Capture of industrial CO_2-rich off-gas through optimized cultivation of microalgae

by

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Abstract

The utilization of microalgae to treat carbon dioxide (CO_2)-rich industrial off-gas has been suggested as both beneficial for emissions reduction and economically favourable for the production of microalgal products, such as lipids to produce biodiesel. Common sources of off-gases include coal combustion (2-15% CO_2), cement production (8-15% CO_2), coke production (18-23% CO_2) and ore smelting (6-7% CO_2). However, industrial off-gas also commonly contains other acid gas components (typically nitrogen oxides (NO_X) and sulphur dioxide (SO_2)) and metals that could inhibit microalgae growth and productivity.

To utilize industrial off-gas effectively in microalgae cultivation systems, a number of solutions have been proposed to overcome potential inhibitions. These include genetic modification to improve specific cellular characteristics, chemical additions, bioreactor designs and operating procedures, and bioprospecting to identify suitable acid tolerant strains.

In all this work, one commonly overlooked consideration is the inoculation density of the microalgae culture, which can have significant influence on growth, and possibly lipid production withing the cell. This thesis examines inoculation density and the utilization of bioprospected (acidophilic or acid tolerant) strains to combat the inhibitions caused by acidic conditions, simulating those created through the addition of industrial off-gas.

Inoculation density optimization has shown to significantly affect the growth and biomass production of microalgae. It was found that, for most microalgae strains tested, an inoculation density of 100 ml L^{-1} resulted in optimal growth and biomass productivity. It was, however, determined that there was no

correlation between inoculation density and cellular lipid content. The most successful strain was a bioprospected *Coccamyxa* sp. strain. While the inoculation density affects the growth, the extent is clearly species dependent.

There is a growing need for CO_2 capture, and results of this thesis lead to several possible future directions for further development, including; development of an evaluation matrix for target compounds, repeat experiments with direct application of industrial off-gas, analysis of lipid quality, increasing volumes to test at pilot scale (and eventually, industrial scale), and direct testing of an optimized cyclical harvest approach.

Keywords

Industrial off-gas, Carbon dioxide mitigation, Bioprospected acid tolerant microalgae, Inoculation density, Lipid production

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Chapter 1

Introduction

1.1 Background

Greenhouse gas (GHG) emissions are major components of the off-gas produced from essential industries, such as energy and building materials (Yadav et al. 2015; Ritchie 2022). The industrial off-gas from various industries include carbon dioxide (CO_2), acidic components (SO_2 and NO_X), and particulate metals (Laamanen et al. 2017; Yadav et al. 2019; Cui et al. 2019). These components have many negative effects, such as contribution to climate change, respiratory disease, photochemical smog, and acid rain (Sun et al. 2015). Efforts to mitigate industrial CO_2 emissions are set to continue to grow, as countries worldwide are introducing carbon taxes to reduce the amount of CO_2 emissions. As of April 2022, the highest carbon taxes worldwide include Uruguay (137 USD per metric ton of CO_2-eq), Sweden (130), Switzerland (130), and Liechtenstein (130) (Tiseo 2023).

An economically and technically feasible solution to remediation of industrial off-gas is presented through the use of microalgae. Microalgae are microorganisms that utilize photosynthesis for energy production, and thus are able to convert CO_2 into biomass. Stoichiometrically, microalgae have the capability to convert 1.8 kg of CO_2 into 1 kg of biomass (Chisti 2007). When cultivated without the use of industrial off-gas, CO_2 supply accounts for 8-27% of operational costs (Godos et al. 2014). Through utilizing CO_2-rich industrial off-gas, these costs can be avoided and the economics of the cultivation can be improved (Laamanen and Scott 2020).

The limitation of utilizing industrial off-gas as a carbon source for microalgae cultivation is the inhibitions caused by the major components in off-gas (CO_2, acidic components (SO_2 and NO_X) and metals) (Jiang et

al. 2013; Dineshbabu et al. 2017). When in excess all of these components can have negative impact on the growth of microalgae cultures (Yadav et al. 2015). There are many methods explored to combat these inhibitions, such as strain selection, chemical additives, and process design and procedure (Yadav et al. 2021).

Strain selection is a pertinent part of the process, depending on the overall goal of the process (Desjardins et al. 2021). For example, bioprospecting a strain that has adapted to acidic ecosystems, such as those created in mine tailing ponds, are ideal candidates for applications utilizing acidic off-gases (Eibl et al. 2014; Desjardins et al. 2020). Strain selection also has a significant effect on end use as different strains will produce varying bioproducts in different quantities, such as lipids and antioxidants (Senhorinho et al. 2018).

Microalgae have many options for bioproduction of saleable products, such as general biomass for food and animal feed, antioxidants, and lipids for conversion into biodiesel (Laamanen et al. 2021). The production of a chosen target is affected by many factors, such as microalgae strain, stressors, nutrient levels, cultivation systems, and harvesting (Laamanen et al. 2014; Du et al. 2019; Desjardins et al. 2022). Lipids, a main component of interest from microalgae cultivation, can be converted into biodiesel to produce a saleable product (Hosseini et al. 2018b; Desjardins et al. 2022). In Ontario, Canada, the production of renewable fuels is required by the Cleaner Transportation Fuels regulation, which states that diesel must have a 4% blend of renewable content, while emitting 70% less greenhouse gases than fossil fuel diesel on a lifecycle basis (Government of Canada 2022a).

Utilizing biodiesel as a fuel source can be a net neutral release of CO_2 emissions, as the maximum amount of CO_2 that can be release from the combustion of biofuels is the amount of CO_2 captured to produce the biofuels. This results in no increase of the overall atmospheric CO_2 from combustion (Tamilselvan et al. 2017). Meanwhile, fossil fuel diesel combustion is a net positive release of CO_2 emissions, as it does not capture CO_2 during production. As a result fossil fuel diesel combustion adds to the overall atmospheric CO_2 (Rajendran 2021).

When investigating methods of combatting inhibitions from utilizing off-gas for microalgae cultivation, bioprospected strains coupled with another method that will offer additional benefits such as increased cellular lipid production should be explored (Desjardins et al. 2020). Chen et al. (2012) found that optimizing inoculation density improved biomass productivity. The same study also determined that an inverse relationship between inoculation density and lipid production existed. Coupling bioprospected strains with optimized inoculation density has the capability of combatting inhibitions caused by off-gas cultivation while improving lipid production (Chen et al. 2012; Ahn et al. 2022).

1.2 Thesis Objective and construction

The objective of this thesis is to explore the methods of combatting inhibitions caused by utilization of industrial off-gas as a CO_2 source for microalgal cultivation. This will lead to further understanding the effects of individual components of industrial off-gas on microalgae cultures and the options available to combat the inhibitions to these components. This will further lead to determining options of coupling multiple methods of combatting the inhibitions of industrial off-gas with a focus of smelter off-gas.

The thesis is organized as an introduction followed by Chapter Two, a submitted paper, that provides a literature review on considerations and solutions of utilizing industrial off-gas for microalgae cultivation. Chapter Two provides critical background for Chapter Three, a submitted paper, which provides experimental results for the coupling of bioprospected strains with inoculation density to optimize growth while utilizing industrial off-gas. Chapter Four concludes the results of the thesis and introduces considerations for future work.

Chapter 2

Paper #1 – Literature Review

Utilizing industrial off-gas for microalgae cultivation: considerations and solutions

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Abstract

The utilization of microalgae to treat carbon dioxide (CO_2)-rich industrial off-gas has been suggested as both beneficial for emissions reduction and economically favourable for the production of microalgal products. Common sources of off-gases include coal combustion (2-15% CO_2), cement production (8-15% CO_2), coke production (18-23% CO_2) and ore smelting (6-7% CO_2). However, industrial off-gas also commonly contains other acid gas components (typically nitrogen oxides (NO_X) and sulphur dioxide (SO_2)) and metals that could inhibit microalgae growth and productivity.

To utilize industrial off-gas effectively in microalgae cultivation systems, a number of solutions have been proposed to overcome potential inhibitions. These include bioprospecting to identify suitable strains, genetic modification to improve specific cellular characteristics, chemical additions, and bioreactor designs and operating procedures.

In this review, results from microalgae experiments related to utilizing off-gas are presented, and the outcomes of different conditions discussed along with potential solutions to resolve limitations associated with the application of off-gas.

2.1.0 Introduction

Activities such as fossil fuel combustion for energy generation and cement production are major contributors to the worlds 37 billion tonnes of CO_2, the main anthropogenic greenhouse gas (GHG) emissions (Yadav et al. 2015; Ritchie 2022). The main gaseous components of concern in most industrial off-gases are carbon dioxide (CO_2), sulphur dioxide (SO_2), and nitrogen oxides (NO_X), which, in addition to their roles in climate change, contribute to noxious effects such as respiratory disease, photochemical smog, and acid rain (Sun et al. 2015).

The stoichiometry of three common industrial reactions producing CO_2 is presented in equations 1, 2 and 3.

Coal Combustion $$C + O_2 \xrightarrow{590-710\ C} CO_2 \quad (1)$$

Natural Gas Combustion $$CH_4 + 2O_2 \xrightarrow{700-1500\ C} 2H_2O + CO_2 \quad (2)$$

Cement Production $$CaCO_3 \xrightarrow{800\ C} CaO + CO_2 \quad (3)$$

Within these systems, there can be also parallel oxidation reactions that result in lower concentrations of NO_x (6.9-550 ppm) and SO_2 (0-300 ppm) (Miller 2011).

Efforts to mitigate industrial carbon dioxide (CO_2) emissions are set to continue to increase, as countries around the world make efforts to reduce CO_2 emissions, typically by introducing carbon taxes and/or incentivizing biofuel production and use of battery electric vehicles. As of April 2022, the highest carbon taxes worldwide include Uruguay (137 USD per metric ton of CO_2-eq), Sweden (130), Switzerland (130), and Liechtenstein (130) (Tiseo 2023). Canada currently has a carbon tax of 50 CAD per metric ton of CO_2-

eq, there is clear intent to steadily increase these rates. Between 2023 and 2030 the carbon tax will increase 15 CAD per year, reaching 165 CAD per metric ton of CO_2-eq in 2030 (Government of Canada 2022b).

The abatement of CO_2 emissions require economic and technically feasible treatment options (Wang et al. 2022). The high rate of CO_2 consumption during photosynthetic microalgal growth could provide one solution by feeding CO_2-rich industrial off-gas into dedicated cultivation systems (Kapoore et al. 2022). Stoichiometrically microalgae will convert 1.8 kg of CO_2 into 1 kg (dry weight) of biomass (Chisti 2007) and are 10-50 times more efficient at taking up CO_2 by volume than terrestrial plants (Yen et al. 2015; Toledo-Cervantes et al. 2013). It has been estimated that utilizing CO_2-rich off-gas can cut the cost of microalgae cultivation by 8-27% compared to purchasing the necessary CO_2 (Godos et al. 2014). Whilst experimental results are divided on whether the presence of NO_X and SO_2 act as nutrients or inhibit the growth as toxic components at varying concentrations (Dineshbabu et al. 2017), these components do contribute to the acidification of the medium (Wang et al. 2016). This, in turn, affects microalgae growth rates and related CO_2 uptake (Palanisami et al. 2015).

Lipids, a main component obtained from microalgae cultivation, can be converted into biodiesel to produce a saleable product (Hosseini et al. 2018a; Desjardins et al. 2022). In Ontario, Canada, the production of renewable fuels is required by the Cleaner Transportation Fuels regulation (Government of Canada 2022a), which states that diesel must have a 4% blend of renewable content that emits 70% less greenhouse gases than fossil fuel diesel on a lifecycle basis. While gasoline must be blended with at least 10% renewable content from 2020-2024, which will further increase to 15% by 2030. This

renewable content must emit 45% (before 2030) and 50% (after 2030) less greenhouse gases than fossil fuel gasoline on a lifecycle basis (Government of Canada 2022a).

The most common alternative industrial CO_2 emission reduction system is liquid absorption, using amine-based chemicals or ionic liquids (Hasib-ur-Rahman et al. 2010). In these systems, the absorbent solution is applied countercurrent to the CO_2 rich gas in a system that can be modified through addition of solid materials or membranes. Unfortunately, two common problems remain for these systems, namely high energy demand for regeneration and waste management of degraded solvent (Hack et al. 2022). In general, factors for CO_2 reduction strategies to consider are the environmental impacts, capital cost, variable costs, and operational downtime (Leimbrink et al. 2017). Compared to other currently available methods, microalgae cultivation is an environmentally friendly strategy, that can operate with minimal downtime, especially when utilizing cyclic harvesting procedures.

This review examines different sources and compositions of industrial off-gas that could be utilized in microalgae cultivation, along with potential impacts and potential solutions to overcome the limitations of off-gas application.

2.2.0 Microalgae cultivation – key considerations

Photosynthetic microalgae are single-cell aquatic plants that utilize CO_2 as a carbon source and sunlight to produce energy. Microalgae have a faster growth rate than terrestrial plants, meaning for the same productivity they require a substantially smaller footprint compared to terrestrial plant cultivation (Bolaños-Martínez et al. 2022). Microalgae also do not require arable land for cultivation and can utilize

brackish or wastewater, thereby reducing clean water consumption (Tredici 2010; Tsukahara and Sawayama 2005; Lam and Lee 2012; Rastogi et al. 2018; Laamanen et al. 2017).

An important economic consideration is the microalgae's ability to produce high levels of secondary metabolites, which can be profitable or at least serve to off-set the cost of treatment. These include lipids for biofuel (biodiesel) (Elisabeth et al. 2021) and antioxidants (Senhorinho et al. 2018; Eibl et al. 2014; Laamanen and Scott 2020). By exposing the microalgae culture to different environmental conditions, secondary metabolite accumulation can increase as a stress response (Skjånes et al. 2013; Borowitzka 2018; Hossain and Mahlia 2019). To best utilize the metabolic pathways for end goal production, a two-stage system is typically chosen (Münkel et al. 2013; Chen et al. 2018a). The first stage is used to maximize biomass production by creating optimal growth conditions and the second stage to increase production of one or more target secondary metabolites by introducing a stressor (Odjadjare et al. 2017; Desjardins et al. 2020; Saravana et al. 2022).

Major parameters that are monitored and controlled to obtain optimal growth conditions include CO_2 (Chisti 2007), light (V. Singh and Mishra 2022), nutrients (Abinandan et al. 2018), pH (Aguirre et al. 2013), temperature (Pongsurapipat et al. 2017) and dissolved oxygen (Kazbar et al. 2019). The interconnectivity of off-gas components, culture conditions, products and common solutions is shown in Figure 1.

CO_2 availability is the most important variable in biomass production and stoichiometrically for every kilogram of biomass produced 1.8 kilograms of CO_2 is captured, which commonly makes CO_2 the limiting

factor in growth (Chisti 2007; Grobbelaar 2003). Atmospheric CO_2 concentration is approximately 0.0415%, and this relatively low concentration combined with high surface tensions, means that dissolution of atmospheric CO_2 into water is at a low of 0.1 g $(L\ h)^{-1}$ (Zimmerman et al. 2011; Laamanen and Scott 2020; Lindsey 2022). Whereas application of industrial off-gas through a sparger produces fine bubbles and a large surface area resulting in increased mass transfer of the CO_2 into water, commonly to the point where it is no longer a limiting factor for growth (Zimmerman et al. 2011).

Another potential limiting factor is light. Photosynthetic microalgae utilize wavelengths between 400-700 nm (approximately 50% of sunlight's spectrum) as their energy source (Wang et al. 2012). A major consideration in providing adequate light for large-scale microalgae cultivations is self-shading of cells as culture densities increase. If sufficient mixing or appropriate reactor design is not utilized, microalgae can form a dense 15-30 cm layer below the medium surface (Hosseini et al. 2018a) that inhibits deeper light penetration and reduce the culture's productivity (Nguyen et al. 2022).

While nutrients are commonly provided in excess for laboratory experiments, they commonly present a significant economic impact on cultivation systems due to the of lack of low-cost options (Laamanen and Scott 2020). The combination of nutrients, water and CO_2 has been modelled to account for 10-30% of the overall production costs (Rastogi et al. 2018), but the nutrient associated costs can be reduced by utilizing nutrient-rich wastewaters (Abinandan et al. 2018).

Additionally, temperature can become an expensive parameter to control if atmospheric temperature is outside of the optimal cultivation range, which is typically 15-30 °C (Demirbas 2017). This is noteworthy

as a majority of the world, except tropical climates, are outside of the optimal temperature range for most of the year (Chisti 2007; Elisabeth et al. 2021). To overcome this, it has been proposed that in parallel to CO_2 capture, waste industrial heat can be utilized to maintain the optimal temperature (Laamanen et al. 2014).

Understanding dissolved oxygen concentration in microalgal culture is necessary to optimize productivity, as it can negatively affect growth as concentrations increase. There is a limit of approximately 20 mg L^{-1} of dissolved oxygen, before culture productivity starts to be negatively affected (Costache et al. 2013). Notably, the levels for inhibition involve supersaturation of dissolved oxygen, as saturation is at 9.0 mg L^{-1} at 20 °C. Significant accumulation of dissolved oxygen is a common problem in cultivation systems that are not open to the atmosphere, such as closed tubular reactors. In tubular reactors, degassers should, therefore, be utilized to reduce the concentration of dissolved oxygen (Kazbar et al. 2019). Open cultivation systems, such as raceway ponds and open airlift reactors, do not require degassers as long as enough agitation is provided to encourage mass transfer, as the open nature provides the capability for releasing dissolved oxygen (Kazbar et al. 2019).

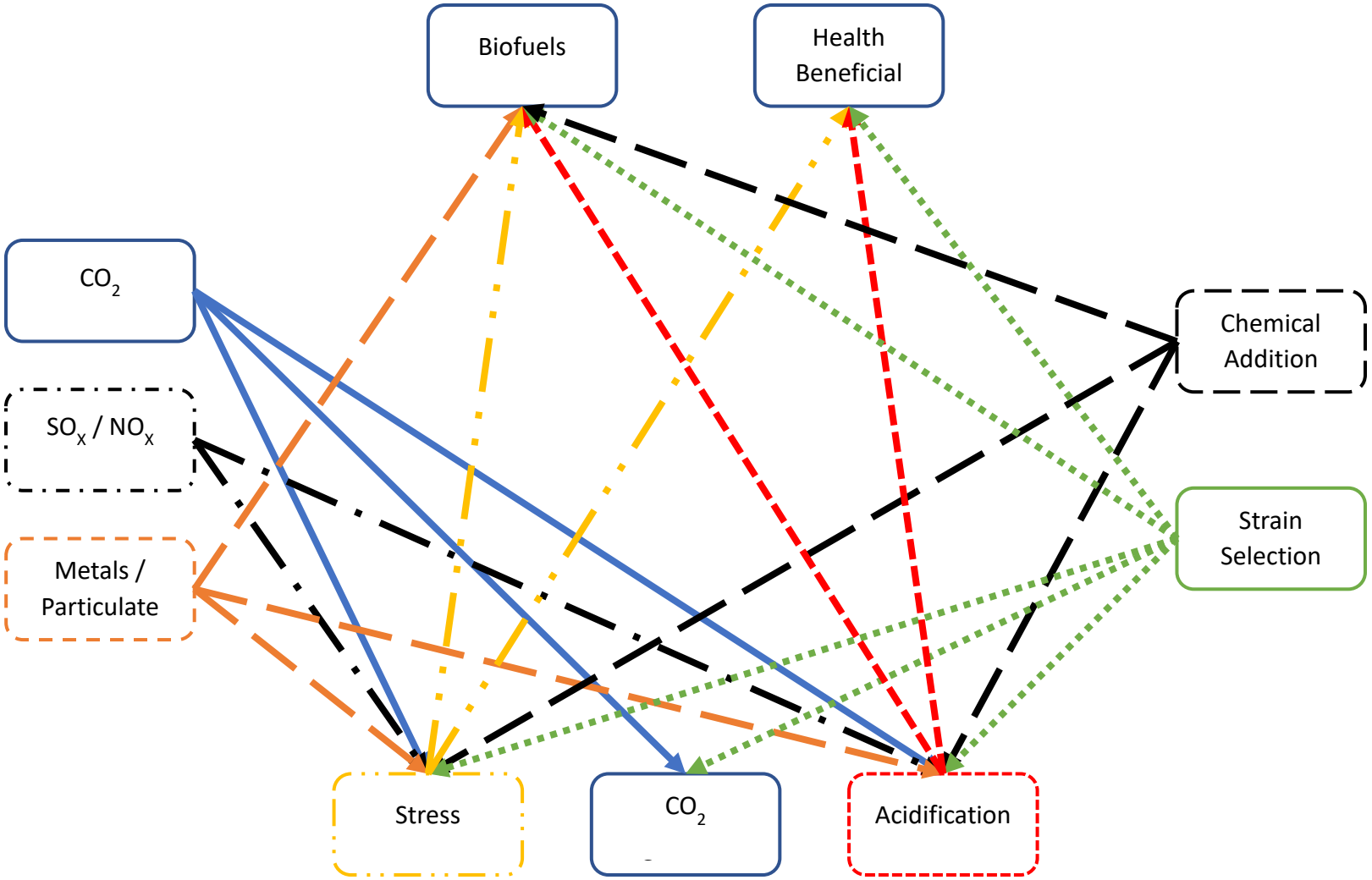

Figure 2.1: interconnectivity of off-gas components (left), culture conditions (bottom), common final products (top), and common solutions (right)

2.3.0 Industrial off-gas for microalgae cultivation

Industrial off-gas as a source of CO_2 for microalgae cultivation is a promising economically and technically feasible option to reducing cultivation costs and increasing bioproductivity (Srimongkol et al. 2022). Industrial off-gas components are mainly CO_2 and acidic components (SO_2 and NO_X) (Yadav et al. 2015). Different sources of off-gas will contain different ratios of the main components mentioned which will have different effects on microalgae cultivation (Yadav et al. 2021). As the acidic components increase in concentration, an increase in negative effects on cultivation occurs (Borowitzka 2018). While increasing the CO_2 to certain point (approximately 15% CO_2) will have positive effects on cultivation (Yen et al. 2015). Increasing the amount of CO_2 past the optimal range will result in a negative effect on cultivation (Wijayasekera et al. 2020). The effect of these components greatly varies depending on the strain of microalgae being used for cultivation. Table 1 is a breakdown of the common components found in industrial off-gas being used for microalgae cultivation.

There are design factors that arise when examining the viability of scaling up to commercial application of microalgal cultivation including; automation and control of critical variables (including gas flow, light, nutrients, pH, and temperature), even gas distribution of CO_2 and O_2, consistent and reliable measurement, conversion from batch to continuous operations, sufficient mixing, and productivity optimization (Grobbelaar 2003; Benner et al. 2022; McGinn et al. 2011; Penloglou et al. 2018). It is pertinent that these factors are considered when projecting the scale up of laboratory microalgal cultivation. A strategy to, at least partially, mitigate concern over scale up is to replicate the proposed commercial bioreactor at laboratory scale to determine specific limitations to the proposed system (Benner et al. 2022). Keeping consistent methods for monitoring critical variables through each stage of

scale up will allow for recognizing when critical variables stray from the laboratory experiment (Penloglou et al. 2018).

Table 2.1: Compositions of industrial off-gas utilized for microalgae cultivation

Off-gas Source	Location (On site or Laboratory)	Reported CO_2 Range (%)	Reported SO_2 Range (ppmv)	Reported NO_X Range (ppmv)	Citation
Coal combustion	On site	2	---	---	(Palanisami et al. 2015)
		11	220	150	(Moheimani 2016)
		15	---	---	(Dineshbabu et al. 2017)
		11.24	300	260	(Aslam et al. 2018)
		12	50	260	(Cheng et al. 2018)
	Laboratory	10	.3%	61	(Yadav et al. 2015)
		13.3	---	6.9	(Praveenkumar et al. 2014)
		5.25	---	55	(Taştan et al. 2016)
		7	120	210	(Kandimalla et al. 2016)
		10	30	61	(Yadav et al. 2019)
Cement production	On site	15	---	---	(Borkenstein et al. 2011)
		8.86	---	235.6	(Gunasena et al. 2019)
	Laboratory	8.38	193	260	(Rossi et al. 2018)
		13.5	20	515	(Olofsson et al. 2015)
Coke combustion	On site	23	87	78	(Chiu et al. 2011)
		18	200	150	(Li et al. 2011)
Agricultural	On site	9	4	38	(Kaštánek et al. 2010)
Biomass Combustion	On site	3.55	1	107	(Cui et al. 2019)
Oil Production	Laboratory	15.65	---	---	(Kumar et al. 2014)
Paper and Pulp	On site	11.5	3	65	(Ekendahl et al. 2018)
Heat and power plant	On site	3.2	---	22	(Y. Y. Choi et al. 2018)
Silicomanganese Smelter	On site	17.4	1.1%	102	(Mortensen and Gislerød 2015)
Nickel Smelter	On Site	6-7	0.4%	n/d	(Laamanen et al. 2014)

2.3.1 Impacts of carbon dioxide (CO_2), nitrogen oxides (NO_X) and sulphur dioxide (SO_2)

Introducing excess CO_2 into a microalgae cultivation system excess may inhibit growth as a result of pH reduction of the growth medium (Yen et al. 2015). Acidification occurs as CO_2 in the medium forms carbonic acid (H_2CO_3) and bicarbonate (HCO_3^-) (Chen et al. 2018a). In addition to acidification, high CO_2 content (>15%) can inhibit the microalgal CO_2 uptake system and the enzymes involved (Yen et al. 2015). When evaluating a system's carbon uptake capability, factors such as microalgae strain and gas flow rate need to be taken into consideration. For example, Cheng et al. (2018) showed that when the off-gas flow rate was increased the rate of CO_2 capture increased. Increasing the flow rates from 20 m^3h^{-1} to 150 m^3h^{-1} resulted in increased carbon capture (19.2 $g(m^2d)^{-1}$ to 31.9 $g(m^2d)^{-1}$) and biomass productivity (10.3 $g(m^2d)^{-1}$ to 17.1 $g(m^2d)^{-1}$). Strain selection can also have significant influence on carbon capture rates (Ramanan et al. 2010) and the ability to tolerate acidic conditions (Cheng et al. 2018).

Nitrogen oxides (NO_X) and sulphur dioxide (SO_2) are common components within CO_2-rich industrial off-gas. Both components are acidic which can significantly increase the extent of medium acidification (Mondo et al. 2021). For example, the presence of 50 ppm SO_2 resulted in a pH decrease to 6 that did not inhibit microalgal growth, but increasing the concentration to 400 ppm resulted in a pH decrease to <4.0 pH, which resulted in microalgal growth inhibition (Matsumoto et al. 1997). Fu et al. (2022) found that up to 300 ppm SO_2 resulted in normal growth of culture but, when concentrations reached 400 ppm cell destruction occurred (Fu et al. 2022).

This acidification is caused by a multiple step reaction producing sulphate ions (SO_4^{-2}) (Eqs. 4-6).

$$SO_2(g) \overset{O_2}{\rightarrow} SO_3^-(g) \overset{H_2O}{\longrightarrow} H_2SO_4(l) \quad (4)$$

$$H_2SO_4 \overset{H_2O}{\longrightarrow} HSO_4^- + H^+ \quad (5)$$

$$HSO_4^- \overset{H_2O}{\longrightarrow} SO_4^{2-} + H^+ \quad (6)$$

2.3.2 Metals

When utilizing some industrial off-gas as microalgal carbon sources, such as those from coal combustions or ore smelters, metals may also be introduced into the medium (Mehta and Gaur 2005). Common metals contained in industrial off-gases of interest include lead, cadmium, arsenic, nickel and zinc (Hess et al. 2017; Palanisami et al. 2015). The affinity for uptake of different metals is dependent on the concentration of CO_2 in the off-gas (Aslam et al. 2018), along with many other factors, including pH, temperature, competing ions and the metabolic stage (Mehta and Gaur 2005). While metal uptake can decrease microalgal growth, further concerns with respect to metal concentrations in the resulting biomass may eliminate usage as certain end products, such as nutraceuticals or animal feeds (Mohler et al. 2019).

In the medium, metals commonly exist in aqueous form and can be taken up by microalgae by either extracellular and intracellular mechanisms (García-García et al. 2016). An example of an extracellular mechanism is phycoaccumulation, where metals are bound at the cell wall by lipids, proteins and carbohydrates (Danouche et al. 2021). Phytochelatins produced by the microalgae bond with metals to

aid uptake, typically by being compartmentalized in vacuoles, chloroplasts and mitochondria (Danouche et al. 2021).

Metal stress results in increased sulphur uptake due to an increased demand for sulphur-based metabolites and increased nitrogen uptake to produce antioxidants, as a response to combat stress through secondary metabolism (Rijstenbil et al. 1998; Saito 2004). The increase in sulphur uptake due to metal stress is largely to facilitate the production of phytochelatin (Saito 2004), which aids the uptake of metals by creating available ligand bonds (Simmons and Emery 2011). The main precursor for phytochelatin production is cysteine, an amino acid produced by the assimilation of sulphate ions. It has been found that high metal concentration can cause damage to the chloroplast and disturb the endoplasmic reticulum, which are both utilized in CO_2 fixation and lipid biosynthesis (Einicker-Lamas et al. 2002).

Through the production of phytochelatin and subsequent metal ion bonding, the bioavailability of metals is increased, which in turn decreases the negative impact on cultivation by decreasing both the metal and sulfate ion concentrations in the medium (Mehta and Gaur 2005; Aslam et al. 2019). This is also a possible explanation as to why acidophilic microalgal strains, such as *Coccomyxa* sp. (Desjardins et al. 2021), *Chlorella* sp. and *Euglena* sp. (Johnson 2012), were capable of growing naturally in low pH (2.5-3 pH) mine waste environments.

Simulating microalgae growth utilizing coal combustion off-gas containing metals was found to decrease biomass productivity (Hess et al. 2017). Metal salts containing; arsenic (As 77.4 mg mL^{-1}), cadmium (Cd 15.0 mg mL^{-1}), cobalt (Co 15.6 mg mL^{-1}), chromium (Cr 1.29 mg mL^{-1}), copper (Cu 130.0 mg mL^{-1}), manganese (Mn 149.0 mg mL^{-1}), nickel (Ni 251.0 mg mL^{-1}), mercury (Hg 9.8 mg mL^{-1}), antimony (Sb 40.6

mg mL^{-1}), vanadium (V 113.0 mg mL^{-1}), and lead (Pb 54.1 mg mL^{-1}), were added to a medium used for cultivation of *Nannochloropsis salina* UTEX 1776. Exposure to these metals resulted in a 67.5% decrease in biomass productivity and a 77.9% decrease in lipid productivity across a 7 day growth experiment (Hess et al. 2017). This was likely due to the increased uptake of metals, as As, Cd, Co, Cu, Mn, Ni, Pb, and Sb were found in the biomass, which also resulted in a decreased levels of these metals in the medium.

2.4.0 Potential solutions for off-gas limitations

Several solutions have been investigated to combat the inhibitions caused by the application of industrial off-gas, these solutions are summarized in Table 2 and discussed in the following sections.

Table 2.2: Proposed solutions to common limitations associated with industrial off-gas utilization

Considered	**Solution Proposed**	**Limitations**			**Sources**
		CO_2 Uptake	**Acidification**	**Metals**	
Strain Selection	Bioprospecting		X		(Sabrina Marie Desjardins et al. 2021)
		X			(Wijayasekera et al. 2020)
			X	X	(Abinandan et al. 2019)
			X		(Kandimalla et al. 2016)
			X		(Lara-Gil et al. 2016)
			X		(Eibl et al. 2014)
			X		(Praveenkumar et al. 2014)
Genetic Modification	UV Mutagenesis	X			(Li et al. 2011)
	Nuclear Irradiation	X	X		(Cheng et al. 2017)
	Random Mutagenesis	X	X		(Kao et al. 2014)
	Hormesis			X	(Palanisami et al. 2015)
Chemical Addition	Cement Kiln Dust (CKD)		X		(Lara-Gil et al. 2016)
			X		(Talec et al. 2013)
	Phosphate Buffer		X	X	(Aslam et al. 2018)
	Trona Buffer		X		(J. Kim and Lee 2017)
Design and Procedure	Inoculation Density	X	X		(Yadav et al. 2015)
			X		(Yu Chen et al. 2012)
	Photobioreactor	X			(Hosseini et al. 2018b)
	Flow Rate	X	X		(Cheng et al. 2018)
	Off-gas Cycling		X	X	(Lara-Gil et al. 2016)
		X	X		(Kao et al. 2014)
	Off-gas Dilution	X			(Yadav et al. 2019)
		X			(Wijayasekera et al. 2020)

2.4.1 Strain Selection

2.4.1.1 Bioprospecting

Many microalgae strains have been shown to be inhibited at lower pH, but several acidophilic or acid tolerant strains have been identified through bioprospecting. Bioprospecting is the act of locating and isolating microalgae strains with beneficial characteristics, such as the ability to combat low pH and have the potential to produce valuable end products (Senhorinho et al. 2018). Isolating such microalgae can provide strains that can combat the risk of inhibition from exposure to industrial off-gas (Desjardins et al. 2020). Utilizing bioprospected strains for cultivation with industrial off-gas has most commonly been researched as a method to combat acidification. However, one study examined bioprospecting as a means to deal with metal inhibition (Abinandan et al. 2019) and another to deal with CO_2-based inhibition (Wijayasekera et al. 2020).

For example, *Nannochloris* sp. has been shown to grow in acidic conditions produced by off-gas (15% CO_2, 50 ppm SO_2, 300 ppm NO) that produced a medium pH of approximately 5 (Negoro et al. 1991). Other acid tolerant strains, such as *Chlorella caldarium*, have been proposed as ideal for acidic conditions that are produced from the addition of CO_2 and SO_X to microalgal cultures (Pires et al. 2012). Not all cases of bioprospecting strains have positive results for cultivation with industrial off-gas. Table 3 illustrates the use of bioprospected strains for off-gas remediation.

Table 2.3: Bioprospected strains used for off-gas remediation

Identification	**Location**	**pH**	**Results**	**Source**
Scendesmus sp.	Northern Ontario, Canada	4.5	Lig 290 can grow in low pH settings and has increase lipid production.	(Eibl et al. 2014)
Coccomyxa sp.	Northern Ontario, Canada	3	Successfully reacclimated to low pH from neutral pH.	(Sabrina Marie Desjardins et al. 2021)
Chlamydomonas sp.	Northern Ontario, Canada	3	Successfully reacclimated to low pH from neutral pH.	(Sabrina Marie Desjardins et al. 2021)
Scenedesmus sp.	Northern Ontario, Canada	4.5	Successfully reacclimated to low pH from neutral pH.	(Sabrina Marie Desjardins et al. 2021)
Chlorella sp.	Colombo, Sri Lanka	6.25	Was not as successful as *Desmosdesmus* sp. in growth with high CO_2.	(Wijayasekera et al. 2020)
Desmodesmus sp.	Colombo, Sri Lanka	6.25	Had success in growth with high CO_2 compared to *Chlorella* sp.	(Wijayasekera et al. 2020)
Desmodesmus sp.	Callaghan, Australia	3.5	Successful at cultivation with heavy metal presence and pH 3.5.	(Abinandan et al. 2019)
Heterochlorella sp.	Callaghan, Australia	3.5	Successful at cultivation with heavy metal presence and pH 3.5.	(Abinandan et al. 2019)
Chlorella sp.	Daejon, Republic of Korea	6.5	Significant increase in lipid production when cultivated with coal fired off-gas.	(Praveenkumar et al. 2014)
Desmodesmus abundans	Monterrey, Mexico	7.9	Successfully grew with Lead and Arsenic.	(Lara-Gil et al. 2016)
Nephroselmis sp. KGE2	Seoul, Republic of Korea	4.2	Successfully grew in AMD conditions up to 10 mg L^{-1} of Iron.	(Ahn et al. 2022)
*Autodesmus obliquus K*GE17	Seoul, Republic of Korea	4.2	Successfully grew in AMD conditions up to 10 mg L^{-1} of Iron.	(Ahn et al. 2022)

One area of interest for bioprospecting is acid mine drainage (AMD), which is acidic effluent produced from sulfate oxidation during mining operations and usually found in tailing ponds (Abinandan et al. 2019). Due to acidic conditions (e.g., typically at or below pH 4.5), these ponds commonly have high contents of aqueous metals and pose a major problem to surrounding ecosystems (Choi and Lee 2015). Investigating abandoned mine sites is a promising start to finding ecosystems that have adapted long-term to AMD (Ahn et al. 2022). Strains, such as *Nephroselmis* sp. KGE2 and *Autodesmus obliquus* KGE17, were isolated from an AMD pond at the Yong-Dong coal mine in South Korea (Ahn et al. 2022). Similarly, successful bioprospecting of microalgae cultures found around an abandoned lignite mine resulted in the isolation of a *Scenedesmus* spp. as a promising solution for low pH cultivation and high lipid production (Eibl et al. 2014)

Wijayasekera et al. (2020) compared two bioprospected strains, *Chlorella* sp. and *Desmodesmus* sp., for their potential to be cultivated with simulated cement kiln off-gas (15.5% CO_2). *Desmodesmus* sp. was identified as having the most promise and had an average biomass production of 140 mg L^{-1} day^{-1} and CO_2 uptake of 260 mg L^{-1} day^{-1}. Comparatively, *Chlorella* sp. grew best at with diluted off-gas (3.9% CO_2), which resulted in an average biomass production of 70 mg L^{-1} day^{-1} and CO_2 uptake of 140 mg L^{-1} day^{-1}. It is noteworthy that in this experiment, utilizing simulated off-gas, the CO_2 content was the only simulated parameter, which can result in errors compared to a system that directly applies industrial off-gas containing other components (SO_2, NO_X, metals, etc.).

Bioprospected strains *Desmodesmus* sp. and *Heterochlorella* sp. were isolated with the goal of looking for increased tolerance for metals (Abinandan et al. 2019). With cultivation media at pH 3.5, the two isolated strains were tested with variable concentrations of copper (Cu, 0.5-20 mg L^{-1}), iron (Fe, 5-50 mg

L^{-1}), manganese (Mn, 0.5-20 mg L^{-1}), and zinc (Zn, 0.5-20 mg L^{-1}). The tested concentrations of Fe, Mn, and Zn showed negligible influence on the growth of either strain (Abinandan et al. 2019). The lowest Cu concentration (0.5 mg L^{-1}) also showed no inhibition compared to the control, but higher concentrations (20 mg L^{-1}) resulted in inhibited growth for both strains. Comparatively, *Chlamydamonas reinhardtii* cultivation at neutral pH (7) showed negligible changes as a result of copper concentration of 250 µM (1.27 mg L^{-1}) (Jiang et al. 2016). The lower pH studied by Abinandan et al. (2019) is a potential contributing factor to why the cultures were inhibited at higher copper concentrations. Another factor may be the phosphate present in the medium, as increased concentrations of phosphate have been shown to reduce the effects of mixed particulate metals (0.4 mg $(Nm^3)^{-1}$) on microalgal cultivation (Aslam et al. 2018).

2.4.1.2 Genetic modification

For genetically modifying microalgae to be more acid tolerant and/or take up more CO_2, methods that could potentially be considered include chemical mutagenesis, nuclear radiation mutagenesis, and ultraviolet (UV) mutagenesis (Cheng et al. 2019). Table 4 shows examples of successful attempts at genetic modification of microalgae cultures. UV mutagenesis, through exposure to ultraviolet radiation, has been used to improve a microalgae strain's capability to uptake 18% CO_2 (Li et al. 2011). The main components of off-gas discussed (CO_2, acidic gases (SO_X and NO_X), and metals) and the resulting stressors should be examined to determine the best method of genetic modification to utilize as a solution to the stressors at hand (Lu et al. 2021).

Li et al. (2011) found that UV mutagenesis to produce the strain WUST4 *Scenedesmus obliquus* was successful at increasing CO_2 capture from the original strain by 53.5% (40.2 to 62.7% CO_2 capture). CO_2 capture was determined by measuring the inlet and outlet CO_2 concentrations in the gas. The found

increase was determined using actual off-gas with high CO_2. Comparatively, Kao et al. (2014) produced the strain MTF-15 *Chlorella* sp. by the technique of random mutagenesis. It resulted in producing two times the biomass produced with the non-mutated strain.

The genetic engineering of microalgae strains has resulted in many different cellular improvements, including photosynthetic efficiency, carbohydrate and protein storage, and lipid production (Wang et al. 2018). For example, an enzyme that is a common target for genetic engineering is Carbonic Anhydrase (CA), due to its role in converting CO_2 to HCO_3^{-1} and its subsequent consumption. To increase CO_2 capture, overexpression of CA has shown to be a promising strategy (Cheng et al. 2019). To this end, the gene MICA of bacterium *Mesorhizobium loti* was identified as having high CA activity, and Lin et al. (2018) cloned MICA into an expression vector to transform into two microalgal strains, *C. vulgaris* and *C. sorokiniana*. The genetic engineering of the two transgenic algae strains with MICA resulted in improved biomass production, protein content, and lipid accumulation (Lin et al. 2018).

However, public perception of biotechnology, especially when utilizing genetically modified organisms (GMOs), remains an issue. Distribution of information regarding consumer safety has not been historically sufficient to positively impact the public perception of these technologies (Kim et al. 2018). Commonly, the public is susceptible to misinformation regarding the safety of GMOs. Misinformation is spread worldwide, sometimes through social media, or for political or economic gains (Daev et al. 2016).

Table 2.4: Genetically modified strains used for off-gas remediation

Sample	Taxonomic Name	Method	CO_2 Concentration (v/v)	Results	Source
WUST4	*Scenedesmus obliquus*	UV Mutagenesis	20%	Increased CO_2 removal (%) increase from 40.2% to 62.7% (53.5% increase).	(Li et al. 2011)
N/A	*Spirulina* sp.	Nuclear Irradiation	15%	Resulted in no inhibition caused from continuous cultivation with off-gas containing 15% CO_2.	(Cheng et al. 2017)
MTF-15	*Chlorella* sp.	Random	25%	Doubled the biomass concentration but, lipid content stayed constant.	(Kao et al. 2014)
LUCC 002	*Desmodesmus communis*	Hormesis	2%	Showed enhanced growth but, did not combat inhibitions caused by metals.	(Palanisami et al. 2015)

2.4.2 Chemical addition

2.4.2.1 Buffers

Through the addition of buffer solutions to microalgae cultivation systems, control of culture pH can be enhanced to help combat acidic off-gas components (Kim and Lee 2017). Examples of buffers include trona (Kim and Lee 2017), tris (Nguyen et al. 2016), lysis buffer (Chen et al. 2019), and phosphate (Aslam et al. 2019), all of which were shown to stabilize pH and allow for successful microalgae growth. However, while utilizing buffers can be beneficial, the cost associated with their addition for large scale cultivation typically makes them a non-ideal solution (Bartley et al. 2014).

To reduce costs, cement kiln dust (CKD), a basic waste stream from the cement industry, which mainly consists of lime, has been studied as a possible culture medium neutralizing agent (Lara-Gil et al. 2016). Lara Gil et al. (2014) found that adding CKD was effective at combatting the pH decrease caused by off-gas application. The addition of off-gas containing 25% CO_2, decreased the pH from 7.5 to 5 within 11 minutes. At which point, the simulated off-gas was altered to include 800 ppm of NO and 200 ppm of SO_2, which resulted in a further pH decrease from 5 to 3 over 13 hours. In the following hour, 10 doses of 50 ppm of CKD was added in intervals, which increased the pH from 3 to 6 (Lara-Gil et al. 2016).

Talec et al. (2013) examined the effect of metals contained in CKD on microalgae cultures, to see if application would have a negative impact. Four different strains of microalgae were tested (*Chlorella vulgaris*, *Dunaliella tertiolecta*, *Isochrysis galbana*, and *Thalassiosira weissflogii*) utilizing simulated cement off-gas containing CKD. The results showed that normal CKD concentrations had no inhibitory effects on microalgal growth. No inhibitions were observed until concentrations were raised to 680 mg L^{-1}, 2000 times greater than typical industrial levels (Talec et al. 2013; Lara-Gil et al. 2014).

Aslam et al. (2018) showed that adsorption of metals into microalgal biomass resulted in a decrease of the culture pH. To combat the inhibition caused by the pH decrease, 50 mM phosphate buffer was used. However this resulted in a decrease in biomass and lipid productivity by 22.7% and 44.6%, respectively, compared to cultivation with air, and decreases of 6.8% and 30.6%, respectively, compared to cultivation using coal combustion off-gas (11.24% CO_2, 207 ppm NO_x 275 ppm SO_2) without the use of the buffer (Aslam et al. 2018).

2.4.3 Cultivation design and procedure

Reactor designs commonly focus on optimizing the uptake of CO_2 and growth rate. Designing photobioreactors to increase the residence time of off-gas bubbled into the medium will increase the amount of CO_2 diffused into the medium and available for microalgae (Hosseini et al. 2015). Another essential aspect to reactor design is to ensure that light is not the limiting factor in production as CO_2 uptake cannot occur without sufficient light. Examples of improving light utilization include adding a light tube to the reactor (Hosseini et al. 2018a), reducing required light penetration distances by decreasing the width of the photobioreactor (González-Camejo et al. 2020), providing internal lighting (Amaral et al. 2020) and using different light regimes (ex. flashing lights) (Abu-Ghosh et al. 2016).

Factors to consider when designing cultivation systems include minimizing contamination, increasing the control of process parameters, reducing CO_2 losses, reducing water evaporation, and increasing microalgae cell concentrations (Singh and Sharma 2012). While additional considerations also include

even distribution of sunlight (or artificial light), convenient and precise parameter control, minimized capital and operational costs and minimized energy consumption.

The continuous application of off-gas increases the potential for inhibition from high CO_2 concentration, acidic off-gas components (SO_X and NO_X), and other toxic components. High CO_2 concentrations affect the enzymes involved in the Calvin cycle, resulting in a decrease of CO_2 uptake which in turn directly decreases biomass production (Chisti 2007; Cheng et al. 2019). When off-gas was continuously introduced to a *Desmodesmus abundans* culture it could not uptake sulphur and nitrogen fast enough to combat the acidification, resulting in a significant depression of the culture pH compared to cycling off-gas application (Lara-Gil et al. 2016).

Inhibition due to high CO_2 concentrations was mitigated by cycling off-gas application for 3-12 hours a day (Kao et al. 2014; Palanisami et al. 2015). Off-gas containing high CO_2 concentrations (15%) resulted in the inhibition of a wild microalgae strain, *Chlorella* sp.*,* due to the pH reduction to 5.6 pH (Chiu et al. 2008). Following this initial result, similar cycling was then tested with off-gas. The off-gas was bubbled through the culture for 12 hours a day (Kao et al. 2014), resulting in the medium pH not dropping below pH 6.4, which was within the optimal range for the *Chlorella* sp. (pH 6.4-7.4). At the lab scale, rapid cycling of 1 minute on and 30 minutes off, was shown to successfully reduce CO_2 inhibition (He et al. 2012).

The cycling of off-gas application can also have significant influence on microalgal cultivation. When the bioprospected strain *Desmodesmus abundans* RSM was cultivated under continuous off-gas application,

it reached a biomass concentration 2.5 times greater than the initial concentration after 2.5 days at which point it entered the stationary phase. Comparatively, when the off-gas was cycled, 24 hour aeration cycles, the biomass concentration reached 5.75 times the initial concentration over 6.5 days, when the growth began to slow but had not reached the stationary phase (Lara-Gil et al. 2016).

Palanisami et al. (2015) found that introducing coal-fired off-gas (2% CO_2) for 3 hours daily during the cultivation of *Desmodesmus communis* LUCC 002 resulted in a decrease in overall metal bioaccumulation compared to the control which had similar metal levels but, no added CO_2. There was an increase in lead and mercury uptake but a decrease or no effect with the other metals present (chromium (Cr), barium (Ba), selenium (Se), lead (Pb), arsenic (As), cadmium (Cd), silver (Ag), and mercury (Hg)). The microalgae also showed a gradual adaptation to resist inhibition due to metals resulting in an increased biomass concentration when cultivated with cadmium (0.002 mg L^{-1}) and chromium (0.0135 mg L^{-1}) (Palanisami et al. 2015).

Similar to off-gas cycling, diluting off-gas can decrease the inhibitory effects resulting from the components found in the off-gas (Aslam et al. 2018). The required level of off-gas dilution is strain dependent as optimal growth under different CO_2 concentrations has significant variations between strains (Chiu et al. 2008; China and Fujii 2018). Typically the optimal CO_2 concentration range is 2-20% for biomass production (Cheah et al. 2015).

Yadav et al. (2019) and Wijayasekera et al. (2020) both compared variation in CO_2 concentration and the effect on microalgae cultivation. Comparing *Chlorella* sp. in both studies, diluted off-gas to

approximately 5% CO_2 showed optimal growth compared to undiluted (10 and 16 % CO_2) and the control (0% CO_2). Looking at *Chlorococcum* sp., showed similar trends to *Chlorella* sp. that diluting the off-gas results in optimal growth. *Desmodesmus* sp. productivity increased from 4% to 16% CO_2, although minor (20% increase), meaning that determining if dilution is necessary will be a case by case basis. In the non-dilution cases, a lag period occurred, resulting in a decrease in potential productivity. Depending on the strain, the lag period showed to reduce with the dilution of the off-gas (Yadav, Dash, and Sen 2019). The corresponding results of each study is shown in Table 5.

Table 2.5: Bioproductivities in diluted off-gas

CO_2	Bioproductivity (mg L^{-1} Day^{-1})			Source
%	***Chlorella* sp.**	***Chlorococcum* sp.**	***Desmodesmus* sp.**	
0	25	-	22	(Wijayasekera et al. 2020)
0	115	60.5	-	(Yadav et al. 2019)
1	161.5	65.5	-	(Yadav et al. 2019)
2	47	-	56	(Wijayasekera et al. 2020)
2.5	196.5	91	-	(Yadav et al. 2019)
4	62.5	-	125	(Wijayasekera et al. 2020)
5	209	105.5	-	(Yadav et al. 2019)
10	164	94	-	(Yadav et al. 2019)
16	75	-	150	(Wijayasekera et al. 2020)

Inoculation density can be a factor in many different aspects of cultivation utilizing off-gas, but the most common focus is to use it to combat inhibitions resulting from high CO_2 content and acidification early in the cultivation stage (Yadav et al. 2015). Identifying the balance of a high enough density to combat inhibition while ensuring production of biomass and secondary metabolites are optimized, is key when determining the inoculation density for a given culture (Chen et al. 2012; Odjadjare et al. 2017).

It was found that higher inoculation concentrations of 0.06-0.1 g L^{-1} did not adapt well to pH of 5.5, unlike higher densities of 0.15-0.2 g L^{-1} (Yadav et al. 2015). There was a greater decrease in pH at lower inoculation densities, which resulted in a reduced ability to overcome the associated inhibition.

2.5.0 Conclusion

The use of off-gas as a carbon source for microalgae cultivation is a promising solution of off-gas remediation (Lam and Lee 2012). Inhibitions arise, however, from use of off-gas for cultivation, mainly, due to the high CO_2 content, acidification, and metals present. A range of industries have off-gas that

has similar properties including power production from coal and natural gas(Palanisami et al. 2015; Moheimani 2016; Cheng et al. 2018), cement production (Borkenstein et al. 2011; Rossi et al. 2018), and mineral processing (Laamanen et al. 2014; Mortensen and Gislerød 2015).

Studies have given insight into microalgae growth increasing methods, such as bioprospecting strains, chemical addition, genetic modifications, and reactor design (Raja et al. 2008). Strain selection is a vital step to combatting the inhibitions caused by cultivation with off-gas and utilizing bioprospected strains from stressed environments is a promising solution (Desjardins et al. 2021). A bioprospected strain, *Desmodesmus* sp., could cope with high CO_2 (Wijayasekera et al. 2020), a low pH medium (Abinandan et al. 2019), and high heavy metal presence (Lara-Gil et al. 2016; Abinandan et al. 2019).

Although most results came at lab and pilot scale, they are positive and support the potential of scaling up (Alvarez et al. 2021). However, as each bioprospected strain is different, to optimize the capability of off-gas cultivation the combination of multiple methods should be explored to increase the reduction of inhibitions caused. After optimizing strain selection (bioprospecting or genetic modification), utilizing a chemical edition (phosphate, CKD, etc.) and choosing optimal cultivation design and procedure can maximize the capability of combatting the negative impacts occurring from the use of industrial off-gas for cultivation.

Further research to determine economical and technical feasibility, including a detailed cost comparison between microalgal cultivation and other CO_2 mitigation strategies, is required. Other challenges include target product identification and evaluation (e.g., lipids), scale up of laboratory results, and

development of harvesting systems to allow for low cost, cyclic biomass recovery. For any target products, such as lipids in general, or potentially as a sub set, omega-3 fatty acids or C_{12}-C_{24}, further understanding is required to identify the effect of off gas application on microalgal metabolic processes. Quantification of target products will help determine the economic feasibility of microalgal cultivation. Currently, as most available results are from laboratory scale systems, or small pilot scale studies, a major need is greater scale up to determine its effect on operational and growth parameters. At any scale, efficient and economic harvesting remains a limitation of microalgal cultivation systems, and an area also needing further research to identify harvesting efficient technologies and optimize biomass recovery cycle times.

Chapter 3

The effect of inoculation density on microalgal biomass productivity, lipid content and harvesting cycle times at neutral and low pH

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Abstract

The utilization of microalgae to treat carbon dioxide (CO_2)-rich industrial off-gas has been suggested as both beneficial for emissions reduction and economically favourable to produce microalgal products, such as lipids used in biofuels. One example of a potential source of off-gases is ore smelting (6-7% CO_2 and 0.4% SO_2), but similar to other CO_2-rich off-gas, the application to microalgal culture creates inhibiting acidic conditions. This study was designed to examine the use of bioprospected (acidophilic or acid tolerant) strains to combat inhibitions caused by acidic conditions and the impact of inoculation density on growth and lipid production.

Inoculation density was shown to significantly affect the growth and biomass production rates of the microalgae. The influence of inoculation density is clearly dependent on species. It was also determined that there was no correlation between inoculation density and lipid production in the species tested. The most successful strain was a bioprospected *Coccamyxa* sp. Strain, which showed optimized growth at 100 ml L^{-1} inoculation density.

3.1.0 Introduction

Efforts to mitigate industrial carbon dioxide (CO_2) emissions are set to continue to grow, as countries worldwide are making efforts to reduce the amount of CO_2 emissions by introducing carbon taxes. As of April 2022, the highest carbon taxes worldwide include Uruguay (137 USD per metric ton of CO_2-eq), Sweden (130), Switzerland (130), and Liechtenstein (130) (Tiseo 2023). While Canada currently has a carbon tax of 50 CAD per metric ton of CO_2-eq, there is a clear direction of increasing these rates. In 2019, the carbon tax was 20 CAD per metric ton of CO_2-eq and increased by 10 CAD per year to reach 50 CAD per metric ton of CO_2-eq in 2022. These yearly increases are set to increase per year, where between 2023 and 2030 the carbon tax will increase 15 CAD per year reaching 165 CAD per metric ton of CO_2-eq in 2030 (Government of Canada 2022b).

Industrial off-gas components, specifically CO_2 and acidic components (namely, sulphur dioxide (SO_2) and nitrogen oxides (NO_X)), have negative effects on both the environment and health. CO_2 is a major greenhouse gas, that is a contributor to climate change, such as global warming and extreme weather patterns (Sun et al. 2015). While acidic components are the main contributors to acid rain and photochemical smog. Acid rain occurs when SO_2 reacts with water in the atmosphere producing sulphuric acid, which returns to ground level as precipitation (Grennfelt et al. 2020). Acid rain erodes infrastructure (Zhou et al. 2021) and kills vegetation and microorganisms (Liu et al. 2021). And NO_X is a main precursor to photochemical smog, which has negative health effects on humans and animals, causing irritation to eyes and throats, and can further lead to cancer (Chi and Oanh 2021). Photochemical smog also has negative effects on plant life, as it inhibits photosynthesis resulting in inhibition to overall growth (Chaudary et al. 2021).

Microalgae cultivation is a promising method for industrial CO_2 mitigation, which can be coupled with targeted bioproduction of high value-added products to make operations profitable or, at minimum, offset operational costs (Duarte et al. 2017). A wide range of industries, including cement production, ore smelting, and fossil fuel-fired power generation, are sources of off-gas with significant CO_2 content and have the potential for microalgal CO_2 mitigation (Wang et al. 2008; Laamanen et al. 2017; Chen et al. 2018; Laamanen and Scott 2020). However, these industrial off-gases often contain other components which can limit such applications, in particular the acidic gases sulphur dioxide (SO_2) and nitrogen oxides (NO_X), which can drastically decrease culture medium pH and thereby inhibit microalgal growth (Kumar et al. 2014; Desjardins 2020).

Methods to combat this acidification, such as chemical addition and genetic modification of microalgae species, have been shown to produce successful laboratory outcomes, but have significant associated costs (Nguyen et al. 2016; Kim and Lee 2017; Aslam et al. 2019). An alternative solution is bioprospecting and isolation of strains that grow at low pH (acidophilic or acid-tolerant (Desjardins et al. 2021; Ahn et al. 2022)) and still produce the desired biochemical components. For example, by sampling low pH water bodies in abandoned mine sites, high lipid producing, acid-tolerant strains were isolated as promising candidates to be utilized in low pH cultivation (Eibl et al. 2014; Desjardins et al. 2020).

While strain selection is very important, a commonly overlooked factor in cultivation systems is inoculation density, which is the initial concentration of microalgal culture used to start the cultivation (Sen and Swaminathan 2004). Utilizing a larger inoculation density commonly allows for the culture to better combat inhibition due to low pH early in the cultivation cycle, while minimizing the inoculation density can reduce expenses allocated to cultivation (Göksan et al. 2003). Therefore, finding a balance

between inoculation density and culture productivity is essential (Chen et al. 2012). Optimizing the inoculation of an acid-tolerant bioprospected strain could further improve the ability to mitigate inhibitions arising from acidification (Chiu et al. 2008).

While optimizing biomass production can in turn result in better carbon capture, the production of valuable secondary metabolites may determine the overall economic feasibility of the microalgal cultivation process (Senhorinho et al. 2018; Laamanen et al. 2021; Desjardins et al. 2021; Elisabeth et al. 2021). Furthermore, applying one or more stressors to the microalgae culture is a recognized method for inducing biochemical responses in the cells that increase production of secondary metabolites (Wang et al. 2018). Stressors include, for example, pH, limited nitrogen availability and dark stress (Bai et al. 2016; Arguelles and Martinez-Goss 2021; Desjardins et al. 2022) to increase production of lipids that are of interest for biodiesel or health beneficial products.

Lipids, a main component of interest from microalgae cultivation, can be converted into biodiesel to produce a saleable product (Hosseini et al. 2018b; Desjardins et al. 2022). In Ontario, Canada, the production of renewable fuels is required by the Cleaner Transportation Fuels regulation, which states that diesel must have a 4% blend of renewable content, while emitting 70% less greenhouse gases than fossil fuel diesel on a lifecycle basis. While gasoline must be blended with 10% renewable content from 2020-2024, which will further increase to 15% by 2030, while emitting 45% (before 2030) and 50% (after 2030) less greenhouse gases than fossil gasoline on a lifecycle basis (Government of Canada 2022a).

In theory, maintaining high inoculation densities will both result in greater biomass production over time and greater ability for the culture to cope with stressors. Conversely, lower inoculation densities would mean less biomass is required to start the next cultivation cycle and would allow for more of the culture to be harvested per cycle, even if the cycle is longer (Yu Chen et al. 2012). This could also be desirable for economic harvesting and necessary for laboratory experimentation (i.e., to get enough biomass for extraction and testing). These dynamics are highlighted in a model in this paper. To determine the applicability of this simple model, experiments were completed at both uncontrolled pH and at pH 3, which was used as a basis to represent acidification from off-gas application to microalgal cultivation. These experiments examined the effect of strain selection and inoculation density on biomass and lipid production.

3.2.0 Methods

3.2.1 Strain Selection

Three strains were cultured and compared in inoculation density experiments. Two were culture collection strains (*Chlamydomonas reihardtii* CPCC11 (Canadian Phycological Culture Center) and *Scenedesmus dimorphus* UTEX 1237 (Culture Collection of Algae at The University of Texas at Austin)) and the other bioprospected from a polishing pond at an operational smelter located at Sudbury Integrated Nickel Operations (Falconbridge, ON, Canada) in northern Canada (P918, identified as 98% *Coccomyxa* sp.).

The two culture collection strains have been previously shown to be heavily influenced by reducing the pH of the culture medium. *Chlamydomonas reihardtii* CPCC11 (Canadian Phycological Culture Center) showed no significant growth at pH 4.5, and *Scenedesmus dimorphus* UTEX 1237 (Culture Collection of Algae at The University of Texas at Austin) showed no growth below pH 4.5 (Desjardins et al. 2021). Whereas, the bioprospected strain *Coccamyxa* sp. has grown successfully at pH 3 (Desjardins et al. 2021).

3.2.2 Experimental Set up

Three inoculation densities were chosen for screening experiments: 200 ml of microalgae culture (high) diluted into 1 L of Bold's Basil Medium (BBM) (Bold 1949), 100 ml L^{-1} (medium), and 25 ml L^{-1} (low). Each trial was conducted in 1 L Erlenmeyer flasks with a working volume of 500 mL. The BBM was either at uncontrolled pH or modified to keep it at pH 3 depending on the trial. The pH was checked and adjusted three times a week using 1 M sulfuric acid (H_2SO_4) to emulate the application of industrial off-gas containing SO_2. To provide each culture with the same quantity of nutrients, the inoculation was

centrifuged using a Sorvall ST1 (Thermoscientific, MA, USA) to separate from the previous medium and the harvested cells were added to the cultivation flasks containing 500 mL of BBM.

After completing screening experiments with high (200 ml L^{-1}), medium (100 ml L^{-1}) and low (25 ml L^{-1}) inoculation densities, the range was refined to determine a more detailed representation of the influence of inoculation density. Specifically, as the high and medium inoculation densities showed similar results for many of the tests, the range examined in further testing was: 100 ml L^{-1}, 75 ml L^{-1}, 50 ml L^{-1}, and 25 ml L^{-1}.

After the inoculation was completed, the flasks were placed on shaker tables at 125 RPM. The flask were illuminated with a 90W circular grow light (UFO grow quad band (red, blue, orange, white), Ledwholesalers Inc., CA, USA) utilizing a diurnal light cycle of 16:8 (on:off) to emulate natural regional light cycles.

3.2.3 Density Measurements

Three times a week optical density was measured in triplicate using a spectrometer (Agilent BioTek Epoch, CA, USA) at a wavelength of 550 nm.

Once a week, biomass density was measured by centrifuging (Sorvall ST1, Thermoscientific, MA, USA) 50 mL samples and the separated biomass was frozen at -20°C, freeze dried and weighed. At the end of cultivation, the remaining media was harvested and measured to determine final biomass and used for subsequent lipid extraction and analysis.

3.2.4 Lipids Analysis

Lipids were extracted using the Folch method (Folch et al. 1957). Namely, after samples were centrifuged, frozen, and freeze-dried, 10 mg of biomass was mixed with 1 mL of chloroform:methanol (2:1 v/v) and then sonicated for 1 minute using a Sonic Dismembrator Model 500 (Fisher Scientific, Ottawa, Ontario, Canada). Following sonication, 0.2 mL of deionized (DI) water was added and the samples centrifuged with an Allegra X-15R Centrifuge (Beckham, Palo Alto, CA, USA). The chloroform layer was then extracted into a pre-weighed vial. The procedure was repeated in triplicate, with the resulting extracts were combined and dried at room temperature. Once dry, the quantity of lipid was weighed.

3.2.5 Inoculation Density Model

To set a basis for inoculation density experimentation, a simplified model was developed using a specific growth rate of 0.32 day^{-1} found by Hosseini et al. (2015). This specific growth rate is applied to a 1L basis, where the culture is harvested when it reaches 1 g L^{-1}. The amount of culture harvested is varied (80, 90, 95, 97.5%) and the remaining biomass is diluted back to the 1L volume to prepare the solution for the next growth cycle.

The cultivation time for each cycle is provided in Table 1, which results in different time between harvests and different biomass productivities, as summarized in Table 2. The associated growth curves of the lowest and highest inoculation densities (25 and 200 ml L^{-1}) are represented visually in Figure 1 (a), while the cumulative biomass is shown in Figure 1 (b).

Table 3.1: Theoretical time to reach 1 g L^{-1} density

Inoculation Density	Initial concentration	Concentration [g L^{-1}]											
		Day											
[ml L^{-1}]	[g L^{-1}]	1	2	3	4	5	6	7	8	9	10	11	12
25	0.025	0.03	0.05	0.07	0.09	0.12	0.17	0.23	0.32	0.45	0.61	0.84	1.16
50	0.05	0.07	0.09	0.13	0.18	0.25	0.34	0.47	0.65	0.89	1.23		
100	0.1	0.14	0.19	0.26	0.36	0.50	0.68	0.94	1.29				
200	0.2	0.28	0.38	0.52	0.72	1.00							

Table 3.2: Theoretical Amount of Biomass Harvested per Year (1L basis)

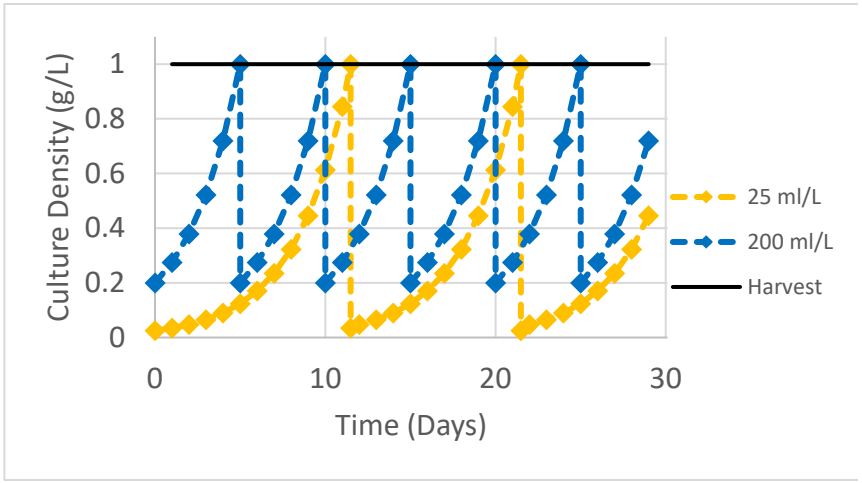
Inoculation	Initial Conc.	Volume / Harvest	Mass / Harvest	Time / Harvest	Harvests / Year	Mass / Year
[mL L^{-1}]	[g L^{-1}]	[mL]	[g]	[days]	[-]	[g L^{-1} $year^{-1}$]
25	0.025	975	0.975	11.5	31.7	30.9
50	0.05	950	0.95	9.4	39.0	37.1
100	0.1	900	0.90	7.2	50.7	45.6
200	0.2	800	0.80	5.0	72.5	58.0

As shown by Figure 1, changing the inoculation density of the cultivation, and thus the harvesting regime, can have a significant effect in terms of biomass productivity. However, there are several important considerations that this simplified model does not consider:

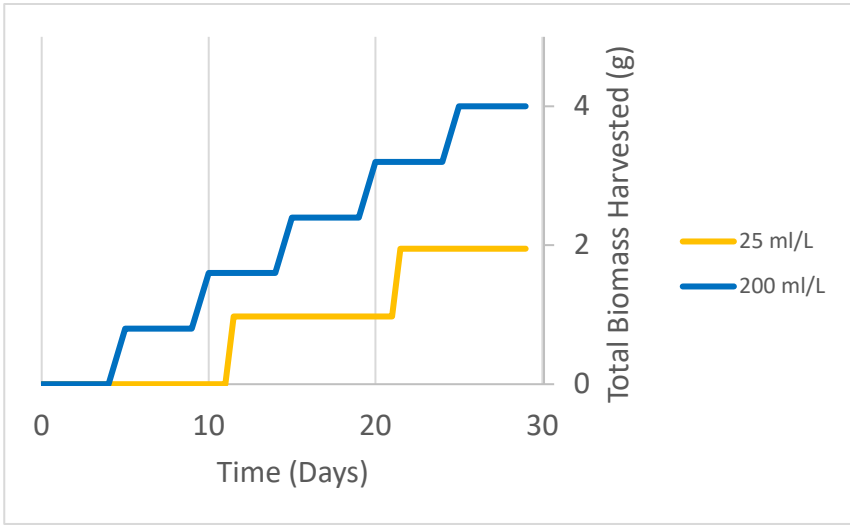
1. The inoculation density may affect growth rate in different ways, including lag phases and non-ideal growth behavior, as a result of self-shading (Hosseini et al. 2018; Nguyen et al. 2022) and competition for nutrients and CO_2 (Chisti 2007; Abinandan et al. 2018)
2. The influence of inoculation density on growth rate may be more significant if the culture is exposed to stressors common to industrial off-gas usage, especially acidification
3. The inoculation density may affect the microalgal metabolism with respect to accumulation of targeted valuable components (e.g. lipids)
4. Counter to continuous industrial growth, the higher harvesting percentage and lower inoculation densities can be very helpful in laboratory settings to ensure sufficient biomass is collected for analysis at the end of an experiment

With the above in mind, several microalgae species were tested to examine growth and lipid content at neutral conditions and at pH 3 to emulate conditions produced by industrial off-gas applications. Chen et al. (2012), the last study we were able to identify that examined the effect of inoculation density, found that 0.98 g L^{-1}, the middle inoculation density tested was the best for their uncontrolled pH experiments. Notably, low pH inoculation density has not previously been reported on, so we hypothesized that the medium or high (100 or 200 ml L^{-1}) inoculation densities would be optimal, as similar limiting factors would exist with the addition of the pH-based stressor. When examining lipid content, as a target compound for biofuel or health beneficial component production, we hypothesize that when cultivating at the lower inoculation density (25 mL L^{-1}), the lipid content (%) would increase

due to the reduced quantity of biomass having to overcoming proportionately more stress. The trend of increased lipid content as inoculation density was reduced was seen in the neutral pH experiments done by Chen et al. (2012).



(a)



(b)

Figure 3.1: the effect of inoculation densities of 25 ml L^{-1} (yellow) and 200 ml L^{-1} (blue) on harvesting cycle over 1 month (a) and the total biomass produced (b)

3.3.0 Results and Discussion

Correlation was determined between density determined by centrifugation and that by optical measurement. Using *Scenedesmus dimorphus* at uncontrolled pH as an example, the correlation graph is shown in Figure 2. Table 3 has correlations for *Coccamyxa* sp. and *Scenedesmus dimorphus* at uncontrolled pH and pH 3, and *Chlamydamonas reinhardtii* at uncontrolled pH only (as it was unable to grow at pH 3).

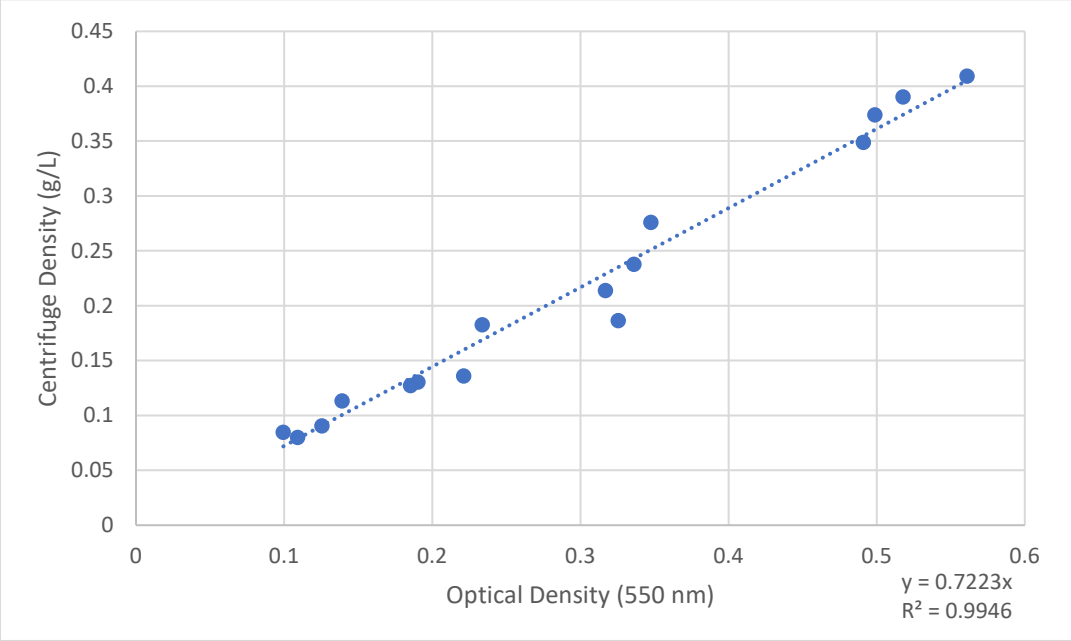


Figure 3.2: Correlation of centrifuge and optical determined density for *Scenedesmus dimorphus* at pH 7

Table 3.3: Correlation of Centrifuge and Optical determined Density

Strain	pH	Correlation (Slope)	R^2
Coccamyxa sp. (P918)	Uncontrolled	0.7852	0.9438
	3	1.0758	0.8301
Scenedesmus dimorphus	Uncontrolled	0.7223	0.9946
	3	1.2716	0.9907
Chlamydomonas reinhardtii	Uncontrolled	1.9204	0.988

By comparing the correlation of biomass (dry weight) densities to optical density measurements, correlation varies between the species and is also influenced by the pH of the system.

To be able to model a cyclic harvesting system, utilizing the inoculation density results over multiple cultivations, a harvesting density was set for each individual species to determine cultivation periods. This allowed for modeling of the effect of inoculation on biomass production and productivity over time.

If it is assumed that specific growth rate is unaffected by inoculation density, higher inoculation densities would consistently result in shorter cultivation periods to reach the harvesting density (as shown in the Inoculation Density Model). However, from our experimental results, the uncontrolled pH experiments did not match this assumption, as medium inoculation densities (100 ml L^{-1}) resulted in cultures quickest to reach harvest density (Figure 3). As a result, the medium inoculation densities (100 ml L^{-1}) would be able to harvest more biomass per year (Table 4). The lowest inoculation densities (25 ml L^{-1}) were also able to harvest more biomass over the year compared to the high inoculation density (200 ml L^{-1}), even though it was harvested one less time per year, as a result of harvesting more biomass per harvest, as less biomass must be retained for inoculation of the next cultivation cycle.

Chlamydomonas reinhardtii (Figure 3 a) at uncontrolled pH followed the expected trend, with 100 ml L^{-1} reaching harvest density 2 days before 200 ml L^{-1}, while the inoculation of 25 ml L^{-1} did not grow sufficiently across the 28 day experiment to reach a harvestable density. The acid tolerant bioprospected *Coccamyxa* sp. (Figure 3 b) strain followed similar trends to *Chlamydomonas reinhardtii* (Figure 3 a), where an inoculation density of 100 ml L^{-1} reached the harvestable density 7-12 days before the other Inoculations. Although in this case, the low density (25 ml L^{-1}) did grow and had a comparable growth period to the inoculation density of 200 ml L^{-1}.

As higher densities showed no additional benefit in trials at uncontrolled pH, *Scenedesmus dimorphus* (Figure 3 c) and *Coccamyxa* sp. (Figure 3 d) at uncontrolled pH were tested at inoculation densities of 25, 50, 75, and 100 ml L^{-1}. For *Scenedesmus dimorphus,* all four inoculations followed similar trends reaching harvestable density within three days of each other. Although each case had similar densities, the lower inoculation correlated to greater recovery of biomass per harvest and thus increased productivity over time. While *Coccamyxa* sp. resulted in 75 and 100 ml L^{-1} reaching harvestable density the fastest, and producing more biomass over time compared to the lower inoculation densities.

Chiu et al. (2008) explored low (8×10^5 cells mL^{-1}) and high (8×10^6 cells mL^{-1}) inoculation density and the influence of CO_2 aeration concentration. Both cases found similar trends, with 2% CO_2 producing the best growth for both inoculation densities, while the higher inoculation had higher biomass production compared to the low inoculation. Low density inoculation produced 1.211 g L^{-1} and high density inoculation produced 1.445 g L^{-1} at 2% CO_2. These results coincided with the results found in this study, the low inoculation cases did not produce as much biomass as the high inoculation cases over the same period of growth.

Coccamyxa sp. (Figure 3 b) was used as an example of application into a cyclical harvesting system, and the results given in Figure 5. Figure 5 (a) shows the culture density over two months of cyclic harvesting, where the equivalent of the initial inoculation is left in the cultivation system to initiate the next cycle. Based on these harvesting cycles and quantities, Figure 5 (b) represents the accumulation of biomass harvested over two months, which shows how a lower inoculation density can produce more biomass over time from less frequent harvests. The results confirm that the medium density (100 ml L^{-1}) is the optimal inoculation density for *Coccamyxa* sp..

Compared to the uncontrolled pH experiments, the pH 3 experiments resulted in considerably decreased growth rates. As *Scenedesmus dimorphus* (Figure 3.4 a) is neither acid tolerant nor acidophilic, it did not generate any appreciably amount of biomass over the 28 day cycle. *Coccamyxa* sp. (Figure 4 b) showed minor growth with the high (200 ml L^{-1}) and medium (100 ml L^{-1}) inoculation densities, but it was unable to grow from the low inoculation density. The lower range of inoculation densities (25, 50, 75, and 100 ml L^{-1}) when tested at pH 3 for *Coccamyxa* sp. (Figure 3.4 c), showed no considerable growth other than at 75 ml L^{-1}, which can likely be regarded as an anomaly. When under pH 3 conditions, cultures had minor increases in density, which can be attributed to acidity killing the culture or inhibiting the growth.

To compare strains, Table 3.4 shows the relationship of the inoculation densities and biomass production over a yearly cultivation while comparing uncontrolled and pH 3 cultivation. It further shows the lipid production over a year of operation. Overall, the trends show that at pH 3 the optimal inoculation density for both strains that grew, *Coccamyxa* sp. and *Scenedesmus dimorphus*, is 100 ml L^{-1}.

While the trends for these strains at uncontrolled pH showed that inoculation density was not a significant factor over this range. Conversely *Chlamydamonus reinhardtii* showed significant influence as a result of inoculation density, where 100 ml L^{-1} resulted in a 3x and a 5x increase compared to 200 ml L^{-1} and 25 ml L^{-1}, respectively.

Not only can the cases be compared with biomass and lipid production, but it can also be compared by the amount of CO_2 captured. CO_2 capture is directly correlated to the amount of biomass produced, resulting in parallel trends. As shown in the biomass comparisons, *Coccamyxa* sp., *Scenedesmus dimorphus*, and *Chlamydamonus reinhardtii*, all resulted in an optimal CO_2 capture with an inoculation density of 100 ml L^{-1}.

As a key component of interest, lipid content in the dry biomass (%) as a function of inoculation density was explored (Figure 6). For *Chlamydomonas reinhardtii,* insufficient biomass was generated through the experiments to provide sufficient lipid data. Figure 6 shows the lipid content for *Coccamyxa* sp. and *Scenedesmus dimorphus* at both uncontrolled pH and pH 3. There were no significant differences in lipid content between inoculation densities. These results for *Coccamyxa* sp. show that inhibitions from pH 3, regardless of inoculation density, did not result in a metabolic change to increase lipid production to protect the culture. Meanwhile, for *Scenedesmus dimorphus* lipid content at pH 3 shows an average increase of 49% compared to uncontrolled pH experiments. These results match the theory that when the culture experiences stressors throughout growth, protective metabolic changes, such as lipid production, are a response to these stressors (Chen et al. 2020). As such, it is likely that *Coccamyxa* sp. did not increase in lipid production at pH 3 as the strain was bioprospected from an ecosystem at pH 3 and has adapted to these conditions.

Chen et al. (2012) determined inoculation density was an important aspect of biomass productivity and lipid production for *Nannochloropsis* sp.. Their study found that at uncontrolled pH cultivation, the medium inoculation densities had the largest biomass productivity, while as the inoculation density increased the lipid concentration decreased. The biomass productivity results match the uncontrolled pH experiments in this paper, where the medium inoculation (100 ml L^{-1}) had the highest biomass production. However, it was found that there were no trends between inoculation densities and lipid content, unlike Chen et al. (2012).

The trends throughout each trial at both uncontrolled and pH 3 show that the effect of inoculation density on culture growth and biomass accumulation is strain dependent. Generally, at uncontrolled pH the medium inoculation had the most successful results, while the other strains had continuous positive upwards growth. The medium inoculation density had the most successful growth is likely due to the best balance between cell numbers and CO_2 and nutrients availability, compared to the high density inoculation.

Comparatively, the pH 3 trials only showed successful growth for *Coccamyxa* sp., which as a bioprospected strain was able defend against inhibitions caused by the acidic conditions. Notably, even for this acid tolerant strain, the results show that lower inoculation densities could not survive in the pH 3 environment. It is hypothesized that over long periods of time being exposed to the harsh conditions of the acid pond allowed for long term genetic modification of the original species to produce the current strain. Further analysis of the culture will need to be conducted to fully understand how the microalgal culture is able to adapt and combat the pH 3 conditions.

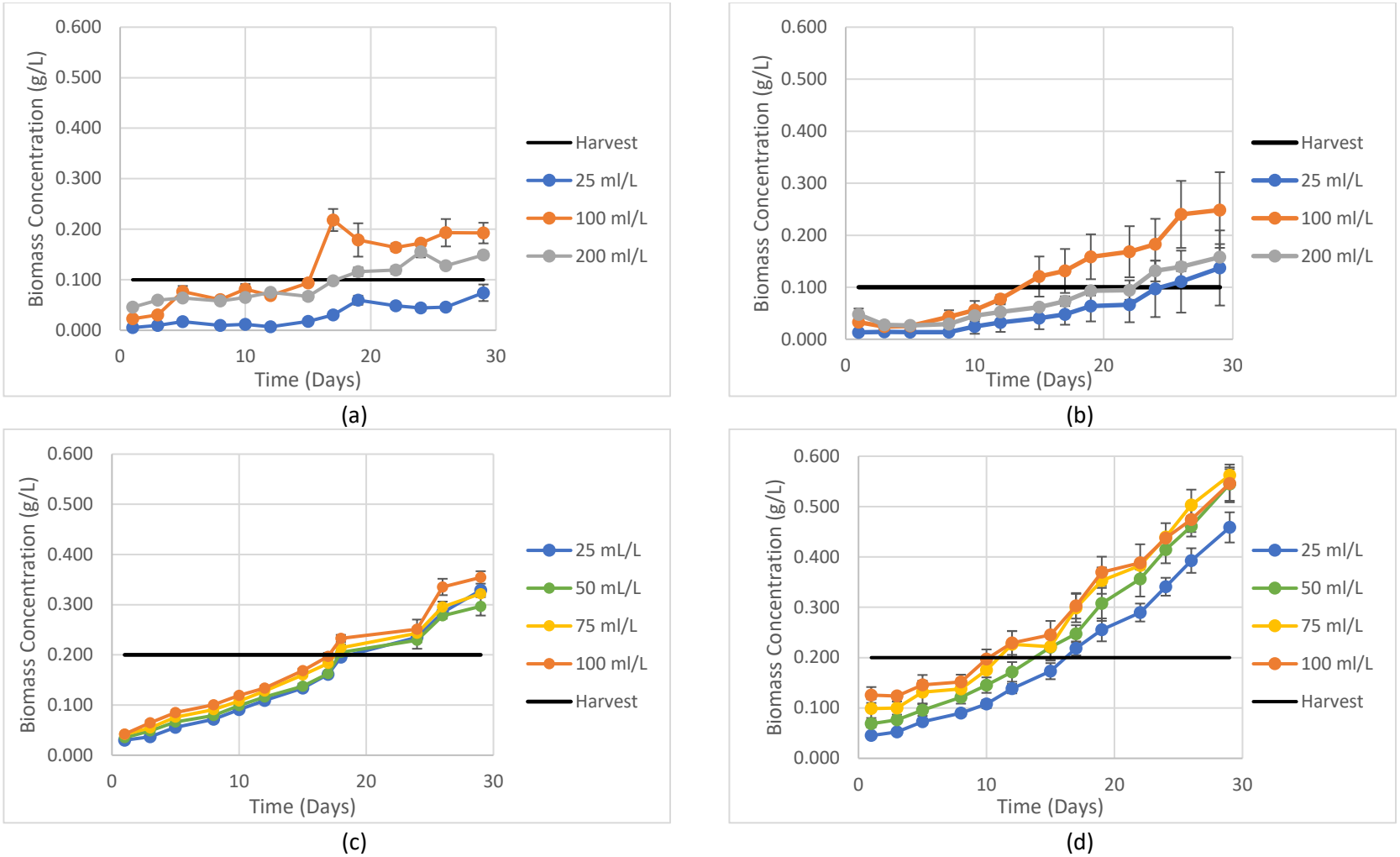
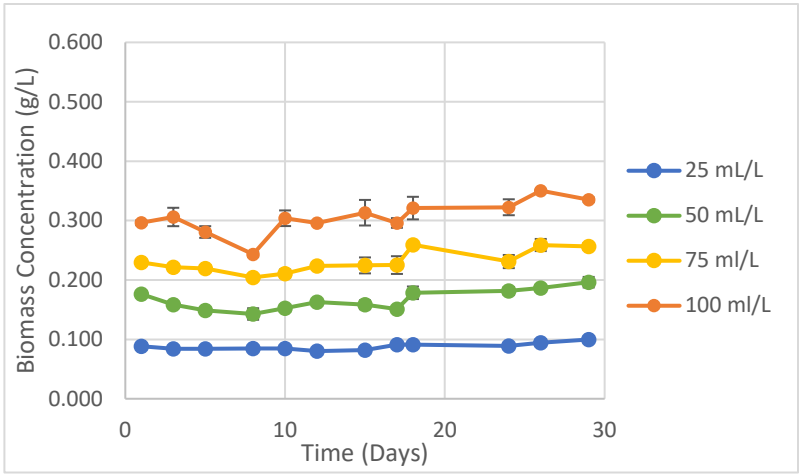
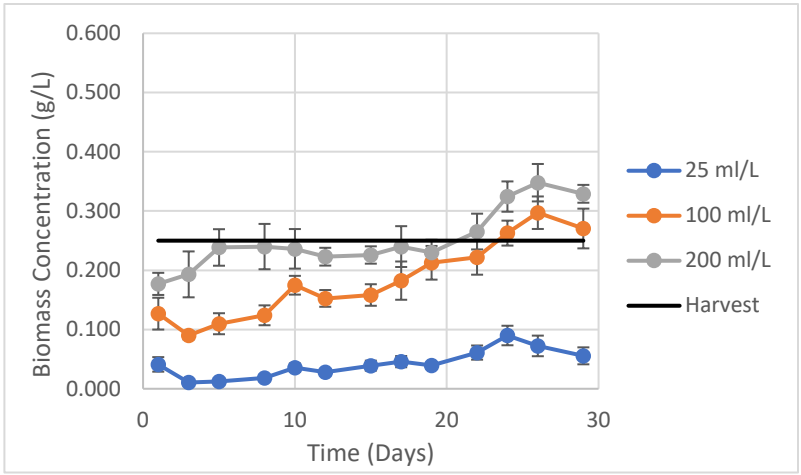


Figure 3.3: cultivation growth curves at uncontrolled pH for *Chlamydamonus reinhardtii* (a), *Scenedesmus dimorphus* (b), *Coccamyxa* sp. (trial 1, c), and *Coccamyxa* sp. (trial 2 (lower inoculation density range), d)



(a)



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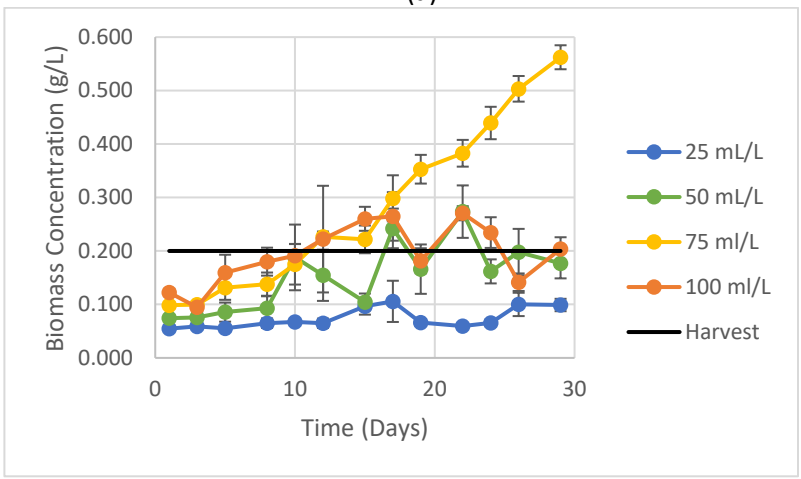
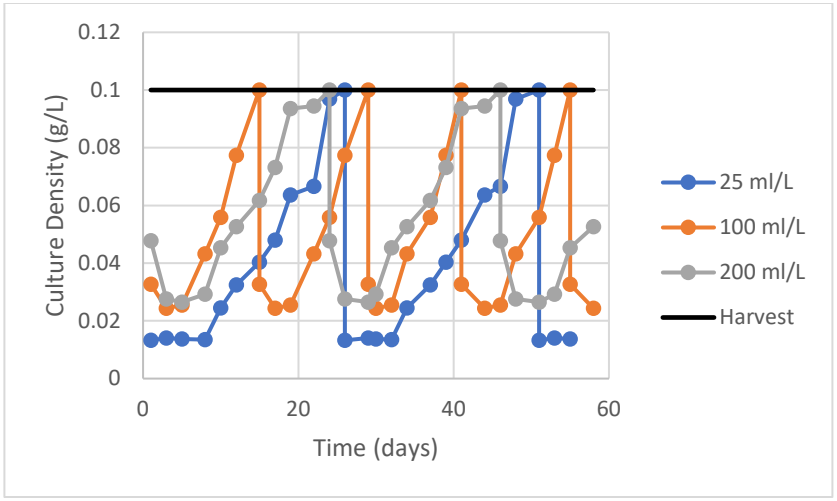
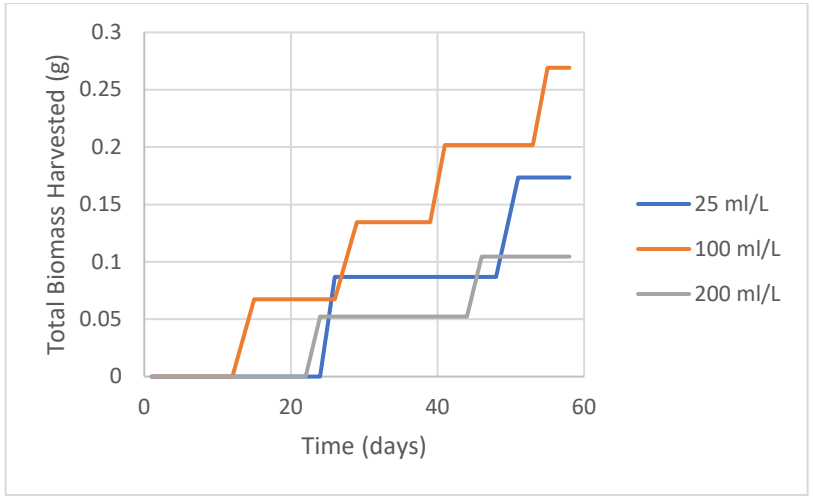


(c)

Figure 3.4: cultivation growth curves at pH 3 for *Scenedesmus dimorphus* (a), *Coccamyxa* sp. (trial 1, b), and *Coccamyxa* sp. (trial 2, c)



(a)



(b)

Figure 3.5: the effect of inoculation densities for *Coccamyxa* sp. of 25 ml L^{-1} (blue), 100 ml L^{-1} (orange), and 200 ml L^{-1} (grey) on harvesting cycle over 2 months (a) and the total biomass produced (b)

Table 3.4: comparison of biomass and lipid production at uncontrolled and pH 3

		Uncontrolled pH Annual Production			**pH 3 Annual Production**		
Strain	**Inoculation Density**	**Biomass**	**CO_2-eq**	**Lipids**	**Biomass**	**CO_2-eq**	**Lipids**
	ml/L	g/1000L	g/1000L	g/1000L	g/1000L	g/1000L	g/1000L
Coccamyxa sp.	25	1,218	2,192	294	-	-	-
	100	1,637	2,947	412	1,955	3,519	346
	200	795	1,431	189	1,268	2,283	228
Chlamydamonus reinhardtii	25	-	-	-	-	-	-
	100	1,760	3,169	-	-	-	-
	200	1,102	1,983	-	-	-	-
Coccamyxa sp.	25	3,531	6,355	852	-	-	-
	50	3,423	6,161	817	2,868	5,163	513
	75	3,361	6,050	735	2,185	3,934	484
	100	2,707	4,873	681	2,574	4,634	455
Scenedesmus dimorphus	25	2,738	4,929	707	-	-	-
	50	2,625	4,726	661	-	-	-
	75	2,833	5,100	741	-	-	-
	100	3,029	5,451	818	-	-	-

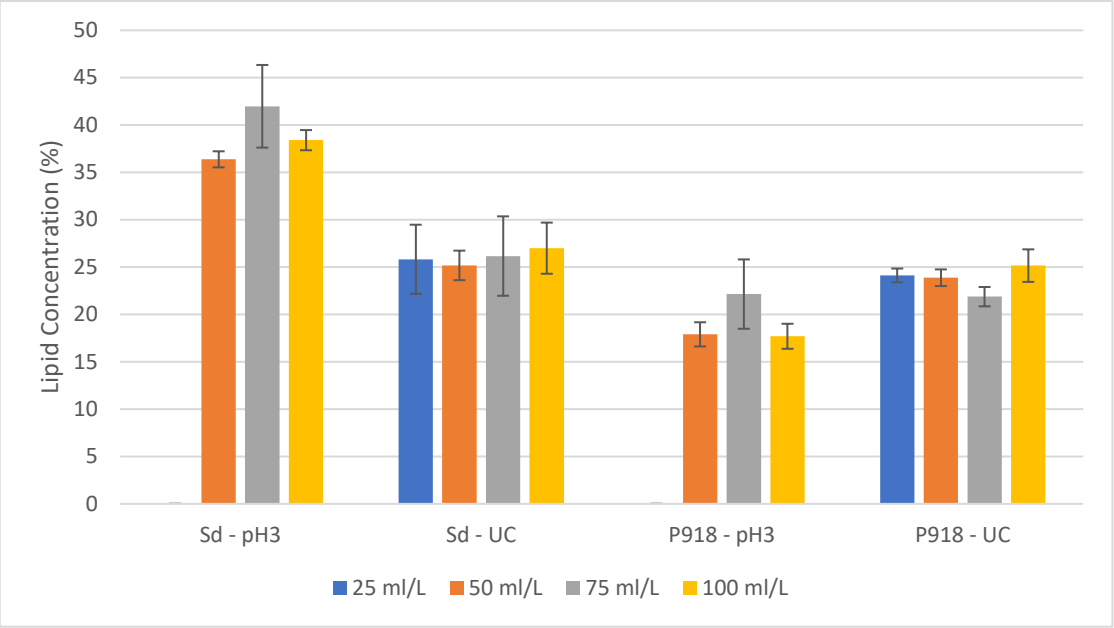


Figure 3.6: Lipid concentration of *Scenedesmus dimorphus* (Sd) and *Coccamyxa* sp. (P918) at uncontrolled pH and pH 3

3.4.0 Conclusion

Transitioning to the use of biodiesel, or biodiesel blends, for transportation fuels increases the need for optimized production of lipids – the main precursor to biodiesel. Inoculation density is shown to have a significant effect on biomass and lipid production.

As such, determining the optimal inoculation density for a strain is an important factor for optimizing biomass and lipid production. In this work, inoculation density had no significant effect on lipid quantity in the biomass and, therefore, maximizing the harvestable biomass will maximize the lipid production.

The highest productivity occurred with the bioprospected *Coccamyxa* sp. at 25 ml L^{-1} inoculation density by producing 3.53 g L^{-1} $year^{-1}$. While at pH 3, which can result from the direct application of industrial off gas, only the bioprospected *Coccamyxa* sp. showed good growth, with the highest productivity achieved with an inoculum of 50 ml L^{-1}, resulting in a productivity of 2.87 g L^{-1} $year^{-1}$. When comparing the CO_2-eq. capture, *Coccamyxa* sp. captured 6.36 $g_{CO2\text{-}eq}$ L^{-1} $year^{-1}$ at uncontrolled pH and 5.16 $g_{CO2\text{-}eq}$ L^{-1} $year^{-1}$ at pH 3.

Chapter 4

4.1.0 Conclusions

The direct application of off-gas as a carbon source for microalgae cultivation is a promising solution to remediate CO_2 emissions (Lam and Lee, 2012). Inhibitions arise, however, from use of off-gas, mainly, due to the high CO_2 content, acidification, and the presence of metals. A range of industries have CO_2-rich off-gas that has similar properties, and limitations, including power production from coal and natural gas (Palanisami et al. 2015; Moheimani 2016; Cheng et al. 2018), cement production (Borkenstein et al. 2011; Rossi et al. 2018), and mineral processing (Laamanen et al. 2014; Mortensen and Gislerød 2015).

Previous studies have provided some insight into microalgae cultivation methods to mitigate the inhibitions associated with off-gas usage, such as bioprospecting strains, chemical buffer addition, genetic modifications of microalgae strains, and reactor design (Raja et al. 2008). Strain selection is a vital step to combatting the inhibitions caused by cultivation with off-gas and utilizing bioprospected strains from stressed environments is a promising direction (Desjardin et al., 2021). One such bioprospected strain, *Desmodesmus* sp., could cope with high CO_2 concentrations (Wijayasekera et al. 2020), low pH medium (Abinandan et al. 2019), and high heavy metal concentrations (Lara-Gil et al. 2016; Abinandan et al. 2019).

Inoculation density is shown to have a significant effect on biomass and lipid production. As such, determining the optimal inoculation density for a strain is an important factor for optimizing biomass and lipid production. In this work, inoculation density had no significant effect on lipid quantity in the biomass and, therefore, maximizing the harvestable biomass will maximize the lipid production.

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4.2.0 Future Work

The direction of this research topic, following the previously discussed research outcomes, is very promising. Possible options for the next steps of research are summarized below, in no specific order.

Target compound identification and evaluation: Understanding the compounds (such as, lipids, antioxidants, etc.) that can be produced from the bioprospected microalgae, and enhanced with the stressors resulting from industrial off-gas application, will allow for an economic analysis of potential combined remediation and production systems. Stressors, such as high CO_2, and acidic components (SO_2 and NO_X), can cause the microalgae culture to produce secondary metabolites to combat the inhibitions. While it is speculated that alterations in inoculation density will affect this stress response in some microalgae strains. Determining what strains produce target compounds, and at appreciable productivities through process decisions, is pertinent for the economics of microalgal cultivation.

Lipid qualification: Determining the quality of lipids (C_{12}-C_{24}, and specific target lipids like omega 3 fatty acids), is another product consideration that can have a significant affect on the economic feasibility of microalgae cultivation. As a result of different stressors, cell metabolism produces different lipid

compounds, so while the total quantity of lipid was unaffected through the inoculation density trials, there could still be interesting interactions between inoculation density and lipid quality.

Direct application of industrial off-gas: This thesis focussed on the low pH associated with industrial off-gas application, however it is recognized that direct application of off-gas can generate a combination of stressors. As such, repeating the experiments in this thesis with the direct application of industrial off-gas will give a more representative approach to large scale industrial production. This will allow for expansion on the promising results obtained through this thesis.

Scale up: Similar to the use of industrial off-gas, to best adapt the results in this thesis to industrial applications, scaled up experiments, such as at pilot scale, will elucidate if the results obtained within this thesis change with scale. Eventually, the same experiments could be completed in large-scale cultivation systems with direct industrial off-gas application.

Cyclic harvesting experiments: As discussed in Chapter Three, inoculation density can be examined through the lens of cyclic harvesting, where rather than harvesting all of the culture and subsequently restarting with a new stock culture, a desired (optimized) amount of culture can be left in the cultivation system for inoculation of the next batch. While this has been proposed in this thesis, a set of experiments exploring the application of this method would be interesting, to prove the concept. While this allows for direct utilization of the explored concepts of inoculation density, an additional major benefit of this experiment would be (insert long term mutagenesis and proven resistance to inhibitions).

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