advantageous.

Table 8 Growth rates ± standard error of *Scenedesmus* with the addition of vitamin B.

Vitamin B Level	Growth rate (mg/L/hr)
0 µg/L	5.19 ± 0.14
25 µg/L	5.10 ± 0.17
50 µg/L	5.06 ± 0.09

There are several micronutrients that could be available from the water source, but there are some trace micronutrients suggested by various media compositions that may not be. Specifically, B, V, and Mo were selected as possible micronutrients that might help increase the algae growth rate. However, there was no significant difference between the control and the medium with each of the elements nor all three (Table 9).

Table 9 Growth rates ± standard error of *Scenedesmus* with the addition of B, V, and Mo.

Additions	Growth rate (mg/L/hr)
none	4.18 ± 0.23
+ B	4.11 ± 0.06
+ V	3.39 ± 0.28
+ Mo	4.26 ± 0.12
+ B, V, Mo	4.14 ± 0.16

In addition to the media ingredients, the pH of the media makes a significant difference. The growth media was buffered to pH values ranging from 5 to 8, fig. 3. The pH of the media will affect the type of CO_2 present and at higher pH values urea is easier to break down, which explains the increase in growth rate as the pH increases. Using a pH of 7 for the urea was deemed appropriate, since this is very close to the pH of the media without buffering.

Conclusions

The influence of media composition and possible flue gas contaminants on the growth rate of two microalgae strains, *Chlorella vulgaris* and *Scenedesmus,* was investigated. These two strains of algae are interesting candidates for large-scale cultivation to mitigate the CO_2 emissions from coal-fired power plants. A

linear programming procedure (LPP) was used in conjunction with the elemental analysis of *Chlorella* to estimate the optimum formulation of the growth medium. The LPP was used to revise the M-8 recipe found in the literature and to develop a new medium based on the use of urea as the nitrogen source. For *Scenedesmus*, the addition of EDTA, vitamin B, or B, V, and Mo did not increase the growth rate. When the urea level was reduced, the growth

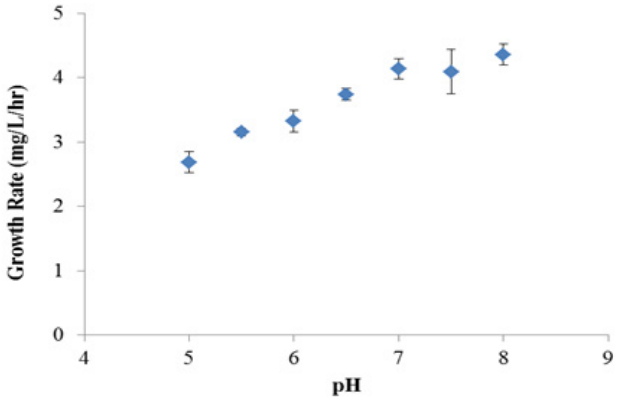

Figure 3: Growth rate versus pH for *Scenedesmus*. Error bars show standard error (n=3).

appeared to increase slightly, showing that the urea level was not the limiting growth factor. The pH of the media had a big influence on the growth rate.

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