Changing operational parameters of a photobioreactor system to culture *Chlorella vulgaris* to maximize carbon dioxide fixation

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Abstract

Managing waste in an efficient and beneficial manner is crucial for NASA to sustain a human population on Mars. A waste management system like BIOSYS is able to convert all water-based waste streams into useful products through mostly biological processes. This system proposes the use of algae photobioreactors to convert carbon dioxide produced from an aerobic digester to oxygen and biomass. The oxygen would be recycled to the aerobic bioreactor or sent to the life support system; the biomass would be used as a source of protein or lipids. A photobioreactor system has been developed to test *Chlorella vulgaris*' ability to remove carbon dioxide from an air stream. Operational parameters that can be changed to study their effects on biomass growth, and subsequently CO_2 fixation, are light intensity, light/dark cycle, gas flow rate, carbon dioxide concentration in the gas, and nutrient addition. A photobioreactor system was operated under semi-batch conditions testing different nitrogen addition concentrations to determine their effect on carbon dioxide fixation. Over 11 days of cultivation, the highest carbon dioxide fixation rate was achieved when 41 mg/L $NaNO_3$-N was added every two days (0.488 g CO_2 L^{-1} day^{-1}). Nitrogen buildup in the reactors with higher nitrogen addition concentrations did not inhibit growth indicating another cause for halted biomass growth. The higher carbon dioxide fixation rate in the early stages of cultivation (0.706 g CO_2 L^{-1} day^{-1}) indicates a need to maintain early cultivation conditions. Future work includes testing different harvesting rates to maintain a biomass productivity conducive to producing constant carbon dioxide fixation.

Introduction

Previous experiments have shown that nitrogen, one of the key nutrient sources of *Chlorella vulgaris*, is completely consumed after two days of cultivation and that biomass growth halts after Day 4 under batch conditions. A nutrient addition strategy (Table 1) was tested to determine if semi-batch operation is beneficial for biomass growth with an overall goal of maintaining a constant biomass productivity leading to steady carbon dioxide fixation.

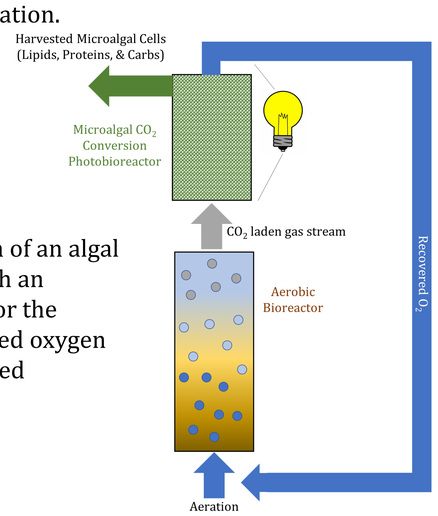
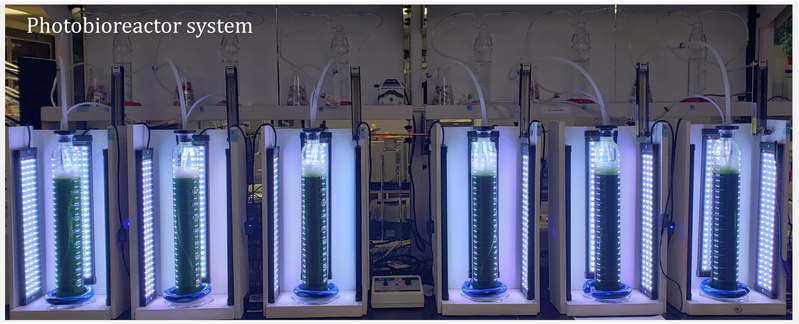


Figure 1: Integration of an algal photobioreactor with an aerobic bioreactor for the production of recycled oxygen and other value-added products

Materials & Methods



Operating & Sampling Conditions

5% CO_2 - 0.556 vvm - 50 mL samples

→ 10 mL freeze dried to determine biomass concentration

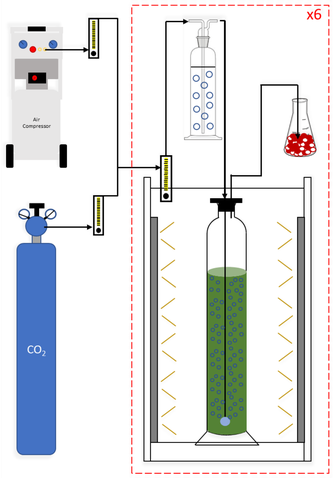
- Samples are centrifuged & the supernatant is tested for pH, nitrogen, and phosphorous concentrations

Table 1: Nutrient addition strategy to study the effect of nitrogen concentration on biomass productivity

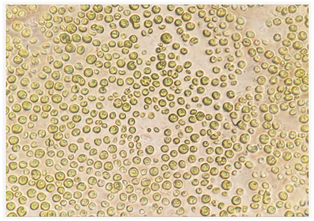
5 mL $NaNO_3$ concentrate solution = 41 mg/L N

	Days 0, 7, 14	Days 2, 4, 9, 11, 16, 18
x1 N	36 mL BBM	5 mL $NaNO_3$
x2 N	36 mL BBM + 5 mL $NaNO_3$	10 mL $NaNO_3$
x4 N	36 mL BBM + 15 mL $NaNO_3$	20 mL $NaNO_3$

Schematic of photobioreactor system



Chlorella vulgaris



- Unicellular, robust, rapid growth rate
- Applications:
  - Biofuels
  - Food source
  - Wastewater treatment

CO_2 fixation

% carbon of dried algae determined through elemental analysis

- Avg carbon % - 41.69%

$$\frac{g\ CO_2}{L * day} = \%C * \frac{g\ biomass}{L * day} * \left(\frac{MW_{CO2}}{MW_C}\right)$$

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Results

Figure 2: Biomass concentration

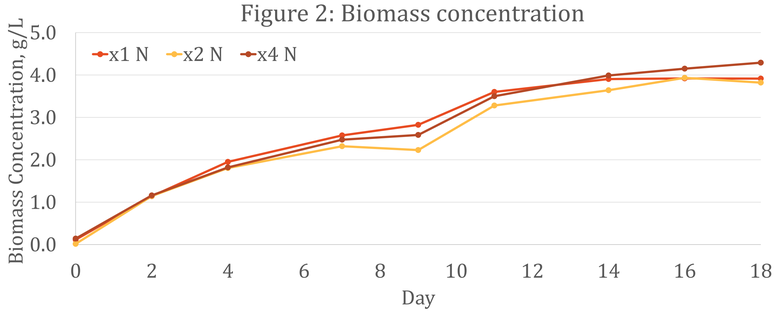

Figure 3: Percent nitrogen consumed

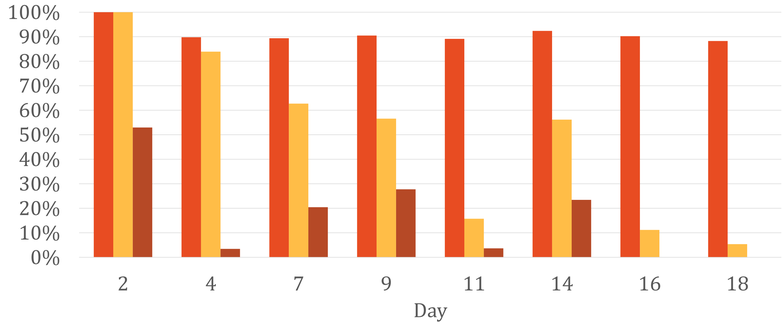

Table 2: Comparing biomass productivity & CO_2 fixation at different cultivation periods

		x1 N	x2 N	x4 N
Biomass productivity (g biomass L^{-1} day^{-1})	Day 0 - 4	0.459	0.448	0.420
	Day 0 - 11	0.317	0.297	0.305
CO_2 fixation (g CO_2 L^{-1} day^{-1})	Day 0 - 4	0.706	0.689	0.647
	Day 0 - 11	0.488	0.457	0.470

Conclusions & Future Work

Adding 41 mg/L NO_3-N every two days allows for at least 90% nitrogen consumption between nutrient additions to the system while adding double or quadruple the amount of nitrogen leads to a buildup of in the reactor. The buildup of nitrogen in the reactor does not inhibit biomass growth indicating other reasons for halted growth. Carbon dioxide fixation was higher in the early days of experimentation leading to a need to sustain the biomass productivity found in the early stages of growth in the photobioreactor system.

Future experiments include testing different harvesting rates, increasing light intensity, and lowering gas feed flow rate to achieve higher biomass productivity leading to higher carbon dioxide fixation.

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Chlorella vulgaris growth from Sodium Bicarbonate produced via Carbon Dioxide absorption using Sodium Hydroxide

AIM-REU Research Symposium – 7/30/21

Andrew Schofield[1], Alex Zappi[2], Sarah Simoneaux[3], William Holmes[3], Mark Zappi[3]

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BACKGROUND

- CO_2 Absorption using Sodium Hydroxide

$$CO_2\ (aq) + NaOH\ (aq) \rightarrow NaHCO_3\ (aq)$$

  - When in solution, the bicarbonate ion (HCO_3^-) is in equilibrium with the carbonate ion (CO_3^{2-}) and aqueous carbon dioxide/carbonic acid
  - The equilibrium concentrations of the three species is determined by [H^+], or pH

$$CO_2\ (aq) \rightleftharpoons H_2CO_3\ (l) \qquad K_1 = 1.7\ e\text{-}3$$

$$H_2CO_3\ (aq) \rightleftharpoons H^+\ (aq) + HCO_3^-\ (aq) \qquad K_2 = 2.5\ e\text{-}4$$

$$HCO_3^-\ (aq) \rightleftharpoons H^+\ (aq) + CO_3^{2-}\ (aq) \qquad K_3 = 4.69\ e\text{-}11$$

- *Chlorella vulgaris*
  - Common versatile algae strain
  - Increased research interest due to applications for flue gas cleaning, carbon-neutral biofuels, dietary supplements, CO_2 removal
  - Current research is for a Mars space camp application for NASA – CO_2 removal for cabin air and aerobic bioreactor effluent.
  - Mars atmosphere contains 95% CO_2, which is a great resource for space camp
  - Project characterized *C. vulgaris* growth from **aqueous carbon sources** (bicarbonates)

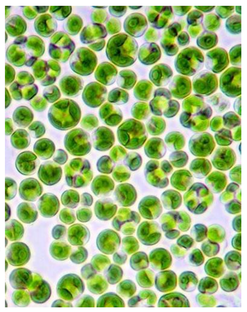


Figure 1: Microscopic image of Chlorella vulgaris

METHODS

CO_2 Absorption

- 500 mL bubble column with sparger.
- Filled with 400 mL solution for most trials
- Bubbled CO_2 rich air (5% and 25%) through solution at 1 L/min
- Tested CO_2 fraction in effluent gas continuously
- Stopped trial once effluent gas CO_2 fraction stopped increasing

Figure 2: 500 mL bubble column

Figure 3: CO_2 analyzer

- Trial Conditions:

Trial(s)	Test Conditions	Data
1	400 mL 0.1M NaOH @ 5%CO_2	Time, CO_2
2	400 mL 0.1M NaOH @ 5%CO_2	Time, CO_2, pH
3	400 mL 0.2M NaOH @ 5%CO_2	Time, CO_2, pH
4	300 mL 0.1M NaOH @ 5%CO_2	Time, CO_2
5	600 mL 0.1M NaOH @ 5%CO_2	Time, CO_2
6,7	400 mL 0.1M NaOH @ 25%CO_2	Time, CO_2
8,9	400 mL 0.2M NaOH @ 25%CO_2	Time, CO_2

Algae Growth

- Filled two bioreactors to 1500 mL with each 225 mL algae media, 30 mL Bold's Basal nutrient solution, one with 480 mL 0.1M $NaHCO_3$ (from Trials 1 & 2), and the balance deionized water.
- Ambient air was bubbled through the bicarbonate-free reactor at 1 L/min; no air was supplied to the other.
- Sampled 10 mL from each reactor; centrifuged at 1932 g for 20 min; measured the pH and inorganic carbon content of supernatant; freeze dried solids to obtain biomass concentration.

Figure 4: Photobioreactor

ABSTRACT

Chlorella vulgaris is a type of algae which can be used for many different applications, including cabin air cleaning for a NASA space camp on Mars. To determine the viability of *C. vulgaris* growth from inorganic carbon sources in solution rather than from gaseous carbon dioxide, a test was conducted comparing algae growth from 1.9 g/L bicarbonate solution and that from bubbling ambient air (0.07% CO_2) at 1 L/min. The bicarbonate solution was prepared by bubbling CO_2-rich air through a sodium hydroxide solution using a bubble column. The CO_2 effluent fraction vs. time and carbon species vs. time relationships can be seen in Figures 5-7. For each situation tested, there was a signature shape of the CO_2 fraction vs. time plot. The stabilization area in the middle is likely where the solution transitions from primarily CO_3^{2-} to primarily HCO_3^-. This can be visualized in Figure 7. The algae in the bicarbonate reactor grew more quickly during days 1-7 but slowed behind the ambient air reactor during subsequent days. The bicarbonate reactor peaked during days 5-8 containing 0.6 g/L algae, while the ambient air reactor reached 0.8 g/L algae at day 11. The inorganic aqueous carbon, pH, and biomass were tracked with time for each reactor and can be seen in Figures 9-11.

RESULTS – CO_2 ABSORPTION

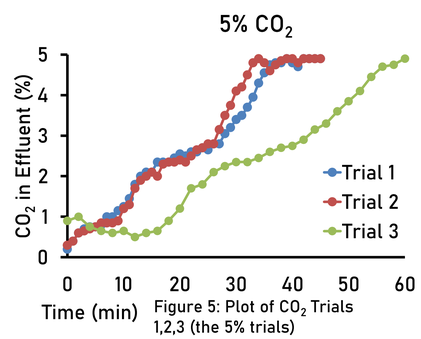


Figure 5: Plot of CO_2 Trials 1,2,3 (the 5% trials)

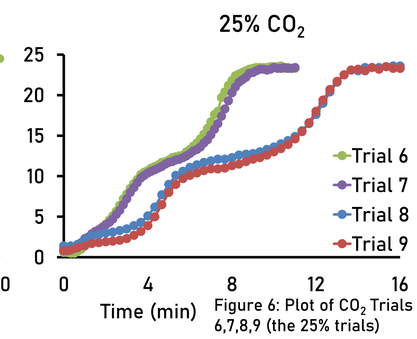


Figure 6: Plot of CO_2 Trials 6,7,8,9 (the 25% trials)

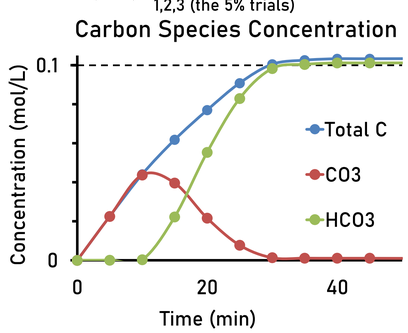


Figure 7: Theoretical carbon species concentrations from carbon dioxide absorption in a basic solution. Based on pH data from Trial 2.

- Characteristic shape of curve present in every trial thus far
- CO_2 data very consistent with expected result from a reaction conservation of mass perspective (visible in Figure 7)
- Figure 7 concentrations based on carbon mass balance and pH data – concentrations are the solution to the following equations, given [H+] and [Total Carbon Added] which can be trivially obtained using measured data:

$$1.7 * 10^{-3} = \frac{[H_2CO_3]}{[CO_2]} \qquad 2.5 * 10^{-4} = \frac{[H^+][HCO_3^-]}{[H_2CO_3]} \qquad 4.69 * 10^{-11} = \frac{[H^+][CO_2^{2-}]}{[HCO_3^-]}$$

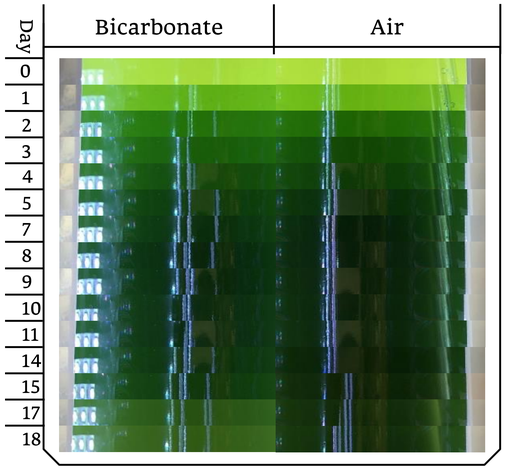
$$[Total\ Added\ Carbon] = [CO_2] + [H_2CO_3] + [HCO_3^-] + [CO_3^{2-}]$$

CONCLUSION

- NaOH is an extremely effective way to absorb gaseous carbon dioxide
- Possible to continuously absorb vast majority of CO_2 using very few stages
- Future work can include tests at varying conditions (notably other temperatures); specifying mass transfer characteristics and reaction kinetics
- Under favorable conditions, algae growth from bicarbonates alone can exceed that of traditional bubbled air
- Future work can optimize bicarbonate conditions for growth, investigate why only half of the bicarbonate was consumed (perhaps pH limited), and test continuous bicarbonate feeding

Combining these two methods has the potential to be beneficial for many applications, not just a NASA space camp. There are many advantages of this style of system in that NaOH is able to absorb the majority of a carbon stream, the algae need not be attached to the carbon stream, and that solutions are easier manipulated for growing conditions than gases. Though currently facing challenges such as high base costs and general economic conditions, once fully developed, this process could provide a viable route of carbon removal without sacrificing economic feasibility, ushering in future generations to the marvels of science and sustainability.

RESULTS – ALGAE



- Visually, growth is very similar through day 10
- In person, Bicarbonate reactor appeared slightly darker through this time.

Figure 8: Visual comparison between bicarbonate-fed photobioreactor and air-fed photobioreactor from startup through day 18.

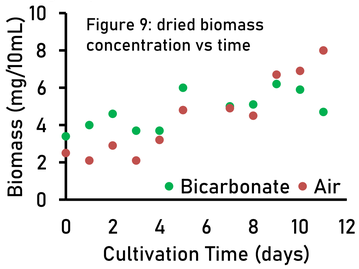


Figure 9: dried biomass concentration vs time

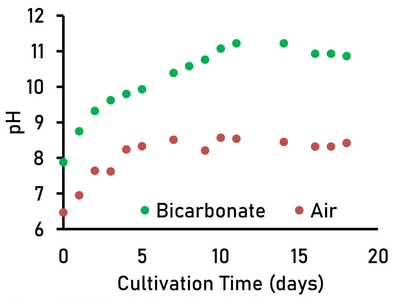


Figure 10: Plot of pH vs time for bioreactors

- pH for bicarbonate increases after growth has slowed (days 6-12)
- pH for air stabilizes during growth period (days 6-12)-counterintuitive

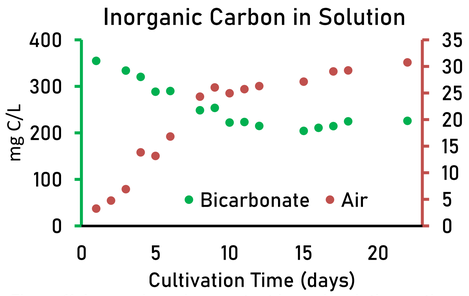


Figure 11: Inorganic carbon content in each solution vs time.

- 60% of Bicarbonate concentration remains – perhaps growth is pH limited

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2. A critical evaluation of CO_2 supplementation to algal systems by direct injection – Langley et al.
3. Carbon dioxide dissolution and ammonia losses in bubble columns for precipitated calcium carbonate (PCC) production – Zevenhoven et al.

Algae Cultivation in a Space-based Biorefinery System

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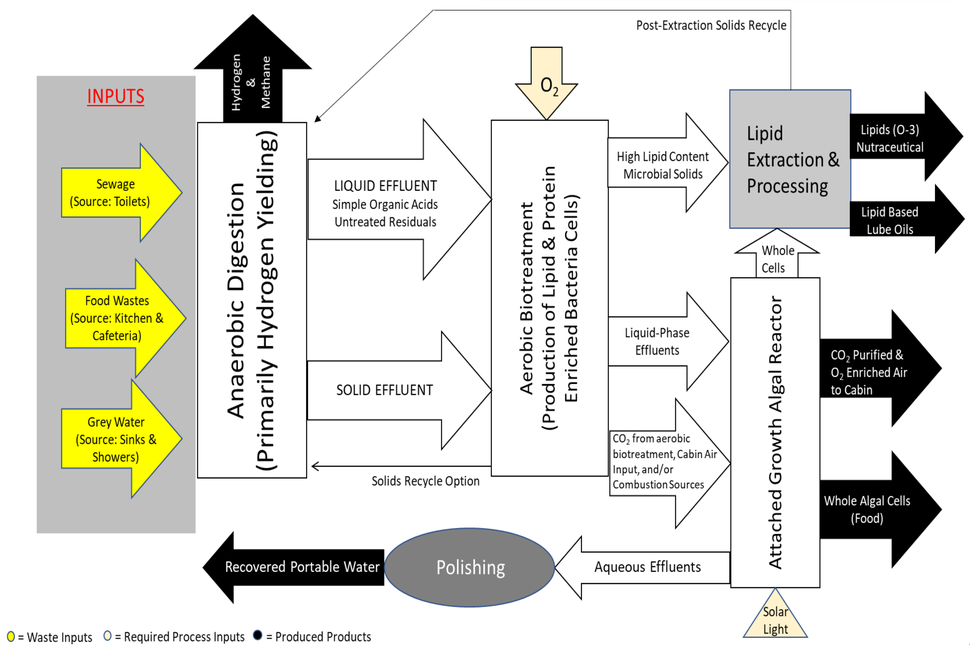
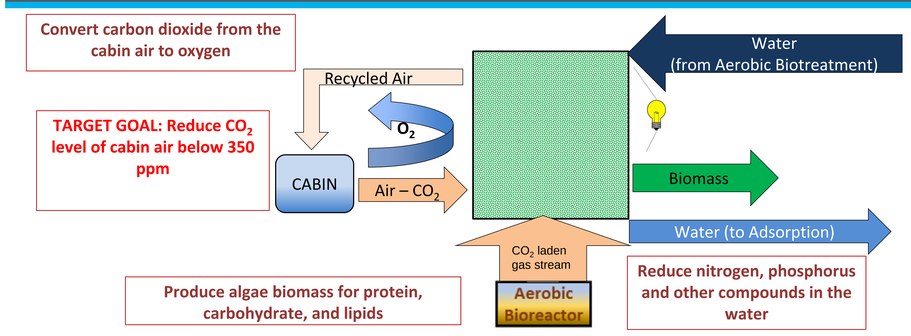
Space-Based Biochemical Conversion System (BIOSYS) Requirements

- Provide life support products from human waste streams
- Be capable of treating water and air to reusable qualities
- Simple to operate but flexible

- Be capable of producing clean water, oxygen-rich air and food for life support
- Carbon dioxide from the space station cabin is passed through algae reactor
- Oxygen-enriched air is recycled to the aerobic digester



Functions of Algae Photobioreactor



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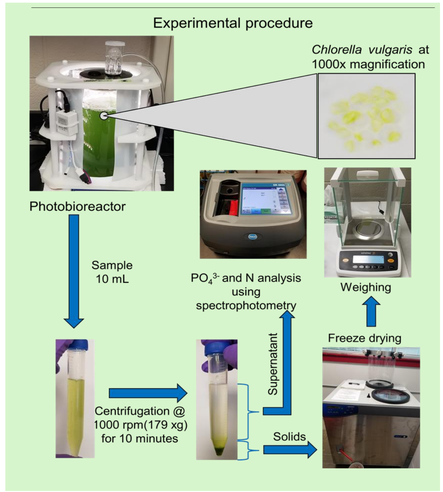
- Biorefinery systems involving microalgae have been demonstrated to simultaneously reclaim wastewater (through nutrient biofixation), provide food supplements (as biomass) and revitalize air (through photosynthetic conversion of carbon dioxide to oxygen).
- Microalgal cultivation in human-derived waste (both gas and liquid), could encounter issues if the nutrient levels in the wastewater surpass system tolerance.

Objectives

- To formulate a maintenance media for acclimation of *Chlorella vulgaris* before feeding the algae with human-derived wastewater
- To obtain the maximum biomass concentrations at high and low nutrient levels
- To produce microalgal biomass for food as a source of nutrients

Materials and Methods

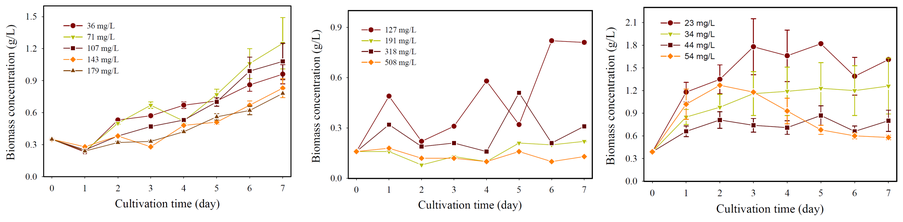


Experiments on the microalgae *Chlorella vulgaris* UTEX 2714 strain at increasing concentrations of macronutrients (C, N and P) were tested at a one-factor-at-a-time approach to determine the inhibitory levels of C, N and P on growth.

A 2^3 factorial study on high levels (from inhibitory levels) and low levels (found in the commercially available media for the seed culture) of C, N and P, was performed to determine the media formulation that obtained the highest biomass concentration.

Results

Experiments on the microalgae *Chlorella vulgaris* UTEX 2714 strain showed the levels of macronutrients (C, N and P) that inhibit growth.



Increasing C levels
Fixed N (low level)
Fixed P (low level)

Fixed C (high level)
Increasing N levels
Fixed P (low level)

Fixed C (high level)
Fixed N (high level)
Increasing P

The highest biomass concentration (1.4 g/L) was obtained at high levels of C, and low levels of N and P. An analysis of the nutritional composition of the harvested *Chlorella vulgaris* grown in synthetic media at these levels of C, N and P, showed that it can be a source of protein, carbohydrates and lipids.

Nutritional composition	% Nutrient Content Ash free dry weight basis	Method of Analysis
Protein	23.5 ± 1.1	Bradford-Lowry assay
Carbohydrates	14.9 ± 1.2	Phenol-sulfuric acid method
Lipids	13.7 ± 3.4	Bligh and Dyer method
	22.7 ± 0.7	Accelerated Solvent Extraction

Summary and Recommendations

- Dilution of the human-derived wastewater in a space station is recommended due to inhibitory levels of macronutrients (C, N and P) to *Chlorella vulgaris*.
- Photobioreactor volumes were estimated based on the inhibitory levels of C, N and P on *C. vulgaris* and baseline values of these macronutrients in urine and flush water in the International Space Station for a crew of six astronauts.

Macronutrient	C	N	P
Available (g/L)*	5.98	8.04	0.05
Low level (mg/L)	1	64	13
Required dilution	1:5,979	1:125	1:3
Reactor volume (L)	12,259	258	8
High level (mg/L)	71	127	34
Required dilution	1:83	1:62	1:0
Reactor volume (L)	173	130	3
Treatment HLL (mg/L)	71	64	13
Required dilution	1:83	1:125	1:3
Reactor volume (L)	173	258	8

*from daily generation of urine and flush water (2.05 L) within the International Space Station per crew member

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