The University Of Sheffield.

Microalgal Bioremediation: An Experimental and Techno-Economic Evaluation.

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A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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Declaration

I, the author, confirm that this thesis is my own work. I am aware of the University's guidance on the use of unfair means (https://www.sheffield.ac.uk/ssid/unfair-means/index#what). This work has not been previously presented for an award at this, or any other university.

Part of this work is published or in peer review, in the following journal articles:

- Leflay, H. Okurowska, K. Pandhal, J. and Brown, S. (2020). Pathways to Economic Viability: A Pilot Scale and Techno-Economic Assessment for Algal Bioremediation of Challenging Waste Streams. RSC: Water Research and Technology 6(12) 3400-3414.
- Leflay, H., Pandhal, J., and Brown, S. (2021). Direct measurements of CO2 capture are essential to assess the technical and economic potential of algal-CCUS. Journal of CO2 Utilization. 52, 101657.

Part of this work has also been presented at conferences:

- The 9th International Conference on Algal Biomass, Biofuels and Bioproducts. Colorado, USA. June 17th – June 19th, 2019. Poster presentation
- CCnet Conference. Nottingham, UK. 10th February – 12th February 2020. Poster presentation

Abstract

In 2020 alone, 2 billion tonnes of solid waste and 40 billion tonnes of CO_2 were produced globally. The release of pollutants from these waste streams into the ecosystem is causing the climate to change. Microalgae offer a solution to clean up these waste streams and produce a usable by-product in the process.

Previously, microalgae have been shown to remediate a variety of waste streams, however, optimisation and evaluation is still required to achieve economically viable processes. Research over the last decade has highlighted that various species can withstand elevated CO_2 concentrations but evidence that the microalgae can be used to remove substantial portions of the pollutant is lacking. Alongside this, many publications suggest that microalgae can be used for the simultaneous bioremediation of leachates/wastewaters and flue gases, with little evidence that both pollutant sources can be cleaned effectively in this way.

This project focuses on evaluating microalgal bioremediation of two key waste streams: flue gas and landfill leachate, both independently and simultaneously, and optimisation for economic viability. Firstly, algal remediation of both waste streams was investigated individually, with techno-economic analysis conducted using experimental results. Further to this, the simultaneous treatment of both waste streams was investigated to ascertain whether there would be an effect on the remediation efficiency and growth of the culture under these new conditions.

We have shown that the theoretical estimation method currently used for assessing algal carbon capture severely underestimates the concentration of CO_2 being removed, and that real-time monitoring should be used. Through the economic analysis conducted, we have shown that improving culture remediation efficiency and reducing growth time should be the focus of future research to lead to the most significant cost savings. Lastly, we demonstrate that concurrent remediation of landfill leachates and flue gases is not as easily applied as considered within the literature. We observed that the organic carbon present within the leachate can have a negative effect on the inorganic carbon assimilation of cultures, negatively impacting the CO_2 abatement. This work can be used as a basis for future optimisation of algal bioremediation, focusing on the areas highlighted as cost sensitive.

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Nomenclature and Abbreviations

3N-BBM+v12	Modified Bold's Basal Media with Vitamin B12
ABECCS	Algae with Bioenergy and Carbon Capture and Storage
Abs	Absorbance
AD	Anaerobic Digestion
AMP	2-amino-2-methyl-1-propanol
AOP	Advanced Oxidation Process
BBM	Bold Basal Media
C. pyrenoidosa	*Chlorella pyrenoidosa*
C. snowie	*Chlamydomonas snowie*
C. vulgaris	*Chlorella vulgaris*
CapEx	Capital Expenditure
$C_{Biomass}$	% of biomass which is carbon
CaCO	Calcium Carbonate
CCAP	Culture Collection of Algae and Protozoa
CCS	Carbon Capture and Storage
CCUS	Carbon Capture and Utilisation or Storage
CGW	Contaminated Ground Water
Chlorella sp.	*Chlorella species*
C_{insure}	Cost of Insurance
CLWW	Coloured Laboratory Wastewater
$C_{maintenance}$	Cost of Maintenance
CoC	Cost of Capture
COD	Chemical Oxygen Demand
COPB	Cost of Producing Biomass (£ kg^{-1})
DEA	Diethanolamine
dH_2O	Distilled Water
DIP	Dissolved Inorganic Phosphate
DMSO	Dimethyl Sulfoxide
DOC	Dissolved Organic Carbon
DV	Depreciation Value
EFF_{CO2}	Efficiency of CO2 uptake
EOR	Enhanced Oil Recovery
FDCO	Facility Direct Cultivation OpEx
g C	grams carbon
GAL	Gallon
GBP	Great British Pound
GHG	Greenhouse Gas
GtC	Giga tonnes carbon
GtC yr^{-1}	Giga tonnes carbon per year
GWP	Global Warming Potential
HA	Hectare

HM	Heavy Metals
HRAP	High Rate Algal Pond
HRT	Hydraulic Retention Time
HVP	High Value Product
I	Item (for MEC)
I	inflation
I_C	Items Cost
IGCC	Integrated Gasification Combined Cycle
IL	Ionic Liquid
I_{LS}	Items Life Span
KWh	Kilowatt Hours
LED	Light Emitting Diode
LF	Landfill
LL	Landfill Leachate
LO	Labour OpEx
LPM	Litres per Minute
M	Municipal
MAP	Magnesium Ammonium Phosphate
MDEA	Methyl-di-ethanolamine
MEA	Monoethanolamine
MEC	Major Equipment Cost
Me_xO_y	Metal Oxide
Mol	Moles
mol CO_2 mol amine^{-1}	Mole of CO_2 per mole of amine
MSW	Municipal Solid Waste
MW_C	Molecular Weight of Carbon
MW_{CO2}	Molecular Weight of CO2
N	Normality (acids)
NCF	Net Cash Flow
NDIR	Non-Dispersive InfraRed
NEED	N-(1-Naphthyl)ethylenediamine
NH_3-N	Ammoniacal-Nitrogen
nm	Nanometre Wavelength
NO_x	Nitrous Oxides
NPV	Net Present Value
N_{staff}	Number of Staff
OD	Optical Density
OD_{695}	Optical Density at 695 nm
OpEx	Operational Expenditure
ORP	Open Raceway Ponds
P	Culture Productivity
PBR	Photobioreactor
P_{CO2}	Culture CO2 uptake
ppm	Parts Per Million

PSI	Pressure per Square Inch
PV	Photo Voltaic
PVA	Polyvinyl Acetate
R	Repurchase required
R (Appendix B)	Raw (leachate or wastewater)
RC	Recirculated (leachate or wastewater)
R_{CO2}	CO2 Removal Rate
rDNA	Ribosomal DNA
RE	Removal Efficiency (%)
RITE	Research Institute of innovative Technology for the Earth
rpm	Rotations per Minute
RR	Removal Rate (mg L-1 d-1)
RT	Room Temperature
S. Obliquus	*Scenedesmus obliquus*
SBR	Sequence Bioreactor
SO_x	Sulfur Oxides
sp.	Species
S_{staff}	Salary of Staff
TAG	Triacylglyceride
tC	tonnes carbon
TCC	Total Capital Cost
TDC	Total Direct CapEx
TDO	Total Direct OpEx
TEA	Techno-Economic Assessment
TIC	Total Indirect CapEx
TIO	Total Indirect OpEx
t_o	time in year = 0
TOC	Total Organic Carbon
TP	Treatment Plant
TRL	Technology Readiness Level
TWh	terra wat hours
t_x	Any given time
USD	US Dollar
v/v	volume per volume
vvm	Volume per Volume per Minute
W%	% by weight
w/v	Weight per Volume
w/w	Weight per Weight
WW	Wastewater
X	Time point being considered

1. Introduction

1.1. Waste: A Global Problem

Waste is a natural product of social and economic development, population growth and urbanisation (Hoornweg et al., 2013; Kaza et al., 2018). If not managed and treated effectively, waste can cause significant harm to both our health and the planet. Unabated greenhouse gas (GHG) emissions are causing the climate to change, resulting in extreme and unpredictable weather and the spread of tropical diseases (Neira, 2014). Solid waste, which is dumped, causes eutrophication of water systems and the spread of disease. Incineration of certain waste can also lead to toxins and particulates causing respiratory and neurological diseases (Kaza et al., 2018). While recycling and green energy are now being utilised, waste numbers are still rising, and the technologies used to stop their production alongside those which reduce wastes harm are required even more urgently.

1.2. Greenhouse Gases: CO_2

GHGs are required to make our planet a habitable temperature; however, their concentrations in the atmosphere are increasing, raising concerns over global warming and climate change. Each gas has a different effect on the climate, related to three factors:

1) The volume of gas present in the atmosphere,
2) Length of time the gas remains in the atmosphere, and
3) The strength with which the gas affects the atmosphere (the global warming potential (GWP)).

Methane and nitrous oxide both have much higher GWP values than CO_2 (21 and 310 at the 100 year timeline, compared to 1 for CO_2 (IPCC, 1996)), however both are present at much lower concentrations and remain in the atmosphere for a fraction of the time that CO_2 does.

CO_2 is an important GHG not only due to its longevity in the atmosphere but also the volume being added annually by both natural and human activities (Figure 1.1). The atmospheric CO_2 concentration fluctuates naturally; however, human activity is adding more and more CO_2 causing a net increase.

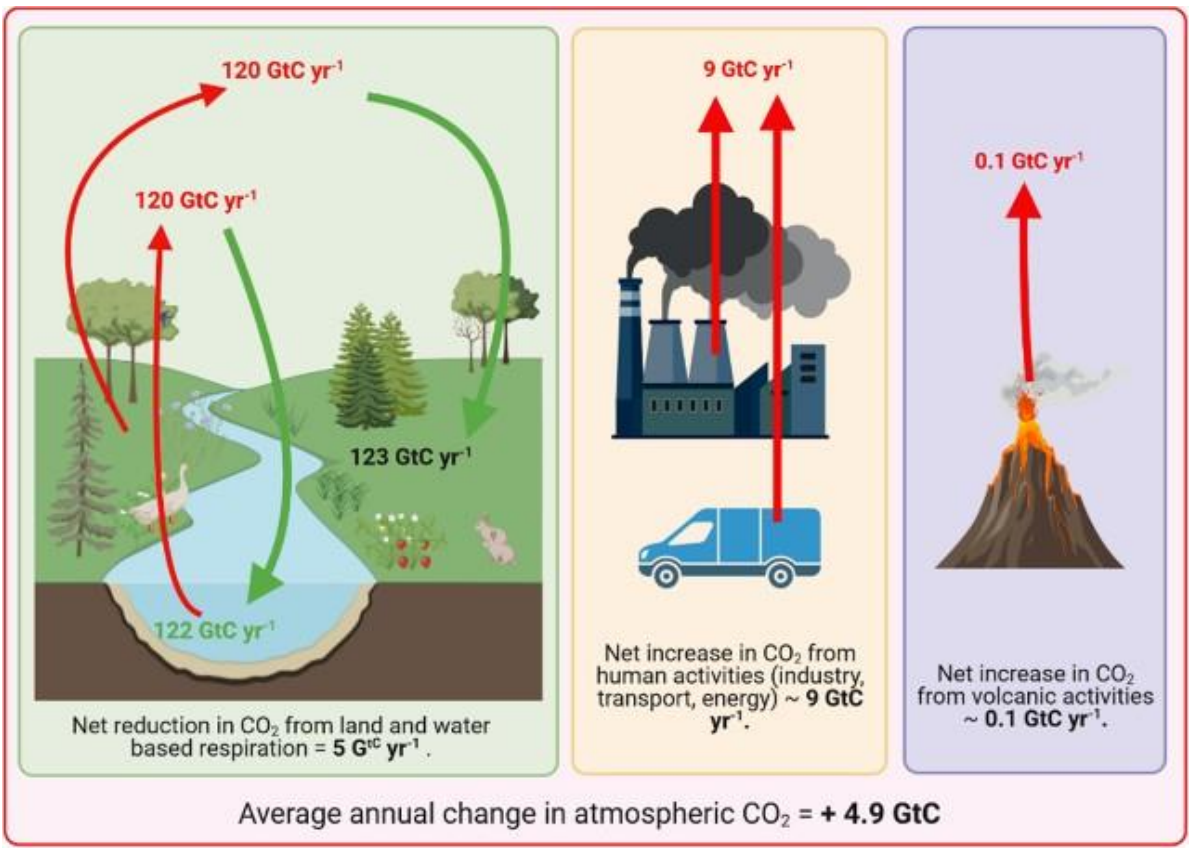

Figure 1.1: *The natural CO_2 cycle and how anthropogenic emissions are affecting the carbon balance. Based on Farrelly et al. (2013).*

Prior to the industrial revolution, CO_2 emissions were very low. Growth in emissions did not begin to occur until the mid-20th Century. In 1950 it is estimated that globally, 5 billion tonnes of CO_2 were emitted, roughly the same as the United States emits today. In 1990, we had reached 22 billion tonnes CO_2 annually and in 2019 it was 36.44 (Figure 1.2) (Buis, 2019).

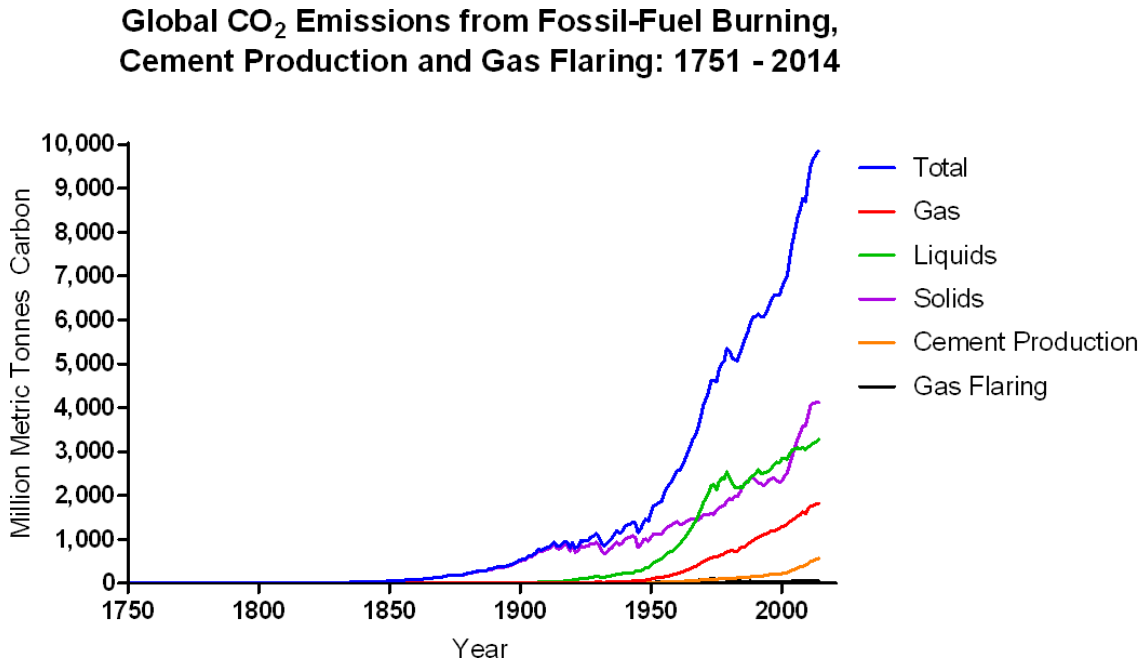

Figure 1.2: *Annual CO_2 emissions globally between 1750 and 2014. Data from* (Ritchie and Roser, 2019).

1.3. Solid Wastes: Landfill

In 2012 it was estimated that 1.3 billion tonnes of municipal solid waste (MSW) was produced annually (Hoornweg and Bhada-Tata, 2012), in 2016, 2.01 billion tonnes were produced and it is expected that without intervention, 3.4 billion tonnes will be the annual production by 2050 (Kaza et al., 2018)(Figure 1.3).

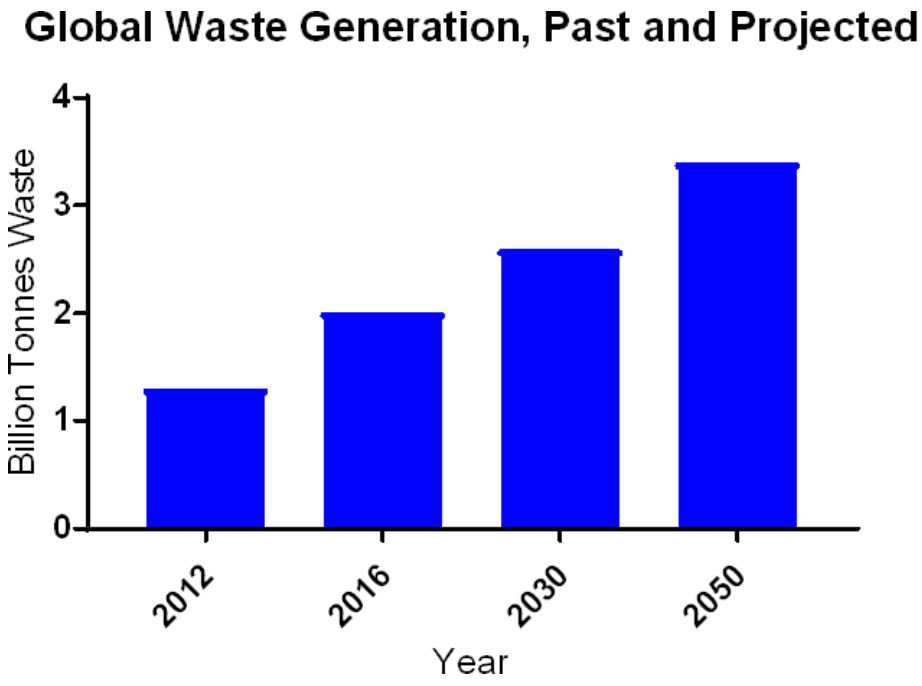

Figure 1.3: *MSW production 2012 & 2016 and projected waste production 2030 - 2050. Based on (Kaza et al., 2018).*

Around 37 % of all solid waste is disposed of in some form of landfill and a further 33 % in open dumps (Kaza et al., 2018). Landfilling and thermal treatment (incineration) are the disposal method of choice in high-income countries, with medium- and low-income countries relying on open dumps (Kaza et al., 2018). In Europe and Central Asia 25.9% of waste is disposed in landfill, presenting a huge concern for the environment. Not only does landfilling require land availability, which is often fought against by neighbouring residents (Hoornweg et al., 2013), but if not managed properly can lead to dramatic environmental consequences. Waste disposed of in this nature produces a toxic liquid runoff – leachate. This liquid often contains high concentrations of organic waste and nutrients (nitrogen and phosphorus) causing contamination of groundwater, soils and rivers and eutrophication of aquatic habitats (Hoornweg and Bhada-Tata, 2012; Kaza et al., 2018; The World Bank, 2016) (Figure 1.4).

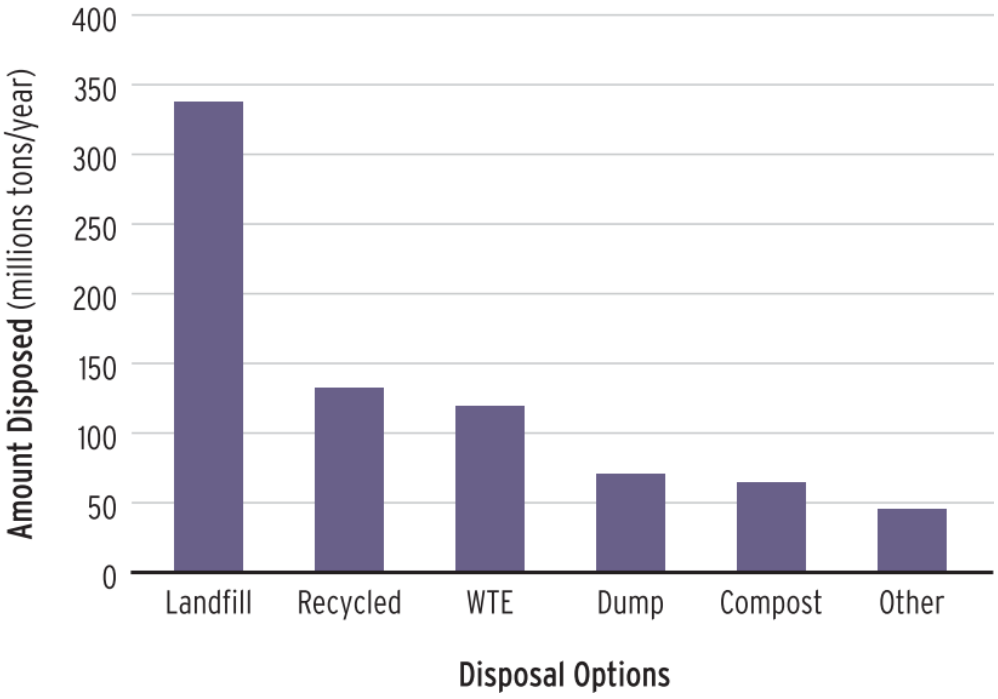

Figure 1.4: *Solid waste disposal methods used across the globe in 2012. Chart from* (Hoornweg and Bhada-Tata, 2012).

1.4. Changing a Throwaway Lifestyle

Developed nations have thrived for decades on a linear economy. We produce something, use it, and then throw it away. Finite sources such as fresh water, land and fossil fuels are often used in the production phase and, as the name suggests, these will eventually run out. A growing population and continued affluence mean these finite resources are being used at increasing rates and they are depleting rapidly.

Momentum is growing behind the switch to a circular economy, particularly in Europe (Kaza et al., 2018) with the new circular economy package (DEFRA, 2016) and the circular economy action plan, part of the European Green Deal (EU, 2015). Alongside tackling MSW, national and international treaties and agreements have been put in place to tackle gaseous emissions. Most notably the Paris agreement (Republique Francaise, 2017), a global initiative to prevent

temperatures rising by more than 2 °C of pre-industrial levels through the reduction in CO_2 and other GHG emissions.

While uptake of a circular economy and reduction in CO_2 emissions will reduce waste production overall, the waste which has already been thrown away or which cannot be recycled needs tacking in an environmentally conscious and economical manner. This includes solid, liquid, and gaseous wastes. Landfills, which have already been filled, will continue to produce gaseous and liquid by-products. Certain industrial manufacturers will also not be able to move away from fossil fuels and so CO_2 emissions from these sources will still require treatment.

1.5. Treating What is Left

There have been vast improvements in recycling technology over the last two decades, which now means hundreds of everyday household items can be recycled and repurposed. While many of these processes are still inefficient and require optimisation, recycling of paper, glass and plastic is readily deployed across the world and contributing to the reduction of rubbish that enters landfills.

There are, however, several waste streams, which are much more complex and difficult to treat. One example would be azo and anthraquinone dyes used in clothing production, which can pollute water streams and are incredibly hard to degrade naturally due to their reinforced structures (Routoula and Patwardhan, 2020).

There are two main waste streams, which will be the focus of this thesis:

- GHG emissions, particularly carbon dioxide emissions, from large point sources such as coal and gas power plants,
- Leachate, primarily from landfill but also from other processes and sources.

Both waste streams have detrimental effects on the environment, current treatment technologies that have a myriad of issues, and have the potential to aid microalgal growth and be remediated in the process, all of which will be discussed in detail in the literature review (Chapter 2).

1.6. Microalgae for Treatment and a Circular Bioeconomy

Microalgae, unicellular photoautotrophs, are the base of most ecosystems across the planet and are responsible for half of global primary productivity (measured as carbon) (Raven and Giordano, 2014). These highly productive cells have a higher oxygen production and CO_2 fixation rate than terrestrial plants and are therefore of high interest for a circular, bio-based, economy. The ability to grow in wastewater and with elevated CO_2 concentrations combined with the production of biomass, which can be used across a variety of industries makes microalgal cultivation a key research topic for sustainable waste treatment and bio-products.

1.7. Research Aims and Objectives

The use of microalgae for the bioremediation of wastewaters and flue gas CO_2 has been proposed numerous times within the literature (Arbib et al., 2013; Bolatkhan et al., 2020; Chang et al., 2020; Cohen et al., 2020; Cuellar-Bermudez et al., 2017; Eze et al., 2018; Gentili, 2014; Ji et al., 2015; Jiang et al., 2011; Mousavi et al., 2018a; Nair et al., 2019; Nayak et al.,

2016; Neves et al., 2018; Shen et al., 2015; Sydney et al., 2021; Yadav et al., 2021), however there is sparse information on carbon capture efficiencies of microalgal species and little to no analyses of how these systems fair economically when considering the major outputs as the treatment of waste and the production of algal biomass.

Therefore, the research presented within this thesis aims to show how microalgae can remediate complex wastewaters and concentrated CO_2 gases using lab scaled experimentation. The experimental data is then used to conduct techno-economic analysis of the process at scale to determine the cost of treatment and cost of algae production under a variety of scenarios. Key parameters are altered to highlight the sensitivity of cost with the aim of focusing future research and optimisation. The project was divided into the following sections:

- Comprehensive literature review surrounding algal cultivation, bioremediation, and economic analyses (Chapter 2).
- The experimental methodologies used to assess algal CO_2 and leachate bioremediation at laboratory scale (Chapter 3).
- The techno-economic methodologies used and how sensitivity analyses were conducted to highlight cost sensitive parameters (Chapter 4).
- Results: How *Chlorella vulgaris* grow under different CO_2 elevations, the real-time CO_2 removal by the cultures, and an economic assessment of the process at pilot scale. This work has been accepted for publication in the Journal of CO_2 Utilisation, pending revisions. (Chapter 5).
- Results: A techno-economic assessment of leachate bioremediation using an adapted consortium at pilot scale (300 L). This work has been published in the Royal Society of Chemistry Journal: Water Research and Technology (Chapter 6).
- Results: Can an adapted algal-bacterial consortium treat two waste streams simultaneously, are there effects on remediation efficiency. (Chapter 7).
- Conclusions and future research (Chapter 8).

2. Literature Review

2.1. Introduction

The aim of this literature review is to describe the issues associated with waste generation and treatment and how microalgae can be utilised to remediate difficult to treat waste streams individually and in combination. The problem of waste generation and the environmental and economic burdens associated with a linear economy were described in Chapter 1. Following on from the introduction, two waste streams, which are the focus of this thesis, will be described in detail: 1) carbon dioxide emissions and 2) landfill leachates. Both waste streams will be described in turn including why their remediation is important and the current technologies used for this along with the advantages and disadvantages associated with each technology. Microalgae will then be introduced as an alternative method of treatment for both waste streams. An in-depth explanation of what microalgae are, their advantages, disadvantages and usage including products gained and remediation potential will be described. This is then followed by an examination of how microalgae have been used previously to treat each of the two key wastes and highlight any gaps in the existing body of knowledge. Penultimately, the use of microalgae to treat both waste streams simultaneously will be described and highlights made where there are gaps in the body of knowledge. Finally, the following chapters and how they aim to address the key gaps identified will be described.

2.2. CO_2 Emission Abatement

There is a wide range of technologies that can be used for carbon abatement, ranging from capture and storage of the purified gas to reforestation allowing an increase in biological uptake from the atmosphere. Van Vuuren (2007) gives an in-depth account of different possible mitigation scenarios available and is referred to for further reading (Van Vuuren *et al.*, 2007).

Ocean fertilisation is a bio-geological strategy where limiting nutrients within the top layer of the ocean are artificially supplemented to improve the photosynthetic productivity of marine organisms (Herzog and Golomb, 2004; Singh and Ahluwalia, 2013; Yang et al., 2008). Increasing the photosynthetic productivity of the environment will not only increase the volume of CO_2 captured by the upper ocean but will also increase food availability for marine animals and fish. The CO_2 is assimilated into the photosynthetic organism and then either consumed by higher organisms or will fall to the bottom of the ocean where it will be sequestered long-term. Iron, an important micronutrient for chlorophyll production (Boyd *et al.*, 2000) has been explored here and advocates for this strategy have claimed cost-effectiveness (USD 1-10 per tonne of carbon) although the risk of adversely affecting the environmental ecology remains high (Herzog and Golomb, 2004).

Terrestrial plants and soils are a net carbon sink of ~ 3 GtC yr^{-1} (Farrelly et al., 2013), with forests considered as long-term sinks due to the extended life-span of trees compared to other plants. The destruction of forests not only reduces the photosynthetic capture rate by reducing plant numbers but actively emits CO_2 through the burning of the carbon rich biomass (Watanabe et al., 1992). Stopping the current deforestation and replanting (reforestation) is a simple solution to this source of greenhouse gas (GHG) emissions. However, this is often

fought against due to the continually increasing need for agricultural land for food production (Garnett, 2009). Research into improving agricultural efficiency goes hand-in-hand with CO_2 and GHG abatement. A variety of options exist for GHG emission reduction from agricultural practices including improving productivity and efficiency and reducing livestock numbers, despite the projected increase in meat and dairy demand over the coming decades (Garnett, 2009; Goodland, 1997).

Switching from fossil fuel derived energy to renewables is probably the most publicly well-known emissions reduction strategy. Mature technologies such as photovoltaics (PV), wind and hydroelectric together contributed to 30 % of the UK's electricity production in the third quarter of 2017, an increase of nearly 5 % over the previous year (National Statistics UK, 2017a). These technologies are well researched and will be major players in the movement towards a carbon neutral society (Lovelace and Temple, 2012). In 2016 the UK produced 79.3 TWh of electricity through renewable technologies (National Statistics UK, 2017b); using the estimated carbon emissions (g C^{-1} KWh^{-1}) figures from Demirbas *et al.* (2007) this is equal to between 7,930 - 18,239 tonnes-Carbon avoided against the same amount of electricity produced from pulverised coal (Demirbas, 2007).

This being said, the move from fossil fuel to renewable is a transition and will take time. The energy system requires inertia produced from the fossil fuel generators to maintain stability and a backup of reliable fossil fuel power is required for when the sun doesn't shine, and the wind doesn't whistle (Duffy et al., 2019).

Carbon capture and storage (CCS) describes a group of technologies with the common goal of reducing CO_2 emissions from fossil fuel utilising industries (Lovelace and Temple, 2012; Pires et al., 2011), such as electricity generation and cement production. There are three phases to

CCS: CO_2 capture from large emitters, transportation to a storage site and long-term storage to prevent its release into the atmosphere. The aim of this is the immediate reduction in emissions without dramatic changes to societal structure. These technologies can be retrofitted onto existing point sources of CO_2, reducing the atmospheric emissions while still allowing the use of fossil fuels. This bridges the move from fossil fuel dependency to sustainable renewable energy production (Haszeldine, 2009) and offers long term solutions for fossil-fuel reliant technologies such as the steel and cement industries.

2.2.1. Carbon Capture and Storage (CCS)

The strategies used for the capture phase of CCS are similar to those used for other pollutants such as sulphurous and nitrous oxides, So_x, and NO_x, respectively. The major difference, however, is the volume of gas to be removed; flue gases contain 15-25 % CO_2 (Herzog, 2001) compared to part per million (ppm) concentrations of NO_x and SO_x. The technologies surrounding CO_2 capture can be split into three broad categories; pre-combustion, post-combustion, and oxy-fuel (Cuellar-Bermudez et al., 2015). Each tackles the carbon content at a different stage of fossil fuel utilisation and post-combustion monoethanolamine (MEA) CO_2 stripping is currently the most popular choice (Luis, 2016; Vaidya and Kenig, 2007).

2.2.1.1. Capture Methods: Pre-Combustion

Pre-combustion capture can be defined by the reacting of steam or oxygen with a primary fuel to give a synthesis gas made of carbon monoxide and hydrogen. The CO is then separated from the H_2 and is converted to CO_2 through a water-gas shift:

$$CO + H_2O \leftrightarrow CO_2 + H_2 \qquad 2.1$$

The hydrogen produced is then used for energy production while the CO_2 is in a pure stream ready for compression and storage (Figure 2.1) (Cuellar-Bermudez et al., 2015; Jansen et al.,

2015). The process is the same for coal, oil, and natural gas, however, more purification stages are required for coal and oil to allow for the removal of ash, sulphur, and other impurities. Theenergy penalty is higher for coal than natural gas due to the higher carbon content in the syn-gas derived from coal (Jansen *et al.*, 2015). Gasification (when considering solid or liquid fuels) and partial oxidation (for gases) have been around for over 90 years now and both are mature technology with the first integrated gasification combined cycle (IGCC) plant integrated with CCS being built in Mississippi in 2015 (Jansen *et al.*, 2015).

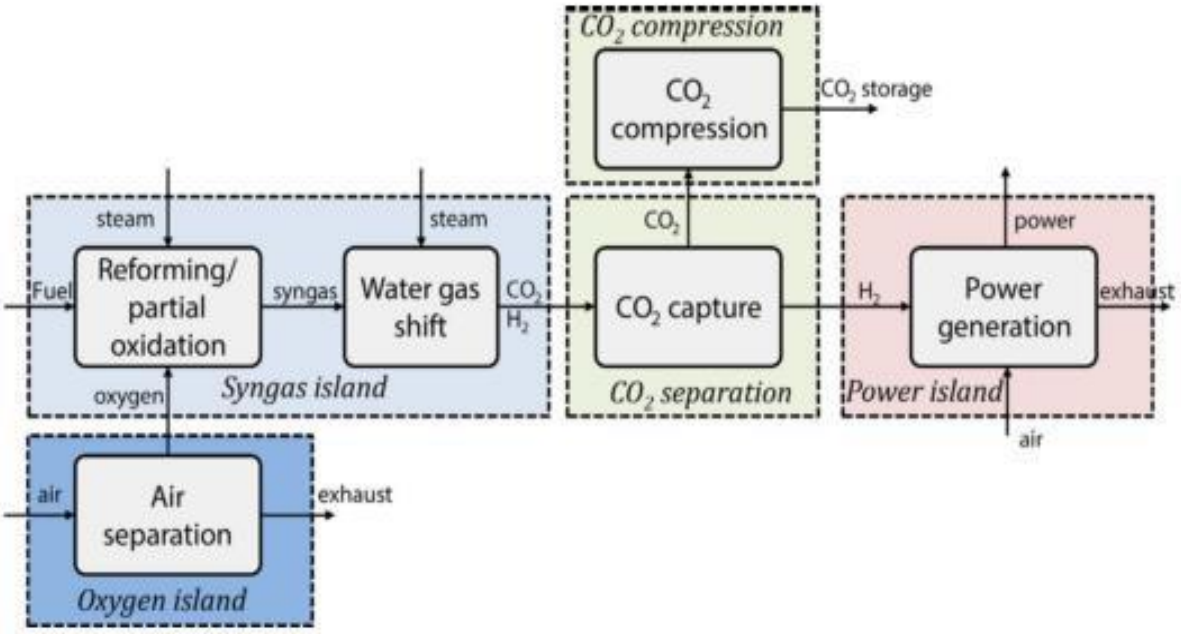

***Figure 2.1**:Schematic of pre-combustion carbon capture for power generation*

2.2.1.2. Oxy-Fuel and Chemical-Looping Combustion

While in gasification and partial oxidation the carbon is removed prior to combustion, oxy-fuel combustion directly burns the fossil fuels in pure oxygen to give a flue gas consisting of predominantly CO_2 and H_2O (Figure 2.2). While this offers the advantage of easy CO_2 capture from a relatively pure flue gas, this technology comes with an energy penalty, associated with the air-separation unit required for O_2 production. Cuellar-Bermudez *et al.* (2015) estimated

that the air-separation unit could consume up to 15 % of the output from the power plant. This being said, the unused by-products from the air separation; nitrogen, argon *etc.* are all marketable and can improve the overall economics (Cuellar-Bermudez *et al.*, 2015). To avoid the potentially high flame temperature caused by burning in pure oxygen, the gas stream is mixed with recycled flue gas (Boot-Handford *et al.*, 2014).

The idea of oxy-fuel combustion was proposed in the early 1980s with the intent of producing highly pure CO_2 for enhanced oil recovery (EOR). The technology is currently at the demonstration phase with commercial concepts expected by 2020 (Boot-Handford *et al.*, 2014). Applications within the UK are, however, limited due to the rapid phase out of coal currently being undertaken, with all unabated coal power stations being closed by 2025 (BEIS, 2018).

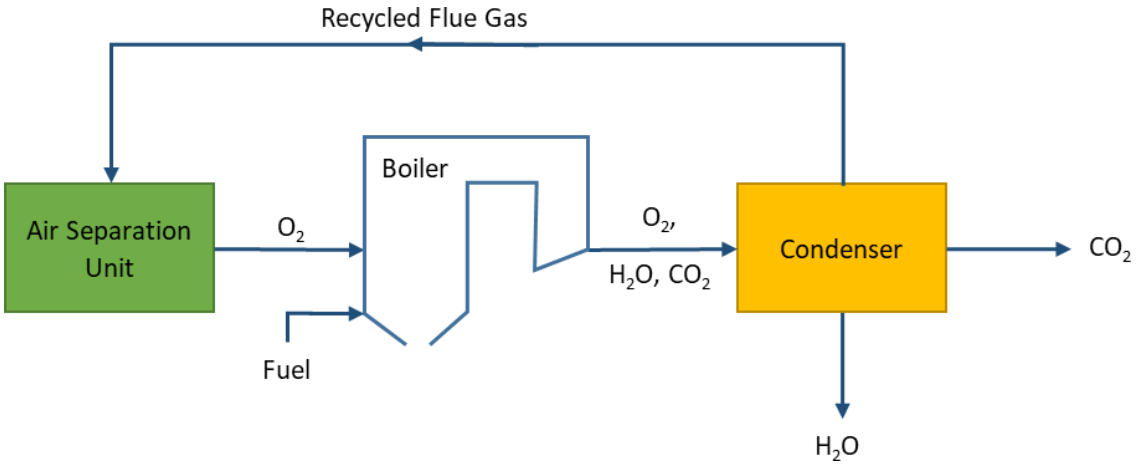

Figure 2.2: *Schematic for oxy-fuel combustion where fuels are combusted in a mixture of O_2 and recycled flue gas to create a flue-gas consisting of CO_2 and H_2O which can be later condensed off.*

Chemical-looping combustion is another technology that aims to produce a pure stream of CO_2 as the flue gas. This technique emerged for CCS as the separation of CO_2 from other pollutants is intrinsic, thus no energy is expended (Boot-Handford et al., 2014; Yang et al.,

2008). Two reactors are required: one air reactor and one fuel (Figure 2.3). The fuel is combusted in the fuel reactor in the presence of a metal oxide (Me_xO_y), resulting in the reduction of the metal oxide and production of CO_2 and H_2O. The reduced metal oxide is transferred to the air reactor where oxygen present within the air regenerates the metal oxide:

$$Me_xO_{y-1}^{-} + \frac{1}{2}O_2 \rightarrow Me_xO_y \qquad 2.2$$

Although the capture of carbon in chemical-looping is intrinsic, the use of this methodology with gaseous fuels would limit the efficiency to the point where the underlying thermodynamics are reduced to that of a steam cycle rather than combined cycle (Boot-Handford *et al.*, 2014).

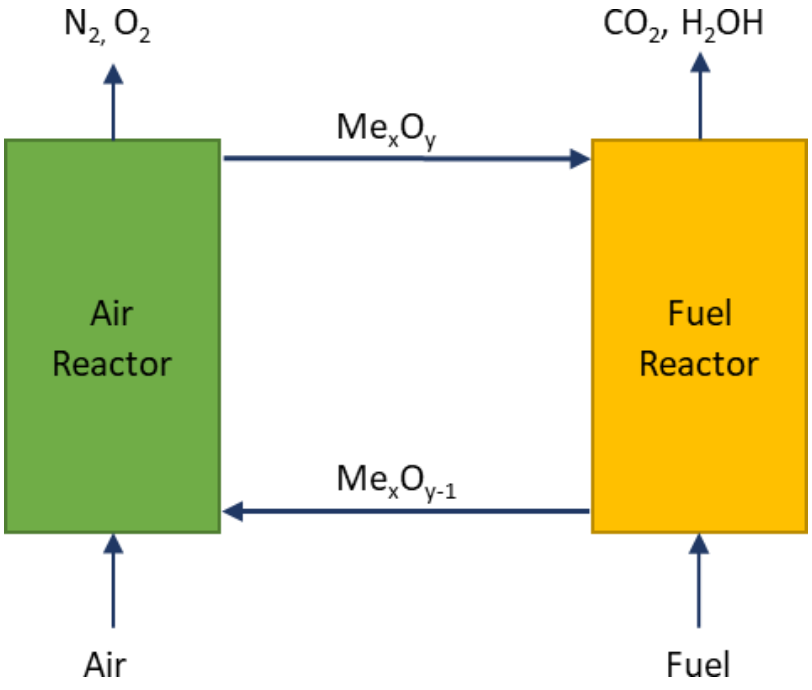

Figure 2.3: *An example of chemical looping combustion for carbon capture and storage using a metal oxide.*

2.2.1.3. Post-Combustion Capture

Post-combustion capture involves the capture of carbon dioxide from flue gases after the fuel has been used for energy production. There are many ways of stripping CO_2 from flue gas including the use of liquid solvents, carbonate, or membrane filters (Vaidya and Kenig, 2007).

Solvent Extraction – Amine Scrubbing

The capture of carbon dioxide from flue gases using amine-based solvents, such as MEA, is the only mature CCS technology used for large-scale post combustion CCS, despite the large equipment size and energy cost associated (de Queiroz Fernandes Araújo et al., 2015; Stewart and Hessami, 2005). The 'lean' solvent is contacted with the flue gas and strips the CO_2 from the gas through zwitterionic interactions:

$$CO_2 + 2R_1R_2NH \leftrightarrow [R_1R_2NCO_2^-] + R_1R_2NH_2^+ \quad 2.3$$

The *'rich*' solvent is then regenerated by heating to 100-150 °C; releasing the CO_2 as an almost pure stream) (Figure 2.4) (Lam et al., 2012).

$$R_1R_2NCO_2^- + H_2O \leftrightarrow R_1R_2NH + HCO_3^- \quad 2.4$$

$$CO_2 + OH^- \leftrightarrow HCO_3^- \quad 2.5$$

MEA is the most popular absorbent because of its high reactivity with CO_2 however the loading capacity of this primary alkanolamine is only 0.5 mol CO_2 per mol amine (Vaidya and Kenig, 2007). Sterically hindered amines have a higher loading capacity of 1 mol CO_2 per mol amine, due to the lower carbamate intermediate stability. There is, however, added cost to these altered chemicals (Vaidya and Kenig, 2007).

The key issue faced with the use of MEA is the high energy requirement for the solvent regeneration (Boot-Handford et al., 2014; Lam et al., 2012; MacDowell et al., 2010; de Queiroz Fernandes Araújo et al., 2015; Stewart and Hessami, 2005; Vaidya and Kenig, 2007; Zheng et

al., 2017). Assuming that the energy used for heating the rich solvent is gained through fossil fuel consumption, an estimated 352 kg CO_2 will be emitted for every tonne captured in the solution (65 % efficiency) (Lam *et al.*, 2012).

Other issues surrounding the use of MEA include the corrosive and reactive behaviour of the compound. MEA can be irreversibly degraded to a salt and precipitated out through reaction with O_2, NO_x and SO_x, all found in typical flue gases (Lam et al., 2012; Zheng et al., 2017). The loss of solvent and corrosion of the machinery, caused by the MEA, increase the capture costs of the process making it less economically favourable (Stewart and Hessami, 2005).

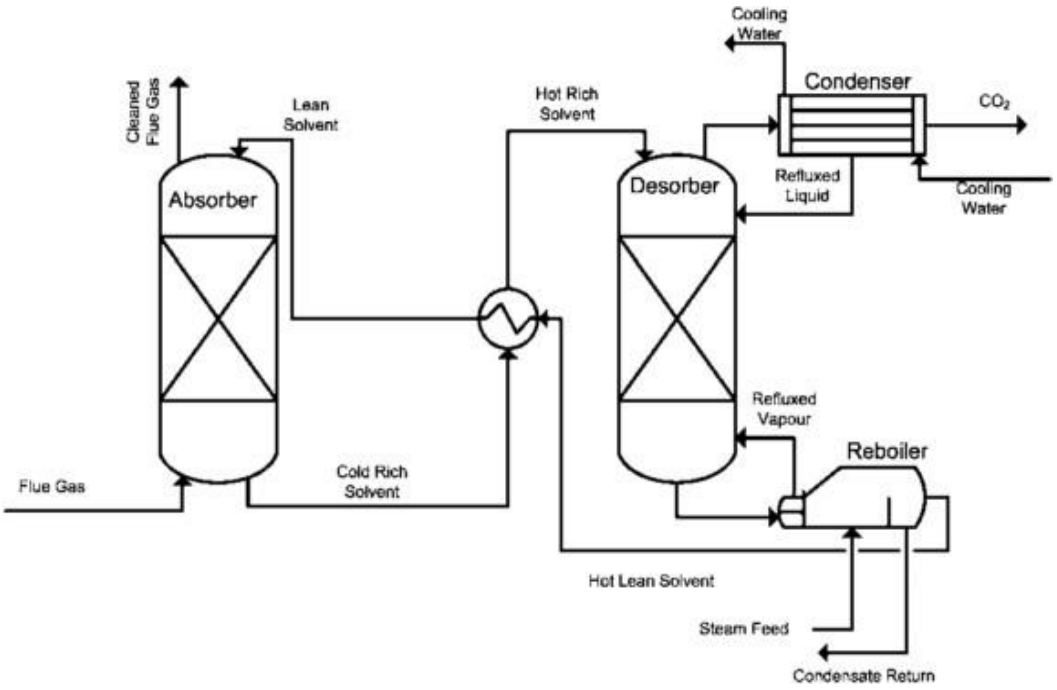

Figure 2.4: *Schematic for an amine based chemical absorption process for CO_2 capture. (MacDowell et al., 2010)*

Other secondary, tertiary and sterically hindered amines are also available for CCS (Table 2.1). MEA remains the preferred compound, due to the high reactivity with carbon dioxide and the low price compared to designer amines (Luis, 2016; Vaidya and Kenig, 2007).

Table 2.1: *Different amines available for carbon capture*

Compound	Structure	Amine type	Reference
DEA (diethanolamine)	$HN(CH_2CH_2OH)_2$	Secondary	Vaidya and Kenig, 2007
MDEA (methyl-di-ethanolamine)	$CH_3N(C_2H_4OH)_2$	Tertiary	Jansen *et al.*, 2015
AMP (2-amino-2-methyl-1-propanol)	$C_4H_{11}NO$	Sterically hindered	Vaidya and Kenig, 2007

Ionic Liquids

The use of ionic liquids (ILs), as an alternative to amines, has gained traction over the last decade (MacDowell et al., 2010; Wappel et al., 2010). The organic cation and inorganic anions (Wappel *et al.*, 2010) can be independently manipulated resulting in an estimated 10^{18} possible variations at room temperature (Wappel *et al.*, 2010). Negligible vapour-pressure, high thermal stability, and low melting temperatures (below 100 °C) (Luis, 2016; MacDowell *et al.*, 2010; Maggin, 2005; Wappel *et al.*, 2010) all give ILs an advantage over the amines traditionally used for CCS. Wappel *et al.* (2010) deduced that a 60 w% ionic liquid had an energy demand slightly lower than a 30 w% MEA reference, leading to a potential saving of 12-16 % in operational costs. The extremely low vapour pressure of ILs gives them the label of a 'green alternative' as there is little chance of the solvent being released into the atmosphere at ambient or higher temperatures (Maggin, 2005). All of this considered, the initial investment into ILs is much higher than primary amines and manipulation of the properties increases this cost further.

Calcium Looping

Calcium looping takes use of the reversible gas-solid reaction between calcium oxide (CaO) and CO_2, shown in Equation 2.6 and Figure 2.5. Calcium looping offers a lower energy penalty

(6–8 %) compared to other capture processes when applied to power-generation due to the recuperation of heat for steam generation from the exothermic carbonation reaction (Florin and Fennell, 2011). Calcium looping has a relatively low scale-up risk due to the use of mature fluidized bed technologies and its synergy with the cement industry (Florin and Fennell, 2011).

$$CaO_{(s)} + CO_{2\,(g)} \leftrightarrow CaCO_{3\,(s)} \qquad 2.6$$

CaO derived from natural limestone, although cheap to source, loses its capacity for carbon capture after multiple carbonation/calcination cycles due to attrition, sintering and chemical deactivation by SO_2 present in the flue gas (Blamey et al., 2010; Florin and Fennell, 2011; MacDowell et al., 2010). Blamey *et al.* (2010) show that natural Havelock limestone CO_2 carrying capacity can be reduced by 50 % or more over just 15 cycles. There are modified sorbents available, which have increased resistance to this loss of capacity, however, all are economically constrained due to the abundance of cheap limestone, making replacement a cheaper alternative.

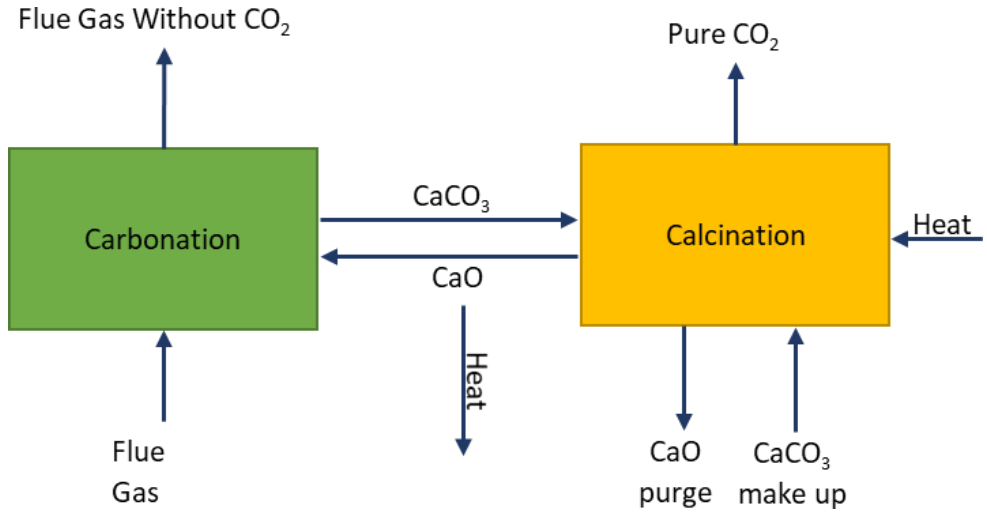

Figure 2.5: *A simple schematic for calcium carbonate looping technologies used for CCS.*

2.2.1.4. Storage

The CO_2 then requires storage and considered sites include geological formations such as saline aquifers and depleted or uneconomical oil fields (Herzog and Golomb, 2004). It is recognised that most of the chosen storage sites will leak small amounts of CO_2 back into the atmosphere over time (Stewart and Hessami, 2005). The ocean is an incredibly large carbon sink, (estimated ~ 40,000 GtC) however the retention time of the CO_2 and the potential long-term effects of localised pH changes on marine ecology have raised concerns. Geological injection into abandoned coal seams and for EOR not only offer longer retention times for the CO_2 (Herzog, 2001) but also the option of a value added process. The porous coal in unused seams can physically absorb the CO_2 making storage pseudo-permanent while the injection also aids the extraction of methane (Stewart and Hessami, 2005).

2.2.1.5. Carbon Capture and Utilisation or Storage (CCUS)

The capture of carbon dioxide is required in order to reduce anthropogenic emissions contributing to global warming, however long-term storage of the product is cost-inefficient unless policy surrounding carbon taxation is improved. Storage of the pure gas means there is no option for the creation of profit; only expense from the processes and the long-term surveillance of the storage site that will be required. One option for increasing the uptake of carbon capture technologies is the utilisation of the pure CO_2 to create products or fuels that can be sold on, carbon capture and utilisation or storage (CCUS).

The market for CO_2 utilisation is, however, currently too small. EOR and the food and beverage industry both utilise pure CO_2, however, the amount of gas required only accounts for 2 % of the CO_2 currently generated by power plants in the USA (Herzog, 2001; Kadam, 2002). Alongside this, many of the applications within these industries, such as the

carbonation of fizzy drinks, lead to ~ 80 % of the CO_2 being released into the atmosphere – it is just a delayed process (Kadam, 2002). The low usage of CO_2 by these industries means, it is unlikely they can resolve the issue. Other options of use for the CO_2 include the supplementation of photosynthetic growth and use in fine chemical synthesis. The issue with the latter being the relative inertness of CO_2, making reacting to produce polymers and value added compounds difficult (MacDowell *et al.*, 2010). Over the past decade, there has been amplified interest in using this increased access to pure CO_2 gases to grow highly productive photosynthetic organisms, algae, in order to produce renewable biodiesel and other high value bio-products.

Beal *et al.* (2017) offer ABECCS (Algae with Bioenergy Carbon Capture and Storage) as a potential for negative carbon emissions. Eucalyptus is used as a biomass fuel and the CO_2 is captured through an amine-based system. A portion of the pure CO_2 is then used to aid algal growth while the rest is sequestered. The financial and lifecycle assessments conducted highlighted two different breakeven points based on the algal biomass being sold for a high value (fishmeal replacement) or low value (soymeal replacement) product alongside carbon credits of $68/t and $278/t, respectively (Beal et al., 2018).

2.3. Landfill Leachate Treatment

Leachate is the toxic liquid run off from landfill and dumpsites (Renou et al., 2008). This liquid is formed from the percolation of rainwater through the layers of landfill and the biodegradation of waste within the landfill itself. Figure 2.6 shows how leachate is formed and how, if not collected for treatment, it can contaminate groundwater (Palmer et al., 2021; Qrenawi, 2006).

Leachate is a major environmental concern due to its high chemical oxygen demand (COD), ammoniacal-nitrogen (NH_3-N) and heavy metal content (Ozturk et al., 2003; Pacheco et al., 2015). If released to the environment, nutrients (nitrogen and phosphorous) can cause eutrophication of water sources, causing a loss to local biodiversity (Eze et al., 2018). Furthermore, heavy metals within the effluent, such as arsenic and mercury, can bioaccumulate within the ecosystems, affecting flora, fauna, and human health (Pacheco et al., 2015; Suresh Kumar et al., 2015).

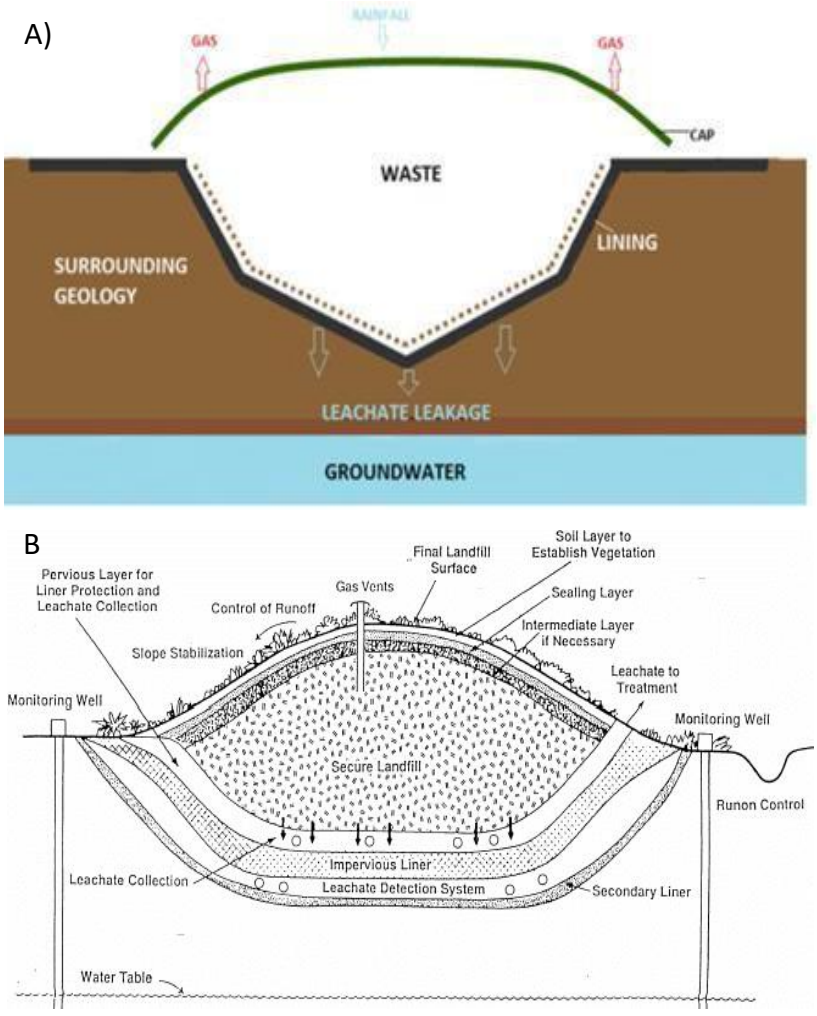

Figure 2.6: *Landfill cross-sections showing how leachate can contaminate groundwater (A, (Palmer et al., 2021)) if not collected for treatment (B, (Qrenawi, 2006)).*

2.3.1. Issues with Treating Leachate

Landfill leachates (LL) are difficult to treat waste streams because of their varying characteristics and flow rates. The original waste composition, geographical location of the landfill (and therefore weather and temperature patterns) and age of the landfill all contribute to the changing composition of leachates and their flow rate (Chang et al., 2018; Ho et al., 1974; Paskuliakova et al., 2018a; Renou et al., 2008). This makes it difficult to define a *'typical'* leachate as even samples from the same site will have different characteristics over time (Talalaj et al., 2019). Data presented in Appendix A highlights the wide variety of leachate and wastewater compositions that can be found within the literature.

This variable composition means one treatment method cannot necessarily be used in multiple instances and limits the efficiency of chemical and physical methods.

Another reason LLs are difficult to treat is the continued stringency placed on them. The contaminant levels acceptable before the water can be released to the environment are continually becoming more stringent, meaning a greater efficiency of the process is required and for many of the pollutants, miniscule amounts need to be removed to comply.

2.3.2. Treatment Methods

As evidenced previously, LL can be complex in nature and therefore a variety of different methods have been employed for their treatment (Dogaris et al., 2020). Biological, chemical, and physical methods can be used to treat leachate (Wiszniowski et al., 2006), although there is no 'most appropriate treatment' available (Renou et al., 2008). Current treatment methods come with both advantages and disadvantages meaning they are often used in combination, Table 2.2. The combination is dependent on both the leachate characteristics and the volumes produced. In the following section, a brief description of different techniques will be

described. Figure 2.7 also highlights the key methods used for treating LL (Dogaris et al., 2020).

Table 2.2: *Advantages and disadvantages of different leachate treatment methods used (Renou et al., 2008)*

Treatment	Pros	Cons
Combining effluent with domestic sewage	✓ Easy maintenance ✓ Low operational cost	× Low efficiency due to inhibition by organics/heavy metals
Recycling back through the landfill	✓ Cheap to run ✓ Shortens the stabilisation time of the site	× Inhibition of methanotrophs × High volumes can saturate the landfill causing ponding
Advanced Oxidation Processes (AOP)	✓ Very high efficiency of COD removal ✓ Improves the biodegradability of recalcitrant organic pollutants	× Mainly used in conjunction with other treatments × High energy demand and capital intensity
Air stripping	✓ High NH_3-N removal	× Requires very high pH × Release of gaseous NH_3
Filtration	✓ Eliminates all macromolecules to the filter size	× High expense from filter replacement and pump operation
Reverse osmosis	✓ High recovery rate of various pollutants	× Membrane fouling × Production of an unusable concentrate
Microalgal growth	✓ Can remove a wide range of pollutants at once ✓ Biomass produced can be sold on for future use	× High expense × Low productivity × Requires pre-treatment - Dilution

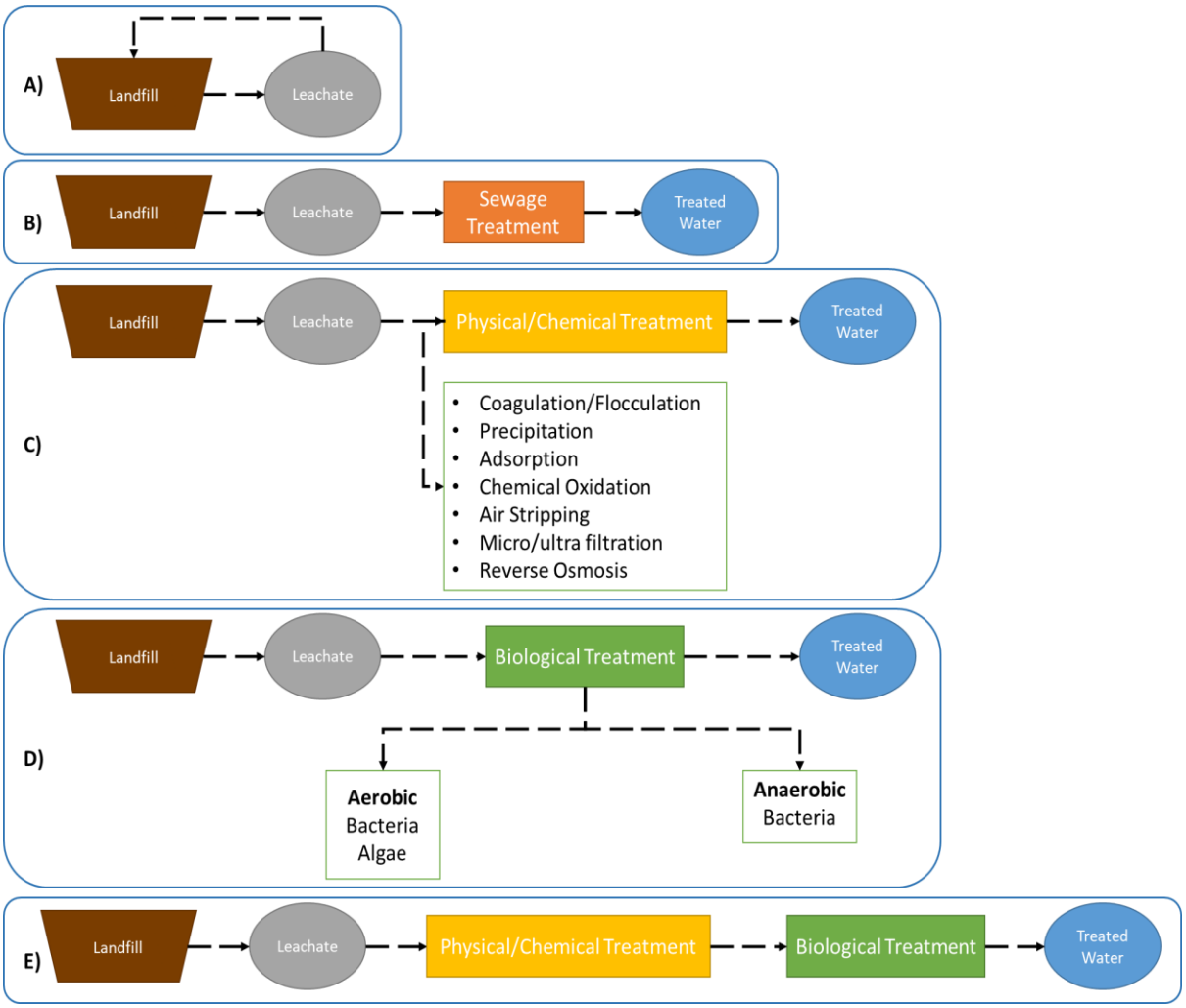

Figure 2.7: *Schematic of different treatment methods for landfill leachate (Dogaris et al., 2020). A) Recirculation of LL back into the site. B) Transfer and co-treatment with sewage. C) physical or chemical methods for treatment. D) Biological treatment methods including both aerobic and anaerobic methods such as activated sludge and sequence reactors. E) Combining of physical/chemical pre-treatments and biological treatment (such as with algae).*

2.3.3. Physical Treatment Methods

Physical treatments do not utilise any additional chemical or biological agents and are a common solution to leachate treatment due to their easy maintenance and low cost (Dogaris et al., 2020; Gao et al., 2014; Renou et al., 2008). Physical methods are often employed to remove the non-biodegradable compounds within the leachates (Gao et al., 2014).

Examples of physical treatment methods include:

- Leachate transfer and recycling
- Sedimentation/flotation and filtering
- Air stripping
- Reverse osmosis and membrane technologies

Transferring landfill leachate for co-treatment with sewage has advantages due to the complimentary nutrients with leachate offering mostly nitrogen and sewage mostly phosphate (Dogaris et al., 2020; Gao et al., 2014). These nutrients complement one another and aid in biological treatment steps. Landfill leachates, however, often contain refractory compounds and heavy metals which inhibit the microbial degradation. Therefore, the ratio of leachate to sewage requires optimisation and studies report that 20 % leachate is the maximum that should be utilised (Cecen and Gursoy, 2000; Dogaris et al., 2019).

Recycling or recirculation of leachate through the landfill is one of the cheapest options (Gao et al., 2014) available and have advantages including lowered COD concentrations in the anaerobic phase (Rodríguez et al., 2004) and shortened stabilisation periods for the site (from decades to 2-3 years (Reinhart et al., 2019)). On the other hand, high recirculation rates can adversely affect how solid wastes are anaerobically digested due to the build-up of organic acids which inhibit methanogenesis. Alongside this, if the volume is too high, saturation and ponding can occur within the body of the landfill.

Sedimentation and flotation methods focus on the large macromolecules which are carried within the liquid and prevent filters used to remove smaller contaminants from becoming blocked easily. These processes are standard primary steps when removing the leachate from the landfill site however, they do not work to treat the chemical composition (COD, nitrogen *etc*.) of the leachate and are mostly utilised in tandem with other treatment processes.

Air stripping is used for the removal of ammoniacal nitrogen. Under high pH conditions up to 89 % of NH_4^+ can be removed within 24 hours (Jiang et al., 2011; Renou et al., 2008). The major concern with air stripping is the release of ammonia into the environment, therefore the gas stream must be treated with H_2SO_4 or HCl (Dogaris et al., 2020; Luo et al., 2020; Renou et al., 2008). Upscaling of this method is also a concern due to the requirement of lime for pH adjustment and large stripping towers (Renou et al., 2008).

Membrane technologies, including reverse osmosis (Figure 2.8), are widely utilised in landfill leachate treatment. Membranes of varying sizes are often used to remove suspended solids, colloidal matter, and microorganisms (Figure 2.9) (Dogaris et al., 2020; Wiszniowski et al., 2006) and predominantly as pre-treatments before other techniques are employed. Reverse osmosis can achieve up to 100 % COD removal (Dogaris et al., 2020; Luo et al., 2020) but is an expensive and energy intensive method requiring downtime for cleaning and maintenance and replacement of expensive membranes due to fouling (Dogaris et al., 2020; Gao et al., 2014). Alongside this, a retentate is produced which is the highly concentrated product of the dewatering which will still require its own treatment or disposal (Luo et al., 2020; Stegmann et al., 2005; Wiszniowski et al., 2006).

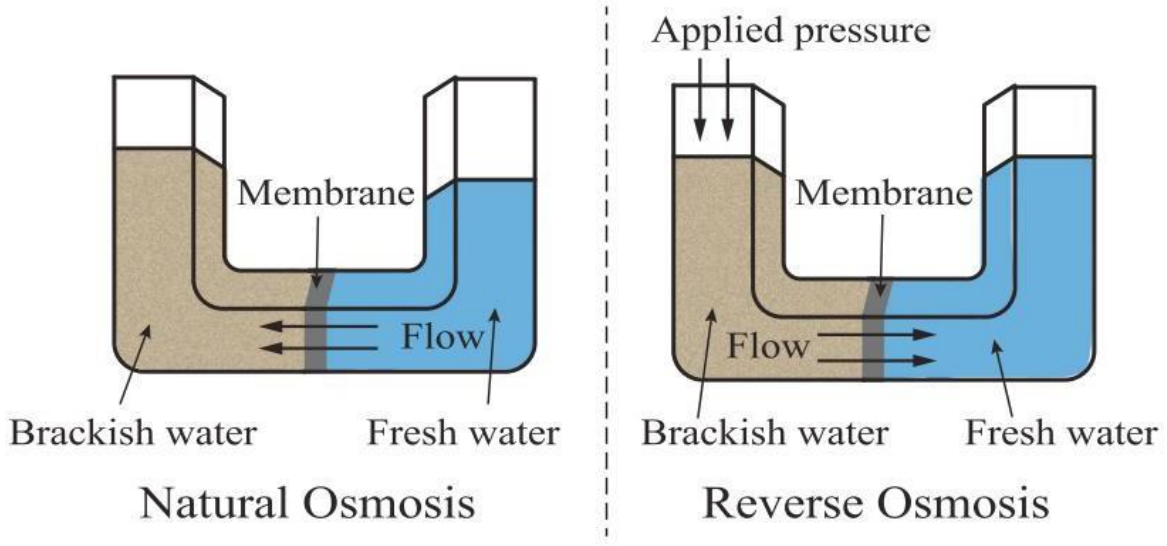

Figure 2.8: *Water migration in natural and reverse osmosis systems for the treatment of brackish water and wastewaters. Image from Duong et al., (2019).*

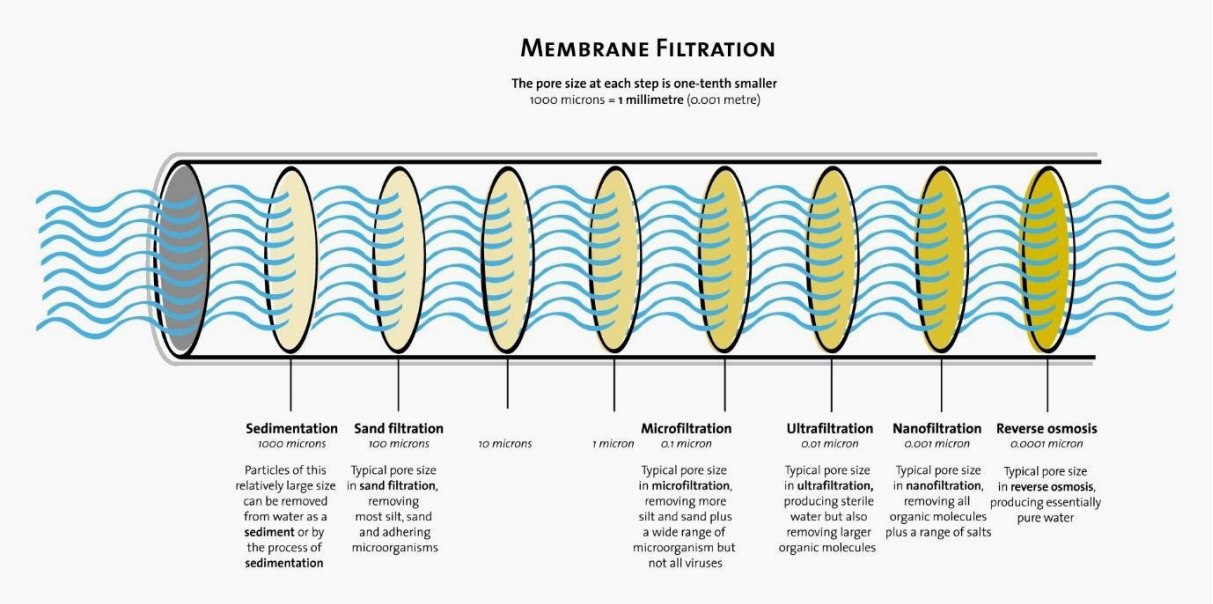

Figure 2.9: *Illustration of different filters and membranes and the pore size associated with each. Image from Kentucky Department of Environmental Protection (2012).*

2.3.4. Chemical Treatment Methods

Chemical treatment methodologies include precipitation using chemical coagulants, adsorption, and oxidation methods. Similarly to physical methods, the main aim is to reduce the non-biodegradable content within the leachate. These methods are more suited for older leachates which have higher loads of refractory compounds and lower concentrations of organic matter (Dogaris et al., 2019).

Precipitation/coagulation/flocculation methods work by adding chemical flocculants, often aluminium or ferrous sulfate (Figure 2.10). As an older treatment method, coagulation/flocculation is a relatively simple and cost-effective process for removing non-settleable colloidal solids including heavy metals, fatty acids, and humic acids (Luo et al., 2020).

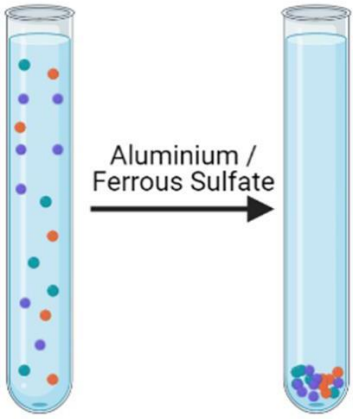

Figure 2.10: *Example of the precipitation of non-settleable colloidal solids using chemical flocculants.*

Iron salts are reportedly more efficient than aluminium, however, increased iron concentration and sludge formation are drawbacks (Abdul Aziz et al., 2004; Amokrane et al., 1997; Dogaris et al., 2019; Gao et al., 2014; Ghafari et al., 2009; Tatsi et al., 2003). Ammonia can be precipitated out in the form of MAP (magnesium ammonium phosphate) with the addition of magnesium chloride and di-sodium phosphate in a molar ratio of 1:1:1 Mg_2^+:

NH_4^+:PO_4^{3-} at pH 8.5-9 (Dogaris et al., 2020; Li et al., 1999). Coagulation or flocculation are often used as a pre-treatment for younger leachates and post-treatment for older, stabilised leachate (Gao et al., 2014).

Compared to other methods, adsorption is widely employed for removing recalcitrant organic compounds and heavy metals (Luo et al., 2020). Activated carbon, in granular and powdered forms, is the most frequently used adsorbents due to the large surface area, controllable pore structure and high removal efficiency of a wide variety of organic and inorganic pollutants (Gao et al., 2014; Luo et al., 2020). The requirement for frequent regeneration of columns and the use of large quantities of activated carbon are major drawbacks to the process (Dogaris et al., 2020).

Advanced oxidation processes (AOP) have been a focus for LL treatment recently (Dogaris et al., 2020). The OH^- radicals produced in AOP are extremely reactive species and disrupt C-X, C-C and C=C bonds (Gao et al., 2014)**.** The oxidised recalcitrant matter has a higher biodegradability and therefore AOP is often applied as a pre-treatment for biological processes. While oxidation allows for the removal of specific organic and inorganic pollutants (soluble organics and recalcitrant compounds) it cannot provide a full treatment for LLs with complex compositions (Gao et al., 2014). Large doses of the oxidising agents are required which results in high energy usage and therefore high treatment costs (Luo et al., 2020).

2.3.5. Biological Treatment Methods

Biological treatment methods are highly efficient and relatively inexpensive compared to other treatments however the presence of toxic compounds or high concentrations of refractory matter can inhibit biological activity (Dogaris et al., 2020; Gao et al., 2014; Luo et al., 2020). Most biological methods utilise consortium or microbial communities which are

adapted to the removal of individual pollutants (Wiszniowski et al., 2006). Biological treatments can be either aerobic or anaerobic, producing either CO_2 or methane (biogas), respectively, and the decision on which treatment to select can be done using the decision model of Forgies (1988) (Dogaris et al., 2020; Gao et al., 2014).

2.3.5.1. Anaerobic Treatment

Anaerobic treatment takes place in the absence of oxygen and it comprises two phases: the acidic phases where facultative microbes convert complex organics to simple organic acids and the methanogenic phase where volatile organic acids are converted to carbon dioxide and methane (Figure 2.11) (Gao et al., 2014). Though anaerobic systems are relatively inexpensive and well-studied they also offer low removal rates and often increases in ammoniacal-nitrogen content (Gao et al., 2014).

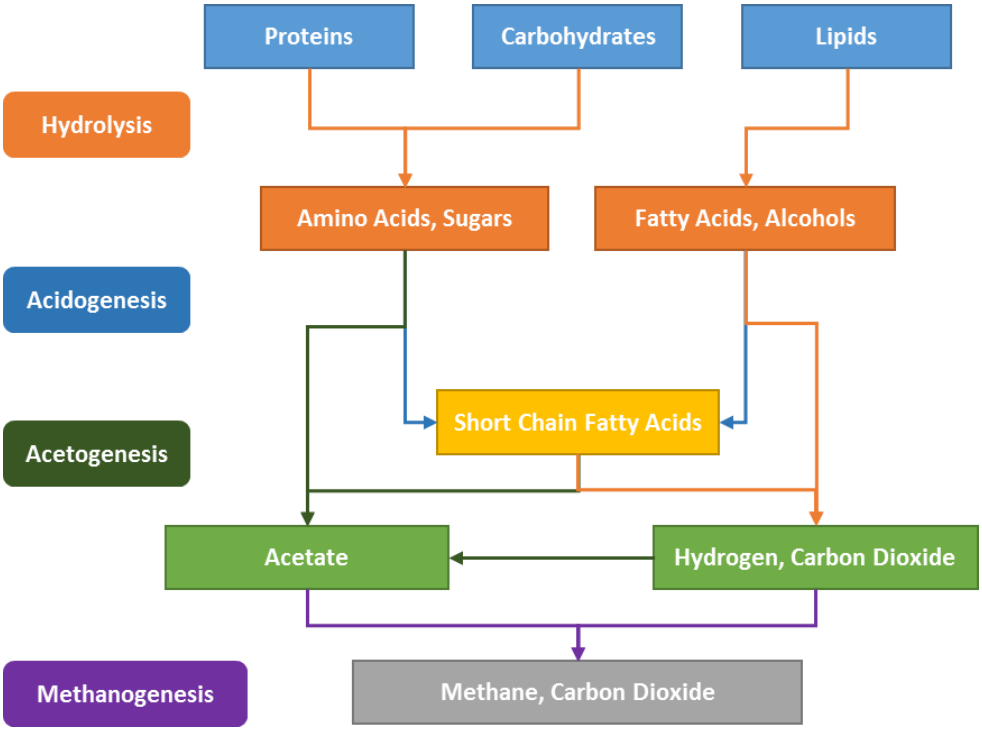

Figure 2.11: *Stages of anaerobic digestion of waste to biogas.*

Anaerobic digestion (AD) leads to the production of biogas (methane, carbon dioxide and trace other gases) which can be utilised as a source of renewable energy (Figure 2.12)(Luo et al., 2020). This is one of the oldest processes for wastewater treatment and has been used since the end of the 19th century (Wiszniowski et al., 2006).

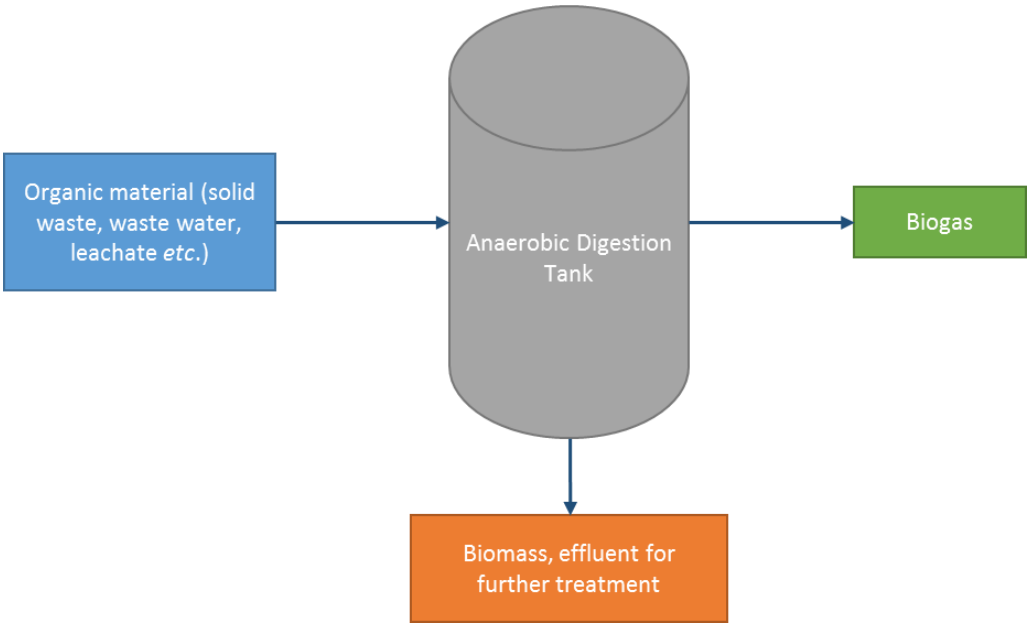

Figure 2.12: *Anaerobic digestion process. Organic waste (solid and liquid) is fed into the anaerobic chamber where temperature and mixing are controlled. The natural microbes digest the organic matter to produce biomass and biogas which can be utilised for electricity generation.*

2.3.5.2. Anammox

Anammox is an autotrophic, anaerobic ammonium oxidation method that uses ammonium as the electron donor and nitrite as the electron acceptor to accomplish nitrogen removal. Anammox eliminates the requirement of organic carbon for nitrification, reduces the energy demand of aeration and produces low amounts of sludge and CO_2 emissions (Luo et al., 2020). This treatment is recommended for mature leachates which have high nitrogen concentrations and low biodegradable COD. Anammox is a novel promising and low-cost alternative to conventional denitrification systems (Wiszniowski et al., 2006) however it only

targets one compound in a complex mixture of pollutants and will therefore require coupling with other treatment methodologies.

2.3.5.3. Lagooning

Aerated lagoons or stabilisation ponds are low maintenance and a popular choice for wastewater treatment (Renou et al., 2008). Lagoons are often made to mimic natural lakes and are 1-2 m in depth with an aerobic upper part and anaerobic lower part (Gao et al., 2014). The limiting factor to lagoons is the temperature dependence of the microbial activity with reduction rates being affected by seasonal variations. The extended hydraulic retention times (HRT) also need to be considered at full-scale treatment (Luo et al., 2020).

2.3.5.4. Activated Sludge

In the application of activated sludge, the reactors microbial community is constantly supplied with organic matter and oxygen (aeration) (Gao et al., 2014; Wiszniowski et al., 2006). This is a more intensive treatment than aerated lagoons due to the intensive aeration and acclimatized bacterial communities. High levels of sludge production and therefore the requirement for disposal, alongside the intensive aeration schedule, make this treatment process more expensive (Luo et al., 2020; Renou et al., 2008).

2.3.5.5. Sequence Batch

Sequence batch reactors (SBRs) allow for aerobic treatment, equalisation, settling of sludge and clarification to take place in the same reactor over a specific time sequence (Gao et al., 2014). This method is more robust than activated sludge and less affected by variations in the leachate composition and ammoniacal nitrogen (Gao et al., 2014).

2.3.5.6. Constructed Wetlands

Constructed wetlands are low-cost and very easily operated and maintained, Figure 2.13 (Luo et al., 2020). They have a strong potential for application in developing nations and in particular for small rural communities. Despite their successful development at lab, pilot and full-scale the poor performance in winter and large land requirements means that developed countries have not extensively pursued this option for LL treatment (Luo et al., 2020).

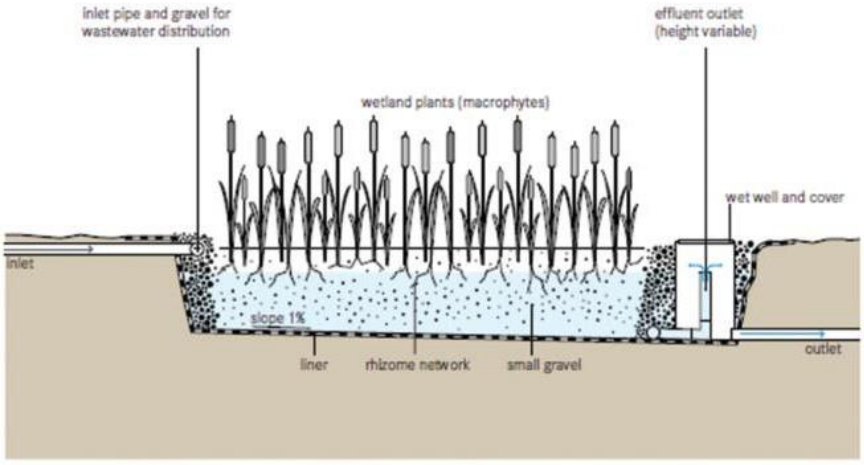

Figure 2.13: *Example of a constructed wetland, image from* (Maiga et al., 2017)

2.3.5.7. Algae for Leachate Remediation

Due to the increasingly stringent discharge limits being implemented on wastewaters and leachates (Dogaris et al., 2019) there is a requirement for competent and novel methods of treatment. The growth of microalgae in leachate and wastewater offers one such novel approach and has been gaining momentum over the past decade although it was originally proposed in 1957 by Oswald (Oswald et al., 1957).

The exploitation of microalgae for wastewater clean-up is twofold: the treatment of a waste stream allowing for water recycling and the production of biomass which can be employed as biofuels or fertilisers, reusing the once wasted and often valuable nutrients found in wastewaters and leachate (Dogaris et al., 2019). A variety of microalgal strains have now been

shown to remove ammoniacal-nitrogen, nitrate and nitrite, organic phosphorous, COD and some heavy metals from leachates with high efficiencies at laboratory scale (Dogaris et al., 2019; Renou et al., 2008). This being said, there are still several limitations to algal bioremediation including issues with the dark colour, sub-optimal phosphate levels, toxic organics, and very high ammonia levels. The dark colour often associated with leachate affects the photosynthetic potential of algae, adversely impacting the biomass productivity (Lin et al., 2007). Algae require both a nitrogen and phosphorous source to grow and leachates can often offer too high of a concentration of NH_3-N and too little phosphorous. Consequently, leachate is often diluted to 10 % (v/v) to increase transparency and reduce the NH_3-N concentration, together with supplementation with a phosphorous source if necessary (Eze et al., 2018; Lee et al., 2016; Lin et al., 2007).

2.4. Algae

The term algae embrace a large, diverse group of photosynthetic organisms. This polyphyletic group includes unicellular diatoms of 5 µM in diameter all the way to multicellular seaweed structures of meters in length (Raven and Giordano, 2014). Although algae and terrestrial plants both convert solar energy to chemical energy through photosynthesis, the former do not have roots, stems or leaves; appendages traditionally associated with photosynthetic organisms (Vidyasagar, 2016). There are estimated to be over 72, 500 species of algae with only a small proportion of these currently known of and exploited (Raven and Giordano, 2014; Sudhakar, 2012). Algae occupy countless different environments, aided by the diversity of the group.

The main focus here will be on the subgrouping of '*microalgae*' which includes unicellular organisms of both eukaryotic and prokaryotic cell types. For simplicity, photosynthetic

cyanobacterium such as *Anabaena* and *Spirulina* species are included in the subgrouping of microalgae within this body of work.

2.4.1. Why are they Important?

Microalgae are the primary producer for most ecosystems across the planet. They play a major role in both the carbon and oxygen cycles and diatoms produce more O_2 than trees and vegetation (Raven and Giordano, 2014). As the base of most ecosystems, algae are hugely important to both animals and humans. As a species, humans have consumed algae as food for thousands of years but there are multiple ways in which they can be exploited, and more are continually being researched.

2.4.2. Products from Microalgae

As previously mentioned, a myriad of products can be extracted from algae and further uses exploited. These include items of high value (HVPs, high value products) such as pharmaceuticals and therapeutics, food supplements, animal feed, fertilisers, and biofuels. Microalgae are a good source of proteins, fats, and complex carbohydrates; however, the biomass does also include ash. Under some circumstances this can be incredibly high, reducing the useable portion of the biomass grown and affecting downstream processing (Liu et al., 2020).

2.4.2.1. High Value Products: Pharmaceuticals and Therapeutics

Most recently, microalgae have been explored as a potential natural source of antiviral drugs to fight against SARS-CoV-2. Algae are one of the richest sources of bioactive compounds that exhibit antiviral properties and are pharmaceutically active (Sami et al., 2020). Flavanones, flavanols and alkaloids are known to inhibit proteins that are involved in the replication cycle of SARS-COV-2.

Other HVPs include carotenoids, a group of photosynthetic pigments with known antioxidant and anti-inflammatory properties. It has been shown that consumption can reduce the risk of some chronic diseases (Zhang et al., 2014) and therefore the global market for these pigments has been growing annually at about 2.3 % and is expected to grow from 1.5M USD in 2019 to 2M USD by 2026 (Singh, 2020). β-carotene is the most well-known carotenoid, essential in the human diet as a precursor for vitamin A (Del Campo et al., 2007). *Dunaliella salina* has been used to produce β-carotene since the 1980s (Borowitzka, 2013).

2.4.2.2. Food, Supplements and Agriculture and Aquaculture

The food industry utilises the high protein and vitamin content of microalgae for protein supplementation, specifically for those following vegan and vegetarian diets. The nutritional benefits of microalgae are also reaped in the agricultural and aqua-cultural industries with microalgal biomass often being supplemented to feeds to improve the nutrition of livestock (Belay et al., 1996). Microalgae have been added to poultry and fish diets since the 1990s as a source of protein and growth proteins (Belay et al., 1996). Becker (2007) estimated that nearly 30 % of algae that were produced at the time of writing were produced to be sold for animal feed (Becker, 2007).

2.4.2.3. Fertilisers

Micro and macro algae can also be used as soil fertilisers in a circular economy. *Chlorella* sp. and *Spirulina* microalgae and *Laminaria digitata* and *Ascophyllum nodosum* macroalgae have been shown to increase nitrate, ammonia, and phosphorous availability at varying concentrations in fields and greenhouses (Alobwede et al., 2019).

2.4.2.4. Energy

One of the major drivers of algal research over the last few decades has been the potential production of biofuels such as biodiesel and bioethanol (Fortman et al., 2008). Average microalgae dry weight contains 15–30 % lipid, a large percentage of this being triacylglyceride (TAG) which can easily be converted to biodiesel through transesterification. This, in combination with their high productivity, means more biofuels can be produced from the same land area than many of the current first and second generation biofuel crops such as soybean and switch grass (Chisti, 2007). The issues associated with first and second generation biofuel crops is the requirement of arable land that would otherwise be used for food production. Microalgae can be cultivated on non-arable land with non-potable water meaning there is no direct competition between food and fuel production. Currently, algal-oil based fuels are too expensive to produce compared to both traditional fossil fuels ($3.77 gal^{-1}) and commercial biofuels ($4.21 gal^{-1}) produced from plant and animal oils (Chisti, 2013; Mata et al., 2010; Zhang et al., 2017). This being said, there is a distinct lack of large-scale production facilities and the range of prices within the literature ($0.9 – $100 gal^{-1} biodiesel (Zhang et al., 2017)) are predominantly gained from theoretical economic analysis making large assumptions on productivity and culture conditions (Amer et al., 2011; Davis et al., 2011; Slade and Bauen, 2013). The main drivers for the high expense are thought to be the energy intensive methods used for harvesting and drying of the biomass (20–50 % of total operational costs (Mata et al., 2010; Sayre, 2010; Ward et al., 2014)) as well as the construction of the complex photobioreactor (PBR) systems used for cultivation which can equal up to 5 times the cost of all other equipment needed (Davis et al., 2011).

2.4.3. Algae Cultivation Systems

There are many different methods of cultivating algae and the technique used will depend on a multitude of factors, including: species used, product and by-products, size of the production facility and geographical location. Once the purpose of and the species to be cultivated have been decided the growth method can be chosen and there are two systems utilised: open pond (Figure 2.14) and closed PBR (Figure 2.15). Open systems are the cheaper and simpler options in practice, but the open environment and lack of control are not suitable for fragile species as contamination can easily occur. Closed PBRs come in a variety of different designs all with enclosed and controllable environments, ideal to produce sensitive species and HVPs such as carotenoids and protein. Both the capital investment and operational cost of these closed systems are much higher than those for the open ponds. Table 2.3 briefly describes the benefits and limitations of both systems.

Table 2.3: *Comparison of different algal cultivation methods*

Closed photobioreactors				**Open raceway ponds**			
✓	Higher culture productivity and therefore lower harvest cost	×	Expensive to construct, operate and maintain.	✓	Large size ponds can be achieved (up to 1 ha)	×	Lower culture productivity, therefore higher harvesting cost
✓	Little risk of contamination allowing sensitive species growth	×	Modular design meaning no economies of scale	✓	Cheap to construct, maintain and operate	×	No environmental control meaning easy contamination to native species or predation, therefore reliant on extremophilic species

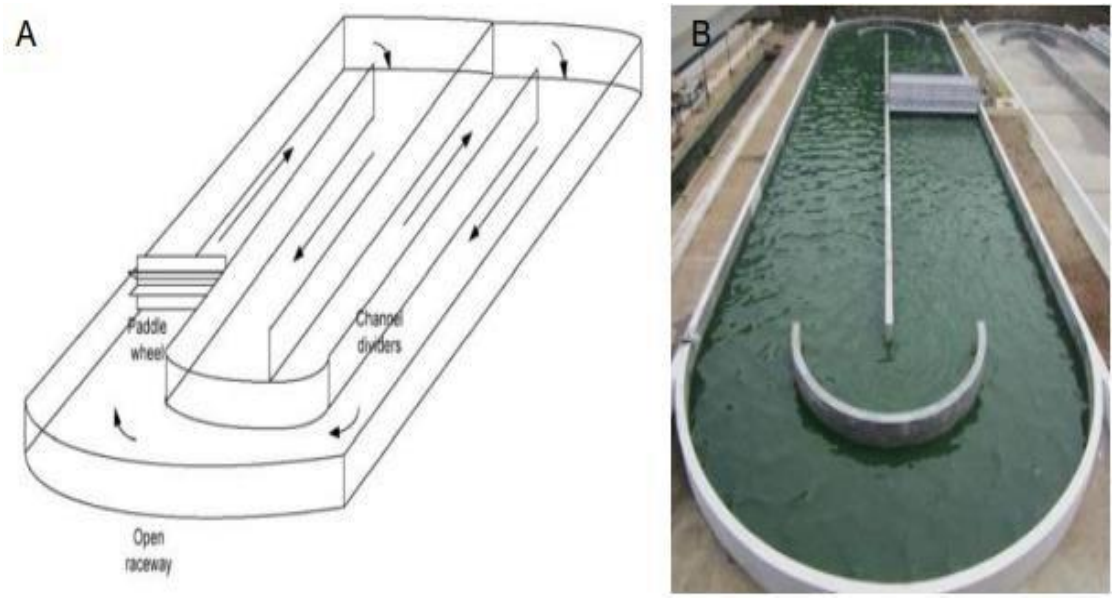

Figure 2.14: *Open Raceway ponds. (A) Schematic for a typical pond* (Jorquera et al., 2010) *and (B) Industrial pond operated by Aban Infrastructure (http://www.aban.com/facilities.html).*

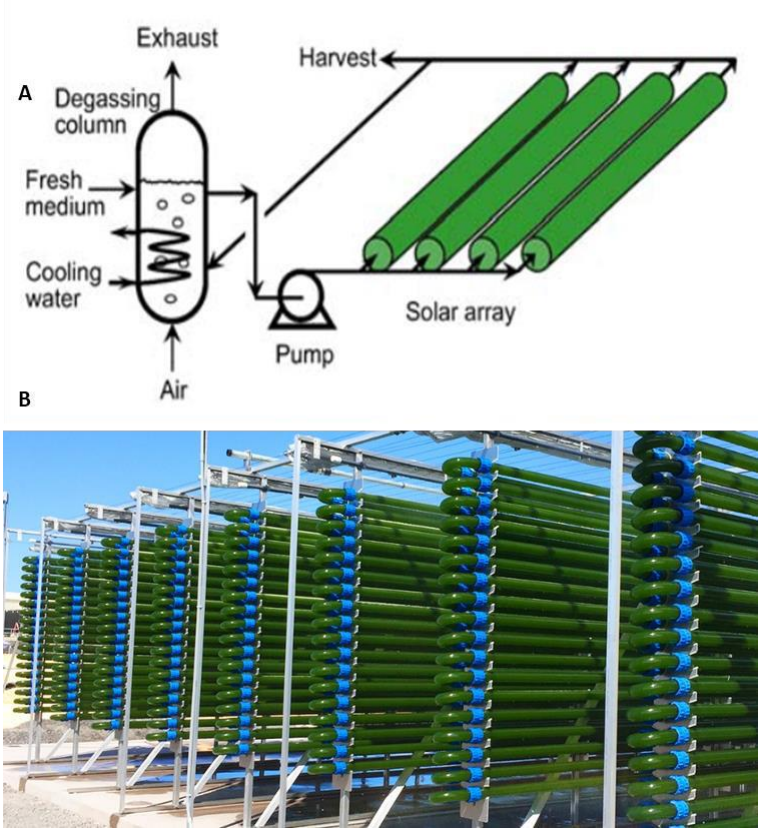

Figure 2.15: *Tubular Photobioreactors. (A) Schematic for a tubular PBR (Chisti, 2007) and (B) A Phyco-flow™ reactor from VariconAqua.*

2.4.4. Harvesting, Drying and Post-Cultivation Modification

Efficient and cost effective harvesting of algal biomass faces many difficulties due to the dilute concentration of cells in the culture (0.5 g L^{-1}), the small size of the cells (< 20 μM), their near neutral buoyancy (cell density = 1.08 – 1.13 g mL^{-1} (Chen et al., 2015)) and the strong negative surface charge on cells preventing flocculation and fall out from suspension. Centrifugation, filtration, and gravity sedimentation are all examples of the options available for harvesting but no singular method is optimised for all microalgae – the choice of technique depends on the species properties, desired end product, capital costs and the cultivation system utilised (Chen et al., 2015; Rastogi et al., 2018).

Open systems result in much lower cell density in the media and therefore energy-intensive centrifugation is prohibitively expensive. Enhancing natural sedimentation or flocculation of cells would be ideal in these cases, allowing mass dewatering and then the potential to use high-efficiency centrifugation on the concentrated slurry. Chemical-, Electro- and Bio-flocculation have been used with a variety of different algal species and is now considered one of the most popular choices for mass-harvesting (Chen et al., 2015).

Closed systems average a higher cell concentration and therefore the utilisation of vacuum filtration or centrifugation has potential. This being said, these processes are very energy intensive (26.5–100 kWh tonne biomass $^{-1}$ (Ventura et al., 2013)) and would therefore still affect the operational costs. For HVPs, the harvesting technique is not of major concern but to produce fuels and commodity products, centrifugation is too expensive to make the products economically competitive with current alternatives.

Milledge and Heaven (2013) reviewed the current techniques used for harvesting microalgal cultures and conclude that there is currently no optimised method or combination of

methods for harvesting the biomass. The review shows that the use of centrifugation is too energy intensive for biodiesel production with the harvesting alone requiring four times the energy of the biodiesel produced. They also state that if efficient harvesting is "*the major challenge of commercialising micro-algal biofuel*" it will have a profound influence on the upstream and downstream processes (Milledge and Heaven, 2013).

Although the biomass concentration will have been improved (up to 200-fold) by the harvesting process, a large volume of moisture (up to 85 %) is still present in the slurry and can spoil the biomass within a matter of hours (Chen et al., 2015; Milledge and Heaven, 2013; Molina Grima et al., 2003). The detrimental effect of moisture on biomass for many downstream processes means in many cases the biomass also requires drying.

Many methods have been utilised for microalgal drying, including spray-, drum-, freeze- and sun- (Del Campo et al., 2007; Cuellar-Bermudez et al., 2015; Juneja and Murthy, 2017; Mata et al., 2010; Milledge and Heaven, 2013; Molina Grima et al., 2003; Mondal et al., 2017). Sun or solar-drying is the cheapest method however it requires a warm climate, large surface area and long drying times (Chen et al., 2015). Open solar drying has issues with end-product quality maintenance but closed solar-systems that trap heat have been shown to reduce this effect and the drying time required to only 3 – 5 hours (Prakash et al., 1997).

The harvested and dried biomass will then be processed to the desired product, be that by extraction of oils and carotenoids or drying and grinding for food powder production. For biodiesel production, the extracted oils will go through a transesterification reaction with methanol to produce methyl esters (Chisti, 2007). There are a variety of different post-harvest applications, however, these are outside the scope of this review.

The techniques mentioned above for harvesting, drying and even the lipid extraction and transesterification for biodiesel production are all adapted from other industries (Zhang, 2015) and therefore not developed specifically for use with microalgae. This means there is an opportunity for improvements in both design and economics by future research. There are many examples of research into new and improved harvesting by methods including: membranes (Eliseus et al., 2017), flocculent-less electro-flocculation (Shi et al., 2017), new chemical flocculants (Noh et al., 2018), coagulation and shear stress harvesting (Zhang et al., 2018) and also by removing the media rather than the cells with polyacrylic superabsorbent polymers (Wei et al., 2018). There is also considerable work towards avoiding the energy-intensive drying step with wet biomass hydrothermal liquefaction (Cheng et al., 2018) and transesterification (Lee and Kim, 2018; Martinez-Guerra et al., 2018).

2.4.5. Reducing the Costs

As previously mentioned, the costs associated with algal biofuels are estimated to be much higher than alternatives and traditional fossil fuels and therefore research in this area has focused on reducing these production costs. Many suggestions have been made including new PBR designs for increased productivity (McGinn et al., 2017; Pruvost et al., 2017), improved harvesting methods (Milledge and Heaven, 2013), utilisation of wet biomass rather than dry (Cheng et al., 2018) and improvement in lipid quantity and quality within the algae (Toledo-Cervantes et al., 2013; Yoo et al., 2010).

Combining biomass production for biofuels with waste-treatment streams is another option for reducing the costs associated. Utilising landfill leachate and other wastewaters which requires bioremediation as a source of ammoniacal-nitrogen and orthophosphate (Craggs et al., 1997) would allow for a reduction in operational costs. This could also potentially increase

revenues through tax avoidance as legislation surrounding toxic leachate discharge becomes more stringent (Zimmo et al., 2004). Current remediation techniques are expensive (Craggs et al., 1997; Xu et al., 2008; Zimmo et al., 2004) and difficult (Ozturk et al., 2003) due to the constantly changing nature of leachate. Combining microalgal growth with bioremediation offers the potential to reduce not only the cost of biofuel production but also the cost of waste-treatment (Richards and Mullins, 2013).

Another waste-stream that can be exploited is flue gas. These waste gases contain high concentrations of CO_2 and some nitrous oxides that can be utilised by microalgae. Many culturing techniques suffer from carbon limitation due to the low atmospheric concentration (406 ppm, roughly 0.04 % (Kumar et al., 2010)) and small increases in CO_2 concentration supplied to the cultures have been shown to be beneficial to cultures. According to Chisti (2007), 183 tonnes of CO_2 is required to produce 100 tonnes of algal biomass and this will be very difficult to effectively source entirely from the atmosphere (Chisti, 2007). A number of reviews and analyses of algae production all suggest the use of industrial flue gases as a cheap CO_2 supply, often stating that there will be no or little cost associated, due to the waste nature of the gas (Packer, 2009).

A further option would be to treat both waste streams mentioned simultaneously. This would reduce the cost of all essential nutritional elements required for algal growth and gain credits for the clean-up of multiple waste streams while producing a by-product of biomass alongside. Many research papers which consider this application will be discussed in Section 2.7.

2.5. Algae and CCUS

The concept of using algae for renewable energy production has been around for decades, and the utilisation of the high CO_2 concentration flue gases to aid this process has been around almost as long (Maeda et al., 1995). In this section, an up to date review of research surrounding algae production with regards to carbon capture will be presented, alongside an account of the current knowledge gaps which should be filled in the near future. Biofuels will be discussed due to their synergy with past research linking carbon abatement and renewable fuel production together. Research into algae production for other purposes such as food and cosmetics is not included but is still relevant to post-harvest applications of biomass.

Although the concept has been around for a long-time, the idea of carbon capture rather than the use of CO_2 merely to supplement growth is rather new. As of current there are no publicly known large scale demonstration plants for this methodology of CCUS, however, flue gases are being utilised within the algae production industry (Henion and Chludzinski, 2017; Sun et al., 2011; Tredici et al., 2016; White et al., 2015).

2.5.1. History of Carbon Capture and Algal Biomass Production

A Web of Science search for *"alga*" and "carbon capture"* gives less than 100 results all from the late 2000s, however, research linking the two was kick-started in the 1990s when the Kyoto protocol was created (UNFCCC, 2019). Although primary (food crops like corn and palm oil) (Chisti, 2007) and secondary (non-food crops like *Miscanthus* (Rastogi et al., 2018)) biofuel crops were still under development, the mass production of microalgae was regarded as a "potentially important technology for biofuel" (Watanabe et al., 1992).

In 1978 the US department of energy began the Aquatic Species Program assessing the production of biofuels from microalgae in open raceway ponds (ORPs) (Sheehan et al., 1998).

Within this body of work, the utilisation of waste CO_2 from flue gas was also considered. The project ran until 1996 with over 25 million US USD in investment (Packer, 2009). Alongside America's efforts, Japan also began RITE (Research Institute of innovative Technology for the Earth) in 1990 which focused on PBR optimisation and algal screening (Murakami and Ikenouchi, 1997; RITE, 2017).

Although at a much smaller, laboratory bench, scale to the previous – publications by Watanabe *et al.* (1992) and Hanagata *et al.* (1992) highlighted that *Chlorella* and *Scenedesmus* species were CO_2 tolerant with *Chlorella* HA-1 having an optimum CO_2 concentration of 5-10 %, much higher than the atmospheric concentration (Hanagata et al., 1992; Watanabe et al., 1992). Due to the biofuel production focus of the time, many of the publications noted focus on the lipid content of the cultures rather than the algal biomass production and CO_2 utilisation rates (Chisti, 2007; Chiu et al., 2009a; Ho et al., 2010; Huntley and Redalje, 2007; Kishimoto et al., 1994; Lam et al., 2012; Ota et al., 2009).

The mid-late 1990s saw an increase in publications in the area, including work from Kurano *et al.* (1995) stating *Chlorococcum littorale* having CO_2 fixation rates of up to 4 g CO_2 L^{-1} d^{-1} (Kurano et al., 1995). Brown (1996) considered not only the carbon dioxide concentration in waste gases, like previous work, but also other potentially harmful pollutants including sulphur and nitrous oxides (Brown, 1996). The work with *Monoraphidium minutum* showed that moderate levels of these pollutants were well tolerated by the microalgae in laboratory cultures but noted that the "bench scale system is far from optimised for flue gas utilisation" (Brown, 1996).

Kadam (1997) assessed the economic viability of using microalgae for flue gas cleaning rather than MEA scrubbing and results found that operations, where flue gas was pumped directly

to the culture were up to 54 % more expensive than MEA extraction. This is largely due to the higher gas volumes required for compression and transportation (roughly 7 times the volume of pure CO_2 due to the concentration of 15 – 25 %) (Kadam, 1997). The paper highlighted that at the time of writing, flue gas 'as is' was not a viable strategy and further research was needed in the delivery methods.

In 2005 – 2010 there was a surge in publications involving algae and carbon capture, with Doucha *et al.* (2005), de Morais and Costa (2007), Chiu *et al.* (2009) and Douskova *et al.* (2009) all considering the CO_2 removal efficiency of different PBR designs. Doucha *et al.* (2005) found that although CO_2, NO_x and CO have no negative influence on the growth of *Chlorella*, the CO_2 utilisation rate was very low, giving an inefficient model for CCS applications. Similar results were experienced by de Morais and Costa (2007a), with up to 100 % efficiency of removal when air was supplied but as low as 0.16 % carbon removal when a concentration of 18 % CO_2 was tested. Chiu *et al.* (2009a) found *Chlorella* sp. could obtain CO_2 removal efficiencies of 16 % (17.2 g L^{-1} d^{-1}) with a supply of 15 % CO_2 (a similar concentration to that found in many flue gases). Douskova *et al.* (2009) established that *Chlorella* has higher CO_2 fixation rates with flue gas than with equal CO_2 concentrations in control gas, suggesting the other compounds found in the gas may be beneficial to the microalgal growth.

More recently, efforts have been focused on improving the economic feasibility of algal growth, with improvements in reactor design and light supply. This being said, Kao *et al.* (2014) carried out large scale experiments utilising real flue gases from different sources (Kao et al., 2014). The 1,200 L (total volume split over 50 individual bubble columns) were set up onsite at the China Steel Corporation site in Taiwan and utilised flue gases from either a:

a) coke oven,
b) hot stove or
c) power plant,

with CO_2 concentrations ranging from 23 – 28 % (Figure 2.16) (Kao et al., 2014). The experiments showed that growth rate of cultures improved, in comparison to growth with air, in all cases tested. The CO_2 removal efficiency was, however, only assessed at laboratory scale and not in the large PBR set up.

Duarte *et al.* (2016) employed synthetic flue gas for the growth of *Chlorella* species finding the best CO_2 biofixation rate was ~64 % efficiency (364.5 mg CO_2 L^{-1} d^{-1}) with the addition of 60 ppm SO_2, 100 ppm NO, 10 % CO_2 and 40 ppm ash. This is only one of many examples of the use of synthetic flue gases, important for assessing the effect the different pollutants have on the biomass production and CO_2 removal efficiency.

Bubbling the CO_2-rich gases through the culture is not the only way of delivering CO_2 to the algae with Zheng *et al.* (2017) opting for membrane separated loaded-MEA which contacts the culture allowing the transfer of CO_2.

Different microalgal species have been grown under varying CO_2 and flue gas concentrations throughout the literature, however, there are still large gaps in knowledge surrounding how the cells utilise the compounds found within the flue gas. Van Den Hende *et al.* (2012) give an in-depth review into what is and is not known about how microalgae interact with the main constituents of flue gas: CO_2, NO_x, SO_x and water (Van Den Hende et al., 2012).

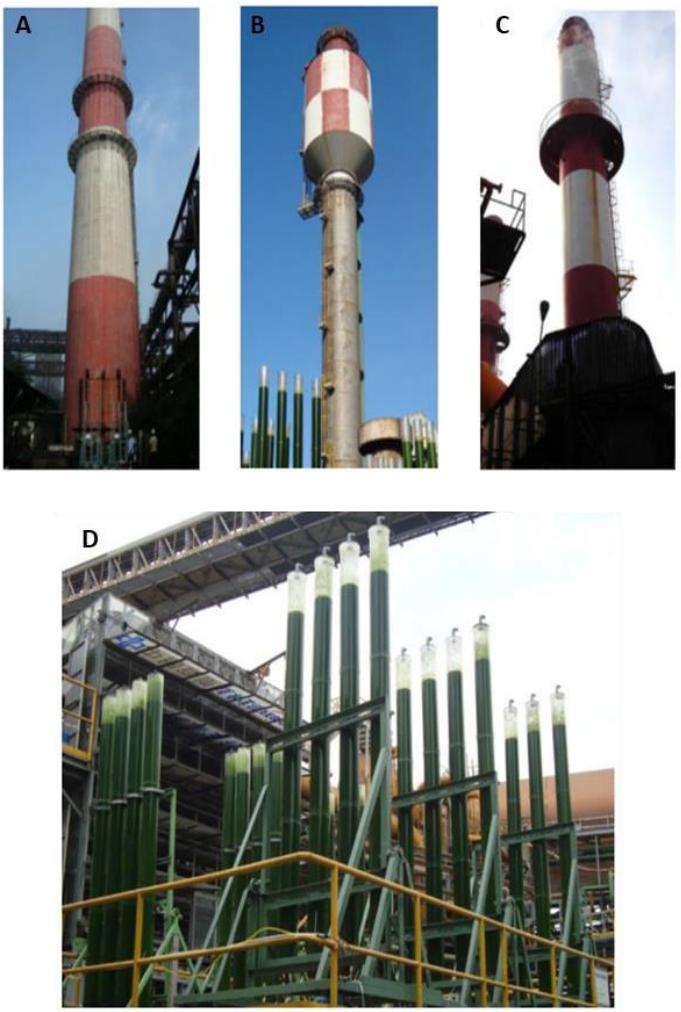

Figure 2.16: *Outdoor trials for algal growth by Kao et al. (2014). (A-C) show the different sources for the flue gases utilised in experimentation; (A) coke oven, (B) hot stove and (C) power plant. (D) shows the setup of the 50 cylindrical PBRs (24 L each) onsite.*

2.5.2. Trends in the Published Literature

Throughout this review, a variety of different experimental systems and analyses have been highlighted, many exhibiting similarities in the experimental design. A comprehensive list of publications and the experimental conditions used can be found in Appendix B.

Although there are estimated to be over 72,500 different species of algae on the planet (Raven and Giordano, 2014), almost half of the surveyed literature utilise *Chlorella* species in their work because of their robustness and ease of cultivation. The large information sets

available for this genus, including uses for the biomass, are important for assessments of scale-up and economics. Although there are advantages of using *Chlorella*, there are so many species unfamiliar to us, which could potentially be better than *Chlorella* both in a financial and CO_2 utilisation sense. Another commonly utilised species is *Arthrospira (Spirulina),* which has a faster growth rate than *Chlorella* as well as the biomass selling for the same purpose (Parada et al., 1998). Other favoured species include *Botryococcus braunii*, known for its high lipid content (Murakami and Ikenouchi, 1997) and *Chlorococcum littorale* known for its robustness to environmental stresses (Ota et al., 2009).

Small scale PBRs or flask experiments are most commonly used within the literature, mainly due to the nature of laboratory research. These small scale cultures however do not scale appropriately to large-scale applications, highlighted in Kurano *et al.* (1995) where a very high fixation rate of 4 g CO_2 L^{-1} d^{-1} was obtained in the smallest vessel with less than a quarter of that in the largest (Kurano et al., 1995). The main reason for the lack of large-scale data is funding – larger scales cost more and with the uncertainty associated with upscaling it is harder to find. This being said, there are now more commonly larger scale, pilot, facilities including those mentioned in the EnAlgae report, an EU initiative to improve algal cultivation knowledge (White et al., 2015).

The light regime used for experiments within the literature differs considerably from that of natural light conditions, the main option considered financially viable for large scale cultivation. The majority of experiments covered in this analysis utilise LED lighting units of varying intensity, and many utilise 24-hour irradiation (Appendix Table B), again not like the natural conditions expected for use. This theme can be assumed to be down to the nature of laboratory experimentation but could affect the assumptions made when considering pilot

scale production under different light intensities and durations. In recent years there has been more focus on research towards the effect of light intensity and regime on algal growth with efforts to improve the photosynthetic efficiency of cultures (Clément-Larosière et al., 2014; Naderi et al., 2015), however, little research is still conducted with natural lighting.

In many cases, pure CO_2 mixed with compressed air at varying concentrations of CO_2 is used rather than flue or synthetic flue gases. This is due to the ease of experimentation and early focus on carbon tolerance rather than utilisation efficiency. The use of pure CO_2 and air mixes does however leave the question of how other pollutants and particulates within the flue gases will affect the microalgae. There are some examples of the use of either flue gas or synthetic flue-gases to assess the tolerance to CO_2 and other pollutants (Borkenstein et al., 2011; Douskova et al., 2009; Duarte et al., 2016; de Godos et al., 2010; Hu et al., 1998; Kao et al., 2014; Zheng et al., 2017). Packer (2009) states that there are a number of reports explaining the potential of utilising waste CO_2 for the production of algal biofuels but that "*there are comparatively few but valuable*" studies actually exploring the possibility of this (Packer, 2009).

Although a large proportion of published literature assumes the use of algae cultivation as a carbon abatement strategy, few measure the CO_2 removal from the gas to the biomass (and media). Many of the published results utilise the assumptions made in Chisti (2007) where 1.83 kg CO_2 is required per kg of algae produced and this is multiplied by the productivity of the cells, P (g L^{-1} d^{-1}), to give a CO_2 utilisation rate, P_{CO2} (g CO_2 L^{-1} d^{-1}) (Chisti, 2007):

$$P_{CO2} = 1.83 \times P \qquad 2.7$$

This methodology has, however, been shown to be inaccurate (Adamczyk et al., 2016), as it makes the assumption that the carbon content of the biomass is stable and that the process

of carbon transfer from gas to biomass is 100 % efficient; both of which are unlikely in a biological system. The carbon content of the biomass is subject to fluctuation due to species differences and environmental stresses. Equation 2.8 can be used where both the carbon content of the biomass, $C_{biomass,}$ and the efficiency of carbon uptake, $eff_{CO2,}$ (gas inlet and outlet) of the system are measured accurately throughout the experimentation and the different growth phases.

$$P_{CO2} = C_{biomass} \times \frac{MW_{CO2}}{MW_C} \times eff_{CO_2} \times P \qquad 2.8$$

Where P_{CO2} is the CO_2 removal efficiency (g CO_2 L^{-1} d^{-1}), MW_{CO2} and MW_C are the molecular weights of carbon dioxide and carbon, respectively and P is the culture productivity (g biomass L^1 d^{-1}). Similarly, to the previous equation, this method is still not accurate as the $C_{biomass}$ is usually assumed at 50 % and not measured throughout the growth period.

2.6. Algae and Leachate Remediation

The use of algae for wastewater/leachate bioremediation was first proposed in 1957 by Oswald *et al.* and since then numerous research groups have studied a variety of species' ability to remove key pollutants from the waste stream (Dogaris et al., 2019; Oswald et al., 1957; Renou et al., 2008). The focus, similar to algae for CO_2 capture mentioned previously, has heavily been on the cost-effective production of third-generation biofuels rather than the remediation of waste. Leachates and wastewaters from a variety of sources are often seen as cheap, if not free, nutrient media which can reduce the operational costs of algal biomass production. The use of wastewater also reduces the requirement for fresh, potable water that is becoming increasingly scarce and therefore of concern worldwide.

This section aims to describe the current literature, trends, and gaps within the knowledge regarding the use of microalgae for the treatment of wastewaters and landfill leachates. Similarly to the previous section, biofuel production will be discussed due to its heavy role in previous algal research.

2.6.1. History of Leachate Bioremediation with Microalgae

Though the idea was proposed in the 1950s by Oswald *et al.*, the first publication referencing both algae and leachate was not until 1981 (Clarivate Analytics, 2021). Apart from a few publications in the early 1980s, the majority of publications referring to both *"leachate"* and *"algae"* were not until the 2000s, with the number of publications trending upwards ever since with the highest number of 19 publications in 2020 (Clarivate Analytics, 2021), Figure 2.17.

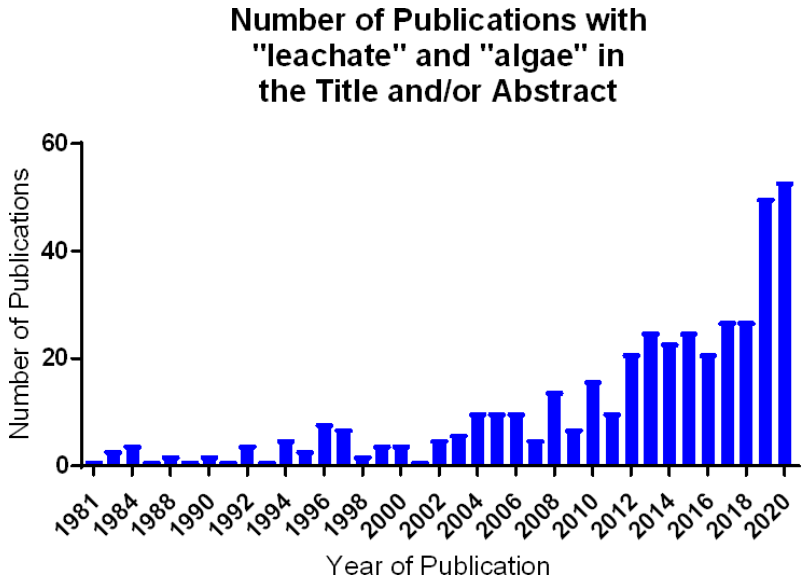

Figure 2.17: *Number of publications including algae and leachate in the title and/or abstract that appear on Web of Science against the year of publication.*

The first recorded paper, in 1981, focused on the toxicity of volcanic-ash leachate on the blue-green algae, *Anabaena flos-aquae*. McKnight *et al.* (1981) found that the leachate was lethal

to the blue-green microalga *Anabaena flos-aquae* even when a 100 times dilution in media was used. Even at a 500 times dilution, the cells presented abnormalities, highlighting that ash from Mt St. Helens could have detrimental effects on aquatic ecosystems.

The first instance of a leachate being used as a nutrient medium or algae being used for bioremediation purposes wasn't published until 1984 by Pryztocka-Jusiak *et al.* (1984). *Chlorella vulgaris* was used as part of a two-stage removal of nitrogen from wastewater. Traditional denitrification processes were followed by algal cultures, leading to a 94 – 99.9 % removal of NH_4^+ from the wastewater. Highlighting how effective microalgae can be at removing key nutrients from wastewaters.

Richards and Mullins (2013) also looked at the bioremediation potential of microalgae. Their focus was on the removal efficiency of metals from wastewaters, rather than the traditional nutrients. The authors found that their microalgal population, enriched from the waters, dominated by *Nannochloropsis gaditana* and *Chlorella muelleri,* removed 95 % of metals from an artificial solution after 10 days of cultivation. They propose the combination of wastewater treatment and lipid production for biofuels, as many of the previously mentioned studies also do.

Lin *et al.* (2007) were one of the first to isolate algae for experimentation from the landfill leachate ponds. Utilising the adapted environmental strains rather than culture collection species. Leachate samples from one of the ponds at the Li Keng landfill in Guangzhou, China were used to isolate two microalgae strains: *Chlorella pyrenoidosa* and *Chlamyndomonas snowiae.* While the growth of both isolated algae was significantly better in controlled media cultures, the isolates grew in 10 % dilutions of the landfill leachate. Lack of growth in higher

concentrations of leachate was attributed to the extremely high ammoniacal-N concentration (1.345 g L^{-1}) found in the leachate.

The use of isolated and enriched consortia containing both algae and bacteria has become more prevalent in the research recently. Lee *et al.* (2016) isolated an algal-bacterial consortia from wastewater which was dominated by *Scenedesmus* sp. algae and *Sphingobacteria, Flavobacteria* and *Proteobacteria* bacterial species. Their research focused on how optimising the photo period using a two-phase regime could improve carbon, nitrogen, and phosphate removal from a 10 % dilution of wastewater.

Microalgal cultivation in combination with other, physical or chemical, leachate treatment methods have also been considered within the literature (Ardila-Leal et al., 2020; Mustafa et al., 2012; Saranya and Shanthakumar, 2020). Mustafa *et al.* (2012) utilised an algal consortium in high rate algal ponds (HRAPs) as a secondary treatment following mechanical aeration. They found that the algae grew well in dilutions up to and including 50 % and could remove 99 % of ammonia, 91 % COD and 86 % ortho-phosphate from the leachate. This outdoor treatment was conducted in Malaysia – highlighting the advantages of algal cultivation which come with warm, sunny countries. *Chlorella* sp. was used as a tertiary treatment step for dye, COD, phosphate, and nitrate removal by Ardila-Leal *et al.* (2020) (Figure 2.18). The *Chlorella* sp. cultivation removed 62.6 % of the remaining blue dye colour, 48.3 % of COD, 41.4 % of orthophosphates and 32 % of nitrates found within the coloured laboratory wastewater (CLWW).

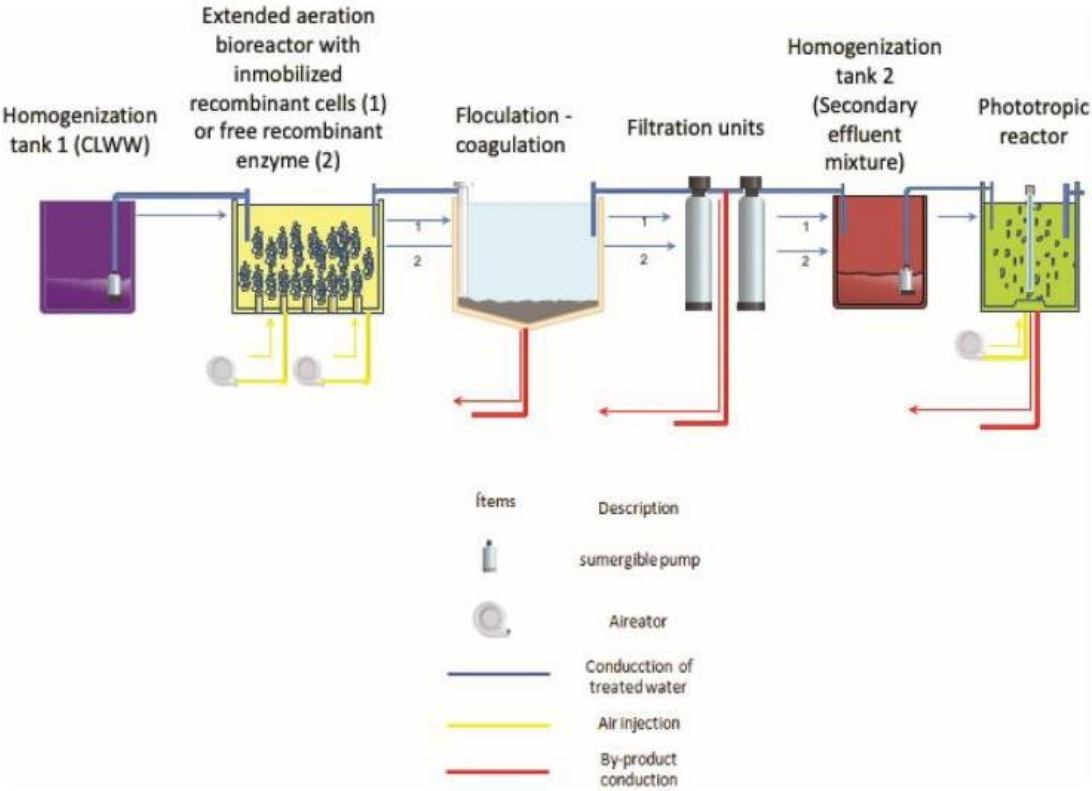

Figure 2.18: *Schematic representation of the treatment plant utilised by Ardila-Leal et al. (2020). Coloured Laboratory Wastewater (CLWW) was treated by immobilised enzymes, flocculation/coagulation and then algae cultures in a three-step process.*

Saranya and Shanthakumar (2020) used *Nanochloropsis oculata* as a secondary treatment for tannery effluent after an initial ozonation step. The combined treatment improved all the measured characteristics of the tannery effluent, including colour, COD and pH. Microalgae were shown to be a beneficial secondary treatment as while ozonation is efficient at removing COD (> 70 %), high ozone dosage can negatively impact the economics of the process. The pre-treated effluent is also less damaging to the cells and allows for greater algal biomass harvest at the end of the process, highlighting that the two processes can complement each other in the treatment of tannery wastewaters.

More recently, research has also considered how PBR alternations could improve both growth and bioremediation. Chang *et al.* (2018) found that the use of a PBR with a membrane, to separate the *C. vulgaris* culture from the toxic leachate, increased nutrient reclamation, growth and lipid production as compared to a traditional PBR. This being said, the nutrient reclamation rates were quite low, 50 % for nitrogen and about 70 % for phosphate. This was attributed to the high values of compounds present within the leachate, alongside the presence of other, toxic compounds such as recalcitrant organic matter and the dark colour.

2.6.2. Trends in the Published Literature

Throughout the previous section, a variety of different strategies for implementing algal bioremediation of wastewaters and leachates were highlighted. Although the major focus is largely the same throughout the literature, there are different approaches used and preferred and these will be discussed next.

Section 2.3.1 has already highlighted the key issues surrounding the treatment of highly toxic, complex, coloured, and changing leachates. The major strategy used to overcome these traits when considering algal bioremediation has been to dilute the leachate with either synthetic medium used to grow cultures in the laboratory or with distilled or tap water (Cheung et al., 1993; Lin et al., 2007; Park et al., 2019; Paskuliakova et al., 2018b; Sniffen et al., 2016; Tighiri and Erkurt, 2019). The dilution of key components such as ammonia and COD removes the inhibition caused at elevated concentrations and allows the cells to grow. Alongside this, most leachates have a dark brown colour due to the refractory compounds within the complex mixture, dilution aids in removing photosynthetic inhibition due to the colour of leachate. Although the level of dilution varies between publications, in most cases it leads to a LL concentration of < 20 %. Lin *et al.* (2007) grew *C. pyrenoidosa* and *C. snowaei* in 10 %, 30 %,

50 %, 80 % and 100 % leachate and found that the algae did not grow well in any of the concentrations above 10 %. It should also be noted that growth in any LL dilution did not pass that in the defined laboratory media control. The authors attribute the lack of growth in higher concentrations to elevated NH_4^+, colour and pH values above 9. Other authors used dilutions to control specific attributes of the LL or wastewater (WW). For example, Park *et al.* (2017) diluted their mixture of wastewater and anaerobically digested piggery water to between 3-5 % vv^{-1} to control the ammonia concentration, similarly to Sniffens *et al*. (2016) who diluted to 5, 10 or 20 % LL depending on the final ammonium concentration. Fan *et al.* (2018) diluted their wastewater because of its high salinity (7 %) which caused inhibition of cell growth.

Another trend seen within the literature is the combination of algal bioremediation with other treatment methods. As mentioned in Section 2.3.2, due to the complex nature of LL, a combination of physical, chemical, and biological treatments is often best suited for the greatest remediation potential. As already mentioned, Ardila-Leal *et al.* (2020) utilised microalgae as a tertiary treatment for tannery wastewaters and Saranya and Shanthakumar (2020) used microalgae as a secondary treatment after ozonation for the same waste type. A variety of papers utilise microalgal cultivation as a secondary or tertiary step after physical methods such as filtering and mechanical aeration have removed large particulates (Mustafa et al., 2012; Pacheco et al., 2019; Villaseñor Camacho et al., 2018). Notably, Arias *et al.* (2018) utilise microalgal growth as a tertiary treatment after activated sludge and anaerobic digestion treatment methods but integrate the biomass produced as a feedstock for the anaerobic digester to improve biogas production, Figure 2.19.

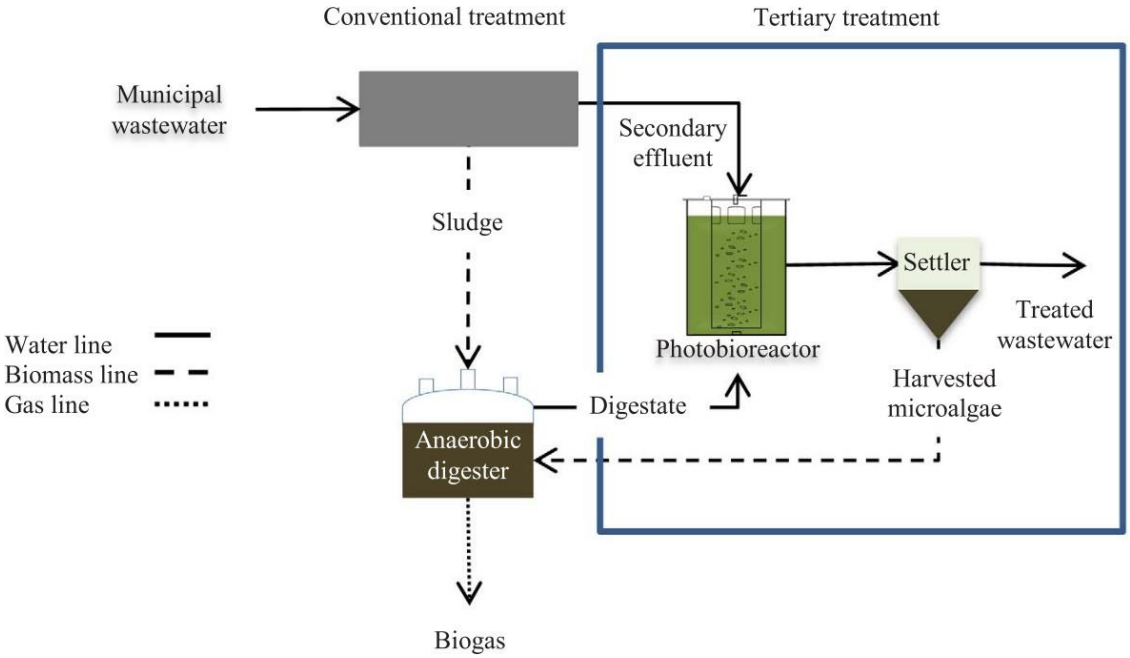

Figure 2.19: *Integration of algal cultivation to conventional municipal wastewater treatments with the biomass supplementing the anaerobic digestion step for biogas production. The picture was taken from Arias et al. (2018).*

A final trend worth mentioning as it is seen throughout the literature is the supplementation of phosphate sources to the WW/LL. While less than 1 % of algal biomass is made up of phosphorous, it is a key and limiting nutrient to their growth (Khanzada, 2020). Many leachates have incredibly high nitrogen loads (ammonia concentrations up to 3 g L^{-1}) and very little phosphorous in comparison. Therefore, many publications have included additional phosphorous sources within their experiments (Fan et al., 2018; Khanzada, 2020; Pereira et al., 2016; Przytocka-Jusiak et al., 1984).

2.7. Algae for Combined Bioremediation: Flue Gases and Leachates

Several articles have stated that "*microalgae cultivation is a promising approach for simultaneous CO_2 conversion and wastewater treatment*" (Eze et al., 2018; Gentili, 2014; Ji et al., 2015). Utilising waste streams for key nutrients required for growth reduces the cost of

cultivation and results in clean effluents as a by-product (Jiang et al., 2011). This section highlights the literature where microalgae have been utilised for the dual remediation of CO_2 gases and nutrient rich wastewater/ landfill leachates. Much of the research surrounding dual bioremediation purposes was not published until the 2010s, with the majority being in the last few years up to 2021 as the focus has moved away from biofuel production and towards cleaning the environment and circular economies.

As highlighted in the earlier sections pertaining to the individual waste streams of focus, earlier research was focused on the production of lipids for fuels rather than the bioremediation potential of algal cultures. Jiang *et al.* (2011) stated in their research that "*in order to reduce the cost of the production of microalgae for biofuel, the feasibility of using the mixture of seawater and municipal wastewater as culture medium and CO_2 from flue gas for cultivation of marine microalgae*" is required. The *Nannochloropsis* species used within this research had the best growth profile with a 50 % wastewater dilution combined with 15 % CO_2 concentration. As the focus of the research was on biomass and lipid production, no evidence of nutrient removal or CO_2 capture was presented.

Similarly, Ji *et al.* (2013) to focused on the production of biodiesel from microalgae (*C. vulgaris* in this case) but coupled the analysis with nutrient removal from the wastewater. Total nitrogen and total phosphorous concentrations decreased below their respective detection limits within 4 days of growth when supplemented with 15 % CO_2. The authors note that tertiary treatment with microalgae is a "*cost-effective and environmentally sustainable method*" for wastewater treatment and algal biomass production.

While the aforementioned papers utilised continual CO_2 feeding to the cultures, many researchers choose to use gas injection as a method of pH control. Arbib *et al.* (2013) utilised

open cultivation in HRAPs, while Eze *et al.* (2018) utilised lab scale polyethylene bottles for cultivation, both with CO_2 controlled pH systems. The ponds were injected with 4-5 % CO_2 and the CO_2 was seen to increase biomass production and improve the efficiency of nitrogen and phosphate removal from the urban wastewater. Tubular PBRs were also tested by Arbib *et al.* (2013) and these showed lower carbon limitations than the HRAPs at the beginning of experiments but this was reversed towards the end of the growth. Eze *et al.* (2018) found that pH control by CO_2 was essential because without a control system the pH increases with algal growth causing ammonia volatilisation and loss to the atmosphere.

Many researchers choose to use purified CO_2 mixed with compressed air in experiments to allow better control over the system. Gentili *et al.* (2014) on the other hand utilised flue gas directly from a combined heat and power unit with a CO_2 concentration of 10 %. The research showed that the addition of CO_2 bubbling improved the biomass yield of algae grown using wastewater. This research also utilised a mixture of different wastewaters from the pulp and paper industry and from the dairy industry. As seen previously, there was some nitrogen stripping from the wastewater due to the bubbling of the flue gasses. If the ammonia stripped to the atmosphere can be efficiently removed, mixing different types of wastewater (municipal and industrial) could make the production of "*algal biomass with high lipid content while at the same time treating the wastewater with added flue gases*" feasible (Gentili, 2014).

S. obliquus has also been shown as able to grow in mixed wastewaters. Ji *et al.* (2015) mixed 0.5 – 2 % municipal and food wastewater with varying concentrations of CO_2 from industrial flue gases. The algae tolerated all of the tested CO_2 concentrations, operating in a mixotrophic manner of using the organic substrates within the waste and the gaseous CO_2 (Ji

et al., 2015). Nitrogen and phosphorous were reduced by more than 50 % in all conditions tested and TOC (total organic carbon) was also reduced in all wastewaters apart from the secondary municipal wastewater where the TOC value was already low.

On the other hand, research by Shen *et al.* (2015) found that, while growing mixotrophically, *S. obliquus* only removes low concentrations of organic carbon pollutants and favours the gaseous carbon source supplied. The nitrogen and phosphorous removals were 97.8 % and 95.6 %, respectively with 256.56 mgL^{-1} CO_2 fixation capacity. Similarly to previous studies, it was shown that the 5 % CO_2 bubbling accelerated biomass and lipid productivity (Shen et al., 2015). *Scenedesmus* species were also used by Nayak *et al.* (2016) for the production of lipids for biofuels with simultaneous treatment of wastewater and 2.5 % CO_2 gas (Nayak et al., 2016).

2.7.1. Trends in the Published Literature

While many papers have considered this dual remediation scheme, many have only focused on the nutrient removal from the wastewater and ignored the CO_2 capture of the systems (Bolatkhan et al., 2020; Chang et al., 2020; Cohen et al., 2020; Mat Aron et al., 2020; Neves et al., 2018). In the same way as presented in Section 2.5.2, many publications still rely on the equation for CO_2 consumption based on productivity and cell composition rather than directly measuring any difference in CO_2 concentration. This becomes an issue when wastewaters contain organic carbon sources which can be consumed in a mixo- or hetero-trophic manner by the microalgae as seen in the research by Ji *et al.* (2015) and Nayak *et al*. (2016).

Both waste streams are complex in nature and therefore so are their treatments. Combining both to reduce the cost of microalgal biomass production is not as simple as some reviews suggest. The interactions between the algae and the waste and each waste stream need to

be considered to avoid potential issues such as ammonia volatilisation as mentioned previously. Alongside this, the reduced growth rates seen where waste is used in comparison to optimised and clean laboratory systems needs to be considered from an economic point of view. The decreased cost of resources may be offset by the reduced efficiency of the system.

2.8. Gaps in the Literature

The trends that are present throughout the literature have been presented throughout and with these come gaps which have been ignored or not thoroughly researched. This section aims to highlight key gaps within the literature which will be later addressed by the research presented within this thesis.

2.8.1. Shifting the Focus from Biofuels to Bioremediation

Biofuel production has been the central research aim of algal cultivation over the last few decades. Only recently has the focus began to shift away from lipid productivity and towards bioremediation. The focus on nutrient removal (nitrogen and phosphorous) from wastewater and leachates has become more frequent in the literature than the focus on carbon capture. This is highlighted, on several occasions, where the benefits of dual remediation are highlighted but only nutrient removal from the leachate is recorded (Bolatkhan et al., 2020; Chang et al., 2020; Gentili, 2014; Ji et al., 2013). Alongside this, where CO_2 capture is considered, the capture rate is expressed as $g_{CO2}\,L^{1}\,day^{-1}$, as seen in Section 2.5.2, not giving a true indication of the efficiency of the process (Arbib et al., 2013; Cohen et al., 2020; Eze et al., 2018; Mousavi et al., 2018b; Nair et al., 2019; Shen et al., 2015). Only a few publications consider the impact each waste stream will have on the efficiency of removal of the other

(Neves et al., 2018), while many simply make the argument for combining waste streams for dual mitigation (Bolatkhan et al., 2020; Cohen et al., 2020; Jiang et al., 2011; Yadav and Sen, 2017). Therefore, it can be seen that investigations considering how algal bioremediation potential is affected when two waste streams are co-treated is a key gap within the literature. Can both streams be remediated simultaneously with the same efficiency or is further optimisation required?

2.8.2. Interactions Between Waste Streams – Organic vs Inorganic Carbon

Leachates often have a high organic carbon load due to the degradation of organic matter within the landfill itself. This supplies a carbon source to the algae and bacteria which can be utilised in treatment. The addition of gaseous CO_2 may aid in the growth of the cultures and lead to higher nitrogen and phosphorous removals but will the same be true for the carbon content? Many papers have only considered nutrient removal when CO_2 has been supplied to the cultures (Bolatkhan et al., 2020; Chang et al., 2020; Cohen et al., 2020; Mat Aron et al., 2020; Neves et al., 2018). Many have also only considered the CO_2 in terms of the equation based on productivity and carbon content, which neglects the idea that the microalgae can growth both autotrophically and mixotrophically and that not all the carbon which is fixed in their biomass will originate from the gaseous CO_2 supplied.

In an optimal scenario, both sources of CO_2 would be utilised by the algae or algal-bacterial consortia used. Nair *et al.* (2019) found that 10 % CO_2 was the optimal gaseous carbon for *Chlorella pyrenoidosa* cultures grown with 5 % LL in nutrient media. Their results show a 91 % decrease in DOC (dissolved organic carbon) and 74 % reduction of COD over an 18 day period and stated that the consumption of "*inorganic carbon from CO_2 was not significantly affected by the presence of organic carbon in the leachate"*, although no analysis of gaseous CO_2

removal was shown to uphold this statement. Direct measurements of carbon reduction, both from organic and inorganic sources, needs evaluating to understand if the COD/TOC present in LL will have a negative effect on the carbon capture potential of the system.

2.8.3. Economic Analysis & Feasibility

The majority of techno-economic assessments (TEAs) or other economic analyses relating to algal culture are focused on biofuel production. There is little focus on other products and rarely any literature surrounding the economics of bioremediation. The major issue surrounding economic analyses focused on remediation is that the benefits are often incalculable as they have no market value. Environmental benefits are difficult to value in a standardised way and on the occasions that economic analyses of these processes are done shadow prices are required (Molinos-Senante et al., 2010). As legislation becomes more stringent, the requirement of economic analyses becomes easier as a market emerges (in terms of cost saving against taxes or fines) and even more valuable.

Alongside this, there are large differences in assumptions made and expectations, due to the theoretical nature of many TEAs within the literature (Beal et al., 2015; Davis et al., 2011). For example, in many assessments, an average growth rate is assumed to be achieved throughout operation outdoors, utilising free sunlight and heat (Beal et al., 2015; Molina Grima et al., 2003; Tredici et al., 2016). In most of the experimental data, this is not reflected due to the controlled laboratory environment. The 24-hour artificial illumination and temperature control will give better biomass productivity than the use of natural sun light in outdoor cultivation that will lead to light and temperature fluctuations with night-to-day and seasonal changes. In many cases, TEAs assume the use of open raceway ponds (Beal et al., 2015), however, the low growth profile showcased by the experimental data suggests this is not ideal

and the use of the more expensive PBRs is recommended, especially where CO_2 capture is the aim as open ponds allow for large volumes of off gassing to the environment.

Another weakness is the assumptions that the use of CO_2 obtained from flue gases and nutrients from wastewaters will:

a) Increase the growth of the microalgae,
b) reduce anthropogenic emissions by bio-fixation of the CO_2, and
c) cost very little or nothing due to the 'waste' nature of the gas and liquid waste streams (Chisti, 2007; Packer, 2009).

The first point is likely true as elevated CO_2 concentrations (to an extent) have been shown to increase the productivity of many algal species (Kao et al., 2014). The same can be seen for use of wastewaters filled with key nutrients and other trace compounds. This being said, the concentration of harmful pollutants must be kept to a minimum to not 'undo' the benefits of the nutrient supplies. The second point – reduction of anthropogenic emissions – is yet to be realised as the use of open systems and traditional gas sparging, suggested within many of the TEAs, would not only lead to vast amounts of the CO_2 being lost to the atmosphere but large financial costs for the compression and transportation of the gases to the algal farm (Zheng et al., 2017). The assumption of little to no cost is also likely not to hold, as costs will still be incurred at some stage in the process due to the need for compression and transportation of the gas and transport and delivery of large volumes of liquid waste.

All this aside, the economic and life-cycle analysis processes are important and required to give insight into areas of large expense or impracticability in which research should be focused. For many of the past analyses conducted, the areas of most concern are the expense for the reactor and / or the harvesting of biomass from rather dilute cultures (Amer et al., 2011; Molina Grima et al., 2003; Richardson et al., 2014; Thomassen et al., 2016). Both are

well studied within the research area with new reactor designs and harvesting methods being suggested frequently.

2.9. Conclusions

The premise of utilising microalgae for wastewater/leachate bioremediation, carbon mitigation and the production of renewable fuel production is a promising one (Chisti, 2007; Eze et al., 2018; Farrelly et al., 2013; Gentili, 2014; Ji et al., 2015; Lam et al., 2012; Shen et al., 2015; Sydney et al., 2010; Thomas et al., 2016; Wang et al., 2008; Zheng et al., 2017), however, there are still many hurdles which require facing. Like any environmental technology, algal bioremediation must not only achieve its main goal (carbon mitigation, nitrogen and phosphorous removal) but must also be done so in an environmentally sustainable way, meaning a positive energy flow, overall net zero or negative emissions footprint and minimum waste production. As of current, algal bioremediation technologies are often far too expensive, with wastewater treatments relying on purified CO_2 sources and CO_2 capture heavily relying on inorganic fertilisers and artificial lighting which ultimately come with a large carbon footprint, reducing the efficiency of their applications (Lam et al., 2012).

A main driver for algal bioremediation technologies is the potential of a profitable system through the production of by-products from food to fuels and high value products. Therefore, research efforts need to be focused on reducing cost and increasing the efficiency of the carbon capture, nutrient removal, and biomass production processes to compete not only with the traditional biomass cultivation techniques but also traditional CO_2 capture and landfill leachate treatments.

3. Experimental Methods

3.1. Introduction

This chapter details the materials and methods used within the experimental work of this thesis. There are two main sections included within this chapter. Firstly, a description of the algal and bacterial strains, chemicals, equipment, and standard analytical methods used throughout the body of work (Sections 3.2 to 3.4). Secondly, the different experimental set ups and conditions used for each set of experiments (Sections 3.5 to 3.9).

The methodologies utilised within this thesis are mainly comprised of those found in Handbook of Microalgal Culture (Abeliovich et al., 2004), Freshwater Algae (Bellinger and Sigee, 2010), and their surrounding literature or based on the equipment manufacturer's recommendations.

3.2. Algal and Bacterial Strains and Media Preparation

This section details the algae and bacteria used and the media preparation for their growth.

3.2.1. Algae

There were two distinct, unicellular green microalgae used within the body of this work, both *Chlorella* species.

Chlorella vulgaris CCAP 211/211B was readily available within the laboratory research group and a 50 mL healthy culture in Bold Basal Media (BBM) was used as the original seed for this work. The strain was originally purchased from CCAP (Alobwede et al., 2019).

During work with leachate, a microalgal and bacterial consortium was isolated from the local environment (Okurowska et al., 2021a). After genetic and microscopic analysis, it was determined that the main algal strain of this consortium was *Chlorella* sp., dominated by *Chlorella vulgaris*. To maintain transparency throughout on which culture is being used, this consortium will be referred to as an "adapted algal-bacterial consortium" throughout the thesis.

3.2.2. Bacteria

The consortium isolated from the leachate also contained within it several bacterial strains capable of degrading various organic molecules. This was a complex mix and strains which could be successfully isolated were identified by 18S rDNA sequencing as *Pseudomonas* sp., *Lysinibacillus* sp., *Pseudomonas fluorescens, Pseudomonas fluorescnes L12, Streptomonas chelatiphage, Streptomyces* sp., *Paenibaceillus* sp., *Alcaligenes* sp. and *Brevundaimonas diminuta* (Hardo, 2016).

3.2.3. 3N-BBM+V_{12} Autotrophic Media

3N-BBM+V_{12}, a modified Bold Basal media (CCAP, 2007), was utilised for the growth of both *C. vulgaris* and consortia cultures. This media is ideal for the growth of autotrophic, fresh water green microalgae. The standard media preparation includes the addition of vitamin B_1, however, during previous work with similar strains of microalgae it was seen that this is not necessary for optimal algal growth (Leflay, 2017). The composition of the media is shown in Table 3.1.

Table 3.1: *Composition of 3N-BBM+V_{12} media used in microalgal growth.*

Stock solution (g / L)		**Stock solutions in 1 L final medium (mL)**	**Concentration in final medium (mM)**
$NaNO_3$	25	10.0	2.941
$CaCl_2 \cdot 2H_2O$	2.5	10.0	0.170
$MgSO_4 \cdot 7H_2O$	7.5	10.0	0.304
$K_2HPO_4 \cdot 3H_2O$	7.5	10.0	0.329
KH_2PO_4	17.5	10.0	1.286
NaCl	2.5	10.0	0.428
Trace Elements (1 L total volume, add in order)			
1. Na_2EDTA	*0.75*	6.0	13.384 µM
2. $FeCl_3 \cdot 6H_2O$	*97.0 mg*		2.153 µM
3. $MnCl_2 \cdot 4H_2O$	*41.0 mg*		1.243 µM
4. $ZnCl_2$	*5.0 mg*		0.220 µM
5. $Na_2MoO_4 \cdot 2H_2O$	*4.0 mg*		0.099 µM
Vitamin B_{12}	0.1 mg	1.0	73.780 pM
dH_2O		913.0	

*0.1 g Cyanocobalamin added to 100 mL dH_2O (solution 1). 1 mL of solution 1 added to 99 mL of dH_2O and filter sterilised.

In Table 3.1, stock solution A was prepared in a volume of 500 mL, B-F were prepared in 250 mL volumes. Stocks A-F were stored on a lab bench at room temperature (RT) for up to 2 months. The trace element solution (Stock G) was prepared by adding each of the components (1-5), in order, to 1 L dH_2O while mixing with a magnetic stirrer. The solution was then stored at 4 °C and added to prepared medium by syringe and 0.45 µm filter (Millex-PES, Merek).

The Vitamin B_{12} stock (Stock H) was prepared in two stages. An initial 100X concentrated stock was created by addition of 0.1 g Cyanocobalamin to 100 mL of dH_2O. 1 mL of this stock was

then diluted in 99 mL dH_2O to produce the working stock H. Due to the light sensitivity of this compound; all stocks were wrapped in foil to protect the solution. The 100X concentrate was frozen at -20 °C to store and thawed when required. The working stock was kept at 4 °C wrapped in foil.

When preparing media all components were added to clean bottles and then sterilised by autoclaving at 121 °C, 15 psi for 30 minutes. Sterile media was kept on bench at RT for up to 3 weeks before discarding. If any sign of contamination or growth was seen in stocks or prepared media, fresh components were made fresh.

For solid media, bacteriological agar was added to prepared media at a concentration of 15 g L^{-1}. The media was autoclaved and then stored for up to 3 weeks on bench at RT. When plates or slants were required, the media was melted by microwaving at 15 – 30 second intervals until completely liquid. The media was then placed in a water bath at 50 °C to allow the liquid to slowly cool to a pouring temperature without setting. Plates and slants were poured into sterile plastic ware in a laminar flow hood (LS90, Envair) and allowed 45 minutes to set and dry thoroughly before storing in the original packaging at 4 °C. Plates and slants were allowed to warm up to RT in the laminar flow hood for 30 minutes - 1 hour before streaking any cultures.

3.3. General Practices and Equipment

3.3.1. Cultivation Equipment

25 mL - 1 L Erlenmeyer flask were used for microalgal cultivation. Flasks with volumes < 500 mL used foam stoppers and those above this volume used either foam or silicon stoppers. Silicon stoppers had a 6 mm diameter glass tubes inserted for aeration and sampling. The gas

was sourced either from an aquarium pump (Aco-9620, Hailea) or from laboratory gas taps. 8 mm diameter silicon tubing was used to connect the gas tap to the glass tubing in the flask's silicon stoppers. 0.22 µm bacterial air-vent filters (Acro 37 TF, Pall, USA) were placed before gas contact with cultures to remove debris and bacteria from the flow. 0-5 $Lmin^{-1}$ flowmeters (Brooks Instruments, PA, USA) were used to control the gas flow to the cultures.

3.3.2. Sterilisation

All media and glassware were sterilised by autoclave: 121 °C, 15 psi, for 30 minutes. Chemicals and reagents which were susceptible to chemical alteration through autoclave (for example the leachate samples) were sterilised by filtration through 0.22 µm syringe filters (Millex-PES, Merek).

3.3.3. Aseptic Technique

To maintain axenic cultures of *C. vulgaris* and to prevent contamination with unwanted bacteria to the adapted consortium, a laminar flow hood (LS90, Envair, Haslingdon, UK) was used for all experimental set up, harvesting and sampling. 70 % (v/v) ethanol was used to clean the hood before and after use to maintain aseptic conditions.

3.3.4. Cell 'Washing' For Sub-cultures and Experimental Set Up

Whenever cells (of either culture) were transferred from one flask to another, be it for sub-culturing or the start of a new experiment, the samples were washed twice to remove extracellular compounds and old media components. The samples were collected aseptically and then centrifuged at 3,500 rpm for 10 minutes. The supernatant was removed and an equal volume to the original sample of fresh media added. Pellets were gently resuspended by pipetting and then the process of centrifugation was repeated. After the second wash, samples were then transferred to their new cultivation flasks.

3.3.5. Stock Cultures: Short- and Medium-term Storage

Liquid stock cultures of both microalgae cultures were maintained in the laboratory throughout this work. Periodic sub-culturing and washing of cells was completed to ensure a healthy culture. Stocks were maintained in 25 – 100 mL volumes in foam stoppered conical flasks of appropriate size. Cultures were kept in 24-hour light conditions (150 – 200 µmol m^{-2} sec^{-1}), RT and where possible, on an orbital shaker (120 rpm). Stock cultures were maintained for 4-6 weeks or until a dark green colour had formed and cells were beginning to floc and settle. Occasionally, 2 mL samples were taken for microscopy to check the health of the cultures (Section 3.3.9).

Solid cultures were maintained for medium-long term storage. Cultures were streaked out on slants of 3N-BBM+V_{12} agar and kept at room temperature under standard light conditions. Slants were kept for ~ 6 months before cells were transferred to fresh agar.

3.3.6. Long-term Storage

For the long-term storage of cultures, samples were cryopreserved and kept at -80 °C. This method utilises a Mr Frosty cryopreservation chamber (Sigma-Aldrich, UK), TS/80-MX cryo-vials (TSC Ltd, Lancashire, UK) and dimethyl sulfoxide (DMSO) as the cryoprotectant. Before implementation:

1) The Mr Frosty was filled with 100 % isopropyl alcohol (according to equipment instructions) and maintained at RT;
2) A 10 % DMSO in 3N-BBM+V_{12} media was prepared and autoclaved to sterilise and
3) The liquid media in the pre-filled cryo-vials was removed by sterile pipette, leaving only the beads.

Cultures were checked under the microscope for cell health and bacterial contamination. Healthy, dense cultures were then sampled (5 mL) and washed using the previously described

method (Section 3.3.4). For the final addition of fresh media, 0.5 mL was added instead of 5 mL, creating a concentrated sample. 0.5 mL of sample was then transferred to the prepared cryovial along with 0.5 mL of the 10 % DMSO 3N-BBM+V_{12} media. Vials were lightly vortexed to mix and allow the algae to enter the beads. Vials were then placed in the Mr Frosty and transferred to the -80 °C freezer for a minimum of 24 hours. After the initial freeze, the vials can be moved to an appropriate storage container for long-term storage.

When cryopreserved cultures were required, the vials were placed in a 37 °C water bath for minimum 1 hour, until thawed. The liquid contents were then transferred to 50 mL of fresh media in a sterile flask. Cultures required incubation for a minimum of 7 days before growth was seen.

3.3.7. pH Analysis

pH of cultures was monitored using an Orion three star benchtop pH probe (ThermoScientific, UK) with premade buffers at pHs of 4.01, 7.00 and 9.21 (Hamilton, Romania). The probe was calibrated using the manual three-point calibration and the previously mentioned buffers.

3.3.8. Spectroscopy

Optical density (OD) of cultures was used to track growth throughout experiments and was determined using a UV-visible spectrophotometer. Spectronic 200E (ThermoFisher Scientific) and UV-10 UV-VIS (Thermo Scientific) spectrophotometers were used throughout this experimental work. OD was measured at 695 nm (OD_{695}), ideal for measuring algal growth as it is outside the range of chlorophyll.

For the Spectronic 200E spectrophotometer, VisionLite software was used to record OD readings on a desktop computer. The ‘fixed’ programme was used. For the UV-10 UV-VIS spectrophotometer, the fixed program was used, and readings recorded by hand.

1.5 mL polystyrol/polystrene cuvettes (Sarstedt) were used with a 1 mL culture sample. A blank comprised of sterile media was used to zero the spectrophotometer before reading samples. All samples were mixed, and bubbles allowed to dissipate before placing in the spectrophotometer. Each samples absorbance was determined three times to give an average absorbance. Absorbance was only recorded if it fell within the linear range of the spectrophotometer (0.1-1 Abs). If the reading fell below this point an additional sample of culture was taken, centrifuged and the pellet resuspended in half the volume before re-reading. If the reading went above an absorbance of 1, the sample was diluted using sterile dH_2O. The reading was then multiplied by the dilution factor to obtain the actual OD:

$$Optical\ Density = Absorbance \times Dilution\ Factor \quad 3.1$$

Due to the unexpected breakdown of the Spectronic 200E spectrophotometer, a calibration curve of the old and new (UV-10, UV-VIS) was not created, therefore for each spectrophotometer used a calibration curve of dry weight vs OD (Section 3.3.10) was established to allow comparison of results. This was because each machine gave a slightly different reading to the other.

3.3.9. Microscopy

Two microscopes were used to monitor algal cultures and cell health: BX51 (Olympus) and Axiostar Plus (Zeiss). A ProgRes C5 lens (Jenopik, Germany) was connected to the BX51 microscope and images were captured using the ProgRes 2.6 CapturePro (Jenoptik, Germany) program.

3.3.10. Dry weight vs Optical Density Standard Curves

Standard curves relating optical density to dry weight were established for each culture and spectrophotometer used. A well-grown culture (of at least 200 mL) was adjusted to an OD_{695} = 1.0 with media. The dilutions shown in Table 3.2 were then created from this. Each dilution was mixed thoroughly and then 1 mL was transferred to a cuvette for OD readings, media was used as a blank. The 1 mL samples were then carefully returned to each dilution and the tubes were centrifuged at 3,500 rpm for 10 minutes. The supernatant was carefully removed, leaving the pellet intact and 10 mL of dH_2O was added. The pellets were resuspended by vortexing and then each dilution was transferred to an appropriately labelled 15 mL falcon tube. The dilutions were then centrifuged again under the same conditions. This time the supernatant was removed, and algal pellets were resuspended in 1 mL of dH_2O before transferring to Eppendorf tubes, which have had their weight pre-recorded. The tubes were centrifuged at 10,000 rpm for 10 minutes and as much supernatant removed as possible without affecting the pellet. The tubes were then frozen at -20 °C for 24 hours before freeze-drying (Lyoquest, Telstar) for 4 hours or until completely dry. The tubes were then reweighed in triplicate and the dry weight was calculated as follows:

$$Dry\ Weight\ (g) = dry\ tube\ weight\ (g) - empty\ tube\ weight\ (g) \qquad 3.2$$

$$g\ L^{-1} = Dry\ weight\ (g)\ \times \frac{1000}{50} \qquad 3.3$$

The OD was then plotted against the dry weight (y-axis) and a line of best fit through (0,0) established, Figure 3.1, Figure 3.2, and Figure 3.3.

Total dry weight (weight with ash) was used for each experiment as downstream processing of the biomass into by-products was outside of the scope of analysis.

Table 3.2: *Dilutions for dry weight vs OD standard curve.*

Dilution number	Concentration (%)	Culture (mL)	Media (mL)
1	100	50	0
2	90	45	5
3	80	40	10
4	70	35	15
5	60	30	20
6	50	25	25
7	40	20	30
8	30	15	35
9	20	10	40
10	10	5	45
11	0	0	50
Total volume required:		275	275

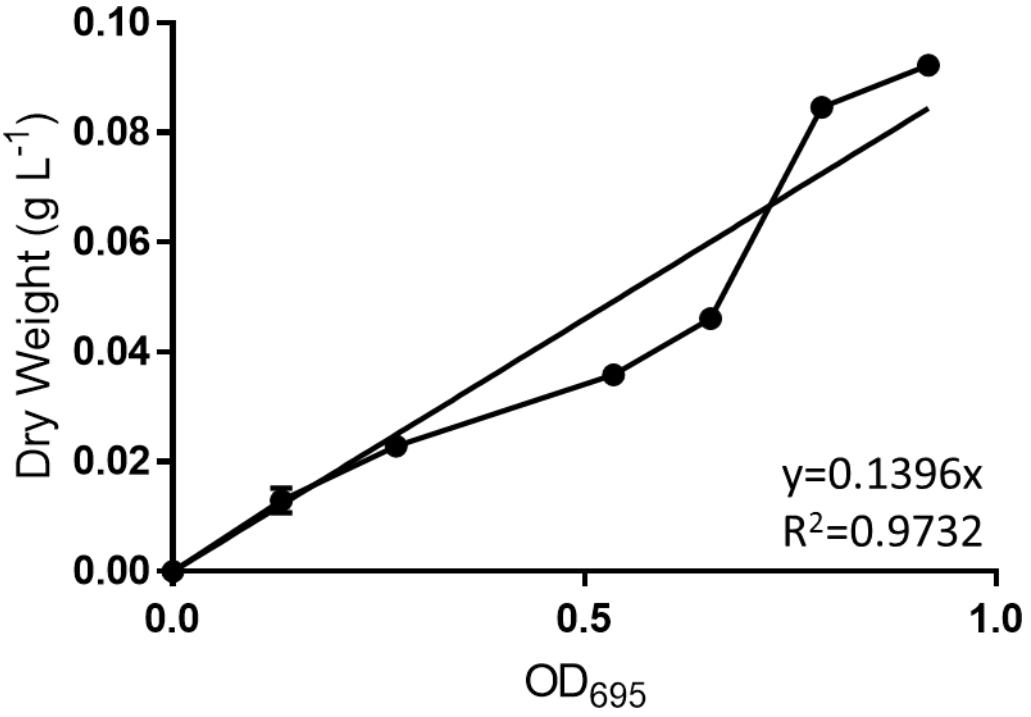

Figure 3.1: *Calibration curve of C. vulgaris dry biomass weight against the optical density (OD) at 695 nm on the Spectronic 200E (Thermo Fisher) photospectrophotometer.*

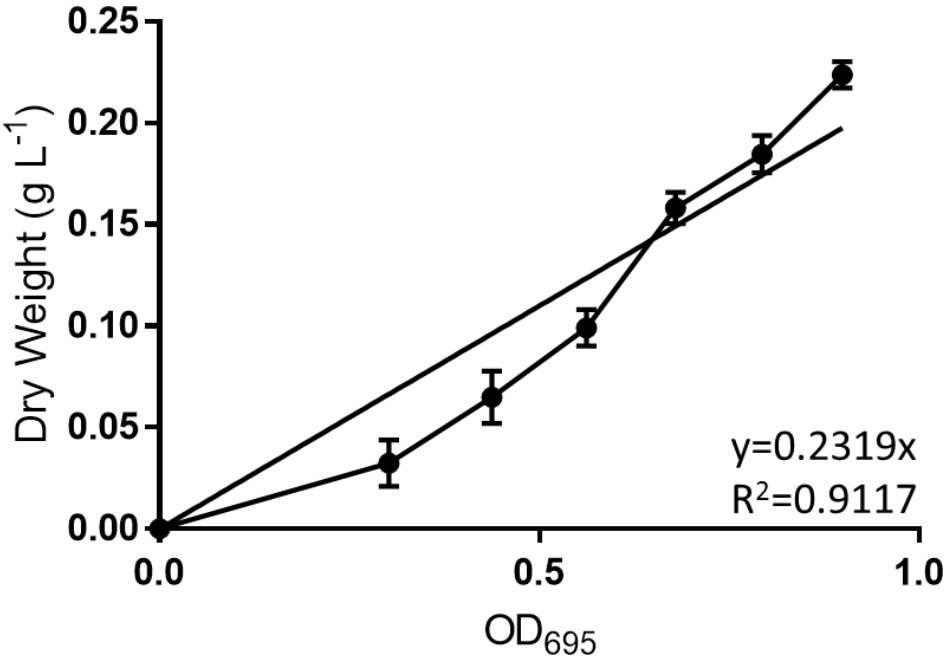

***Figure 3.2**: Calibration curve of C. vulgaris dry biomass weight against the optical density (OD) at 695 nm on the UV-VIS10 (Thermoscientific) photospectrophotometer.*

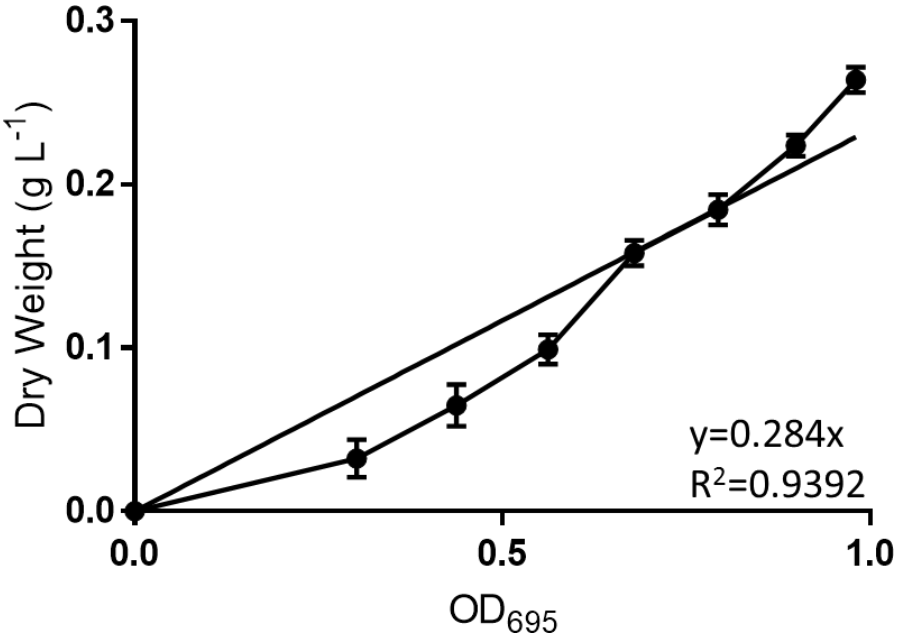

***Figure 3.3:** Calibration curve of dry biomass weight for the landfill leachate algal-bacterial consortia against the optical density (OD) at 695 nm on the UV-VIS10 (Thermoscientific) spectrophotometer.*

3.3.11. Sampling Methods

In small volume flasks sampling was conducted aseptically under laminar flow using sterile pipettes, removing the minimum volume of culture required for processing. Where cultures were aerated, an additional port was present in the silicon stopper to which a sterile 20 mL luer syringe could be attached to withdraw culture without disturbing the gas flow or experimental set up. The glass tube through the port was suspended within the culture volume and the silicon piping to which the syringe attached was clamped shut between samples to prevent loss of culture.

3.3.12. Storage Methods

Any samples (both biomass and media) which required storage before analysis were kept at -20 °C. Viable cell cultures (cryopreserved algal and bacterial cultures) were kept at -80 °C in appropriate cryotubes.

3.4. Analytical Methods

3.4.1. Ammonia Analysis

Ammonia concentration in leachate and algal samples was determined by use of the modified Nessler method, proposed by Jeong *et al.* (2013). The method relies on the production of a yellow coloured-species by the reaction of the mercury potassium iodide within the Nessler reagent and the ammonia within the sample. The colour intensity is directly proportionate to the ammonia concentration. Hardening agents such as chlorine and magnesium can affect the accuracy; therefore, PVA (polyvinyl acetate) and a mineral stabiliser (Cat 2376626, Hach) are added in the modified method.

New PVA was prepared every time the analysis was done alongside a new standard curve using ammonium chloride dissolved in dH_2O. PVA was prepared by dissolving 0.135 g in 100 mL dH_2O (0.135 % w/v solution). The standard solutions were produced by first dissolving 0.294 g of NH_4Cl in 100 mL dH_2O to give a master stock of 1 gL^{-1} NH_4^+. This solution was filtered to sterilise and then stored at RT. Dilutions of ammonia were created using this stock according to Table 3.3:

Table 3.3: *Dilutions for ammonia standards*

Dilution number	Concentration (mg L^{-1})	Ammonia stock (mL)	dH_2O (mL)	Final Volume (mL)
1	10	0.5 from master stock	49.5	50
2	8	8 from #1	2	10
3	6	6 from #1	4	10
4	5	5 from #1	5	10
5	4	4 from #1	6	10
6	2	2 from #1	8	10
7	1	1 from #1	9	10
8	0	0	10	10

1mL of each dilution was pipetted into a clean 2 mL Eppendorf tube. 20 µL of the mineral stabiliser was added to each tube before vortexing to mix thoroughly. 20 µL of PVA was then added and mixed in the same fashion. Finally, 40 µL of the Nessler reagents (mixed thoroughly beforehand) was added and the tubes mixed once again. A yellow colour then develops as the reaction takes place. Each sample was transferred to a clean cuvette and the absorbance at 425 nm was measured in a spectrophotometer against a blank prepared in the same way with dH_2O. Each sample was measured in triplicate. Absorbance was plotted against the concentration and a line of best fit through (0, 0) was established to relate NH_4^+ concentration to absorbance. The R^2 value was used to assess the accuracy of the calibration curve, a value more than or equal to 0.95 or higher was accepted. The equation produced from the line of

best fit was used to convert the absorbance for each experimental sample to a concentration. Figure 3.4 shows one of the calibration curves achieved while testing the analysis method.

The same protocol was repeated for experimental samples, where possible duplicate runs were carried out. In some cases (for example, undiluted leachate) a dilution was required beforehand, and this was done with sterile dH_2O. All samples were filtered through 0.22 µm syringe filters before analysis. Samples were filtered and then stored at -20 °C if analysis was not to be conducted on the same day. Where possible, all samples were stored, and each set was analysed at the same time using a new standard curve produced on the same day with the same reagents.

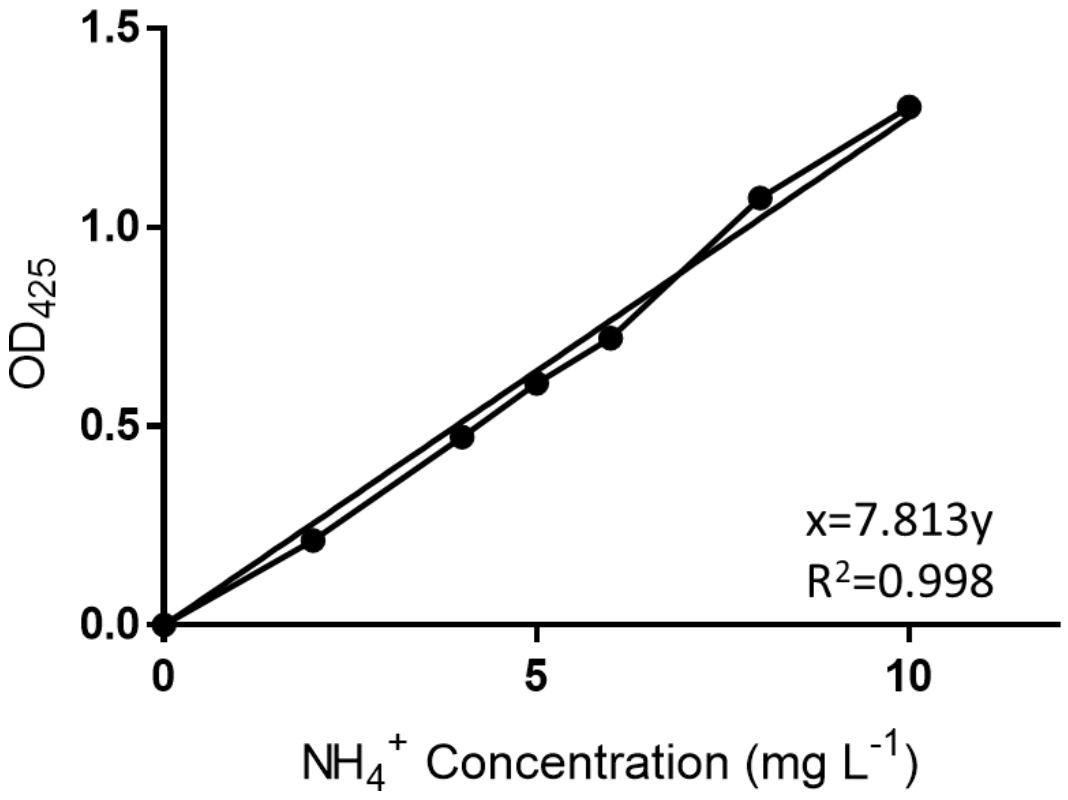

Figure 3.4: *Nessler Calibration curve with ammonium chloride as the standard*

3.4.2. Phosphate Analysis

Dissolved inorganic phosphate (DIP), was measured using the molybdenum blue colorimetric assay from Murphy and Riley (1962). This method utilises the formation and reduction of phosphomolybdic acid, resulting in a blue/purple colour forming which is directly proportionate to the phosphate concentration and can be measured at a wavelength of 880 nm.

This analysis method requires a mixed reagent, which should be prepared on the day of analysis and not stored for longer than 24 hours. This mixture is referred to as the *'cocktail'* and is described in Table 3.4. Master stocks of all the components were produced ahead of time and stored for no more than 2 weeks at RT.

Table 3.4: *Molybdate phosphate assay stock preparations*

Component	**Master Stock Makeup**	**Amount required for the cocktail**
5N Sulfuric acid	70 mL of concentrated H_2SO_4 in 500 mL dH_2O	25 mL
Antimonyl Potassium Tartate	Dissolve 4.39 g in 200 mL dH_2O	2.5 mL
Ammonium Molybdate	Dissolve 20 g in 500 mL dH_2O	7.5 mL
Ascorbic Acid	Dissolve 0.325 g in 20 mL of dH_2O	15 mL
Final Volume of the DIP assay cocktail:		**50 mL**

The standard solution for this method utilises monosodium phosphate. 0.6 g was dissolved in 50 mL of dH_2O to make stock A with a concentration of 100 mM. This was filtered prior to further dilutions. A 100X dilution was then prepared by adding 0.5 mL to 49.5 mL of dH_2O – stock B with a concentration of 1 mM. A further 10 X dilution of stock B was carried out to

produce the final stock C with a concentration of 100 µM. The standard dilutions were then prepared according to Table 3.5.

Table 3.5: *Standard PO_4^{3-} dilutions*

Dilution number	Concentration (µM)	Stock C (mL)	dH_2O (mL)	Final volume (mL)
1	60	6	4	10
2	50	5	5	10
3	40	4	6	10
4	30	3	7	10
5	20	2	8	10
6	10	1	9	10
7	5	0.5	9.5	10
8	0	0	10	10

1 mL of each dilution was added to a clean cuvette. A blank was set up using dH_2O. 50 µL of 100 % ethanol and 50 µL of the cocktail were added to each cuvette before the samples were mixed by pipetting the mixture up and down. The cuvettes were left to develop for approximately 30 minutes. A blue colour develops during this time. Absorbance at 880 nm was measured using the water blank, each dilution was measured in triplicate. Absorbance was plotted against the concentration and a line of best fit through (0, 0) was established to relate DIP concentration to absorbance. The R^2 value was used to assess the accuracy of the calibration curve, a value more than or equal to 0.95 or higher was accepted. The equation produced from the line of best fit was used to convert the absorbance for each experimental sample to a concentration. Figure 3.5 shows one of the calibration curves achieved while testing the analysis method.

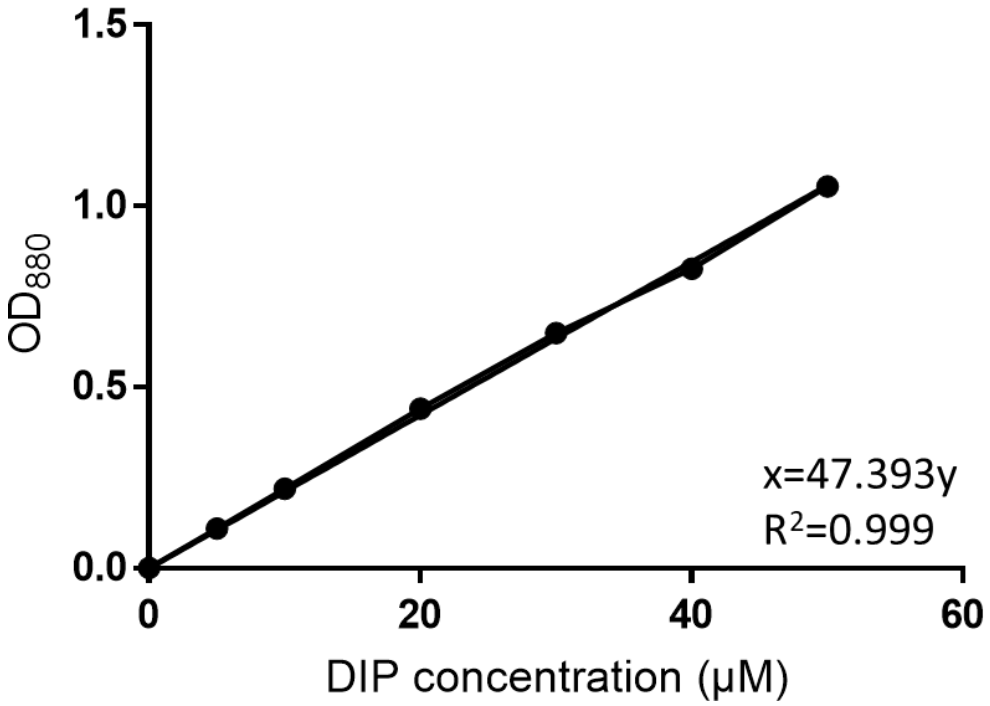

Figure 3.5: *DIP calibration curve using NaH_2PO_4 as the standard.*

The same procedure was repeated for experimental samples, where possible duplicate runs were done. In some cases (for example, undiluted leachate), a dilution was required beforehand, and this was done with sterile dH_2O. All samples were filtered through 0.22 µm syringe filters before analysis. Samples were filtered and then stored at -20 °C if analysis was not to be conducted on the same day. Where possible, all samples were stored, and each set was analysed at the same time using a new standard curve produced on the same day with the same reagents.

3.4.3. Nitrate and Nitrite Analysis

The protocol used for the determination of both nitrate and nitrite in leachate and algal samples is based on the protocols presented in Miranda *et al.* (2001). The protocol works in two stages: 1) vanadium (III) chloride is used to reduce nitrate to nitrite, 2) the total nitrite concentration is then determined through the modified Griess reaction.

This assay required multiple reactive reagents, which can be prepared in advanced. To prepare the vanadium stock, 0.4 g vanadium (III) chloride was dissolved in 100 mL of 1 M HCl. Once dissolved the solution was filtered through a 0.45 µm syringe filter to remove any remaining solids. A NEED (N-(1-Naphthyl)ethylenediamine dihydrochloride) solution was made by dissolving 0.1 g in 100 mL dH_2O. Finally, a Sulphanilamide solution was produced by dissolving 2 g in 100 mL 5 % HCl. All three of these solutions were prepared in advanced and stored wrapped in tin foil at 4 °C for up to 2 weeks. If colour appeared in any of the reagents, they were discarded and remade. When ready for analysis the NEED and sulphanilamide solutions were mixed in a ratio of 1:1 (v/v) to produce reaction mixture A. 500 µL of this is required per cuvette.

Potassium nitrate (KNO_3) was used as the standard for calibration in this assay. A 100 mM NO_3^- stock was produced by dissolving 1.0114 g KNO_3 in 100 mL of dH_2O. This was then diluted 10X in dH_2O to achieve a concentration of 10 mM. This 10 mM solution was then filtered using a 0.22 µm syringe filter. A 250 µM working stock (Stock C) was made by adding 1 mL of the 10 mM stock to 39 mL of dH_2O (40X dilution). The standard dilutions were then made according to Table 3.6:

Table 3.6: *Standard NO_3^- concentrations*

Dilution Number	Concentration (µM)	Stock C (mL)	dH_2O (mL)	Final Volume (mL)
1	250	10	0	10
2	200	8	2	10
3	150	6	4	10
4	100	4	6	10
5	50	2	8	10
6	0	0	10	10

500 µL of each standard (dH_2O in the blank) was added to clean cuvettes. 500 µL of the vanadium mixture was added to each cuvette followed by 500 µL of reaction mixture A. Cuvettes were mixed thoroughly before incubating at room temperature for 30 minutes. Once the incubation time had elapsed, the absorbance of each cuvette was measured at 540 nm against the water blank. Absorbance was plotted against the concentration and a line of best fit through (0, 0) was established to relate the nitrate concentration to absorbance. The R^2 value was used to assess the accuracy of the calibration curve, a value more than or equal to 0.95 or higher was accepted. The equation produced from the line of best fit was used to convert the absorbance for each experimental sample to a concentration. Figure 3.6 shows one of the calibration curves achieved while testing the analysis method.

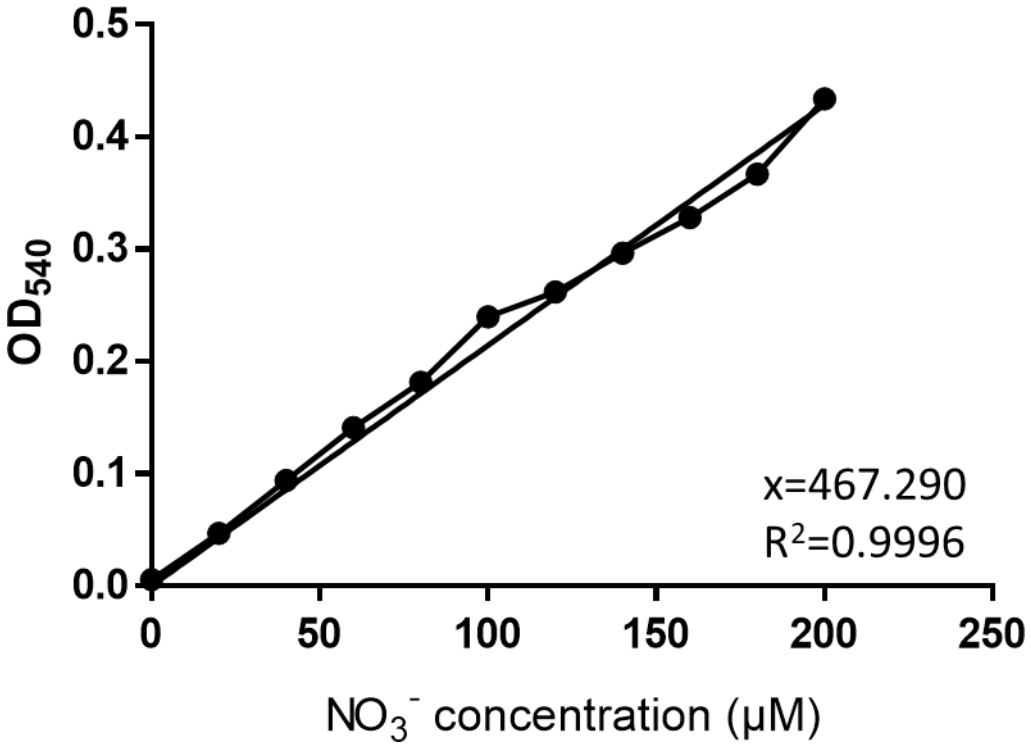

Figure 3.6: *Vanadium/Griess assay calibration curve example*

The same procedure was repeated for experimental samples, where possible duplicate runs were done. In some cases (for example, undiluted leachate), a dilution was required beforehand, and this was done with sterile dH_2O. All samples were filtered through 0.22 µm syringe filters before analysis. Samples were filtered and then stored at -20 °C if analysis was not to be conducted on the same day. Where possible, all samples were stored, and each set was analysed at the same time using a new standard curve produced on the same day with the same reagents.

3.5. *Chlorella vulgaris* Growth Set Up

3.5.1. Growth with No Aeration

C. vulgaris was cultivated under a variety of different CO_2 concentrations to fully assess whether air or CO_2 aeration aided growth. Growth data with no aeration was required for comparison. Cultures were grown in 500 mL working volume in 1 L Erlenmeyer flasks (Figure 3.7). All experiments were carried out in triplicate with a media only control alongside. Stock cultures were used to seed the flasks with the culture being washed (Section 3.3.4). The initial OD_{695} was set to 0.1. To ensure all culture flasks had the same starting cell concentration, a 1.5 L mixture at the desired OD was created by mixing the washed cells and sterile media. The ratio of cells to media was determined using Equation 3.4:

$$C_1V_1 = C_2V_2 \qquad 3.4$$

Where C_1 is the concentration of the cells inoculum (OD) and V_1 is the unknown volume required (mL) and C_2 is the desired concentration (0.1) and V_2 the desired final volume (1500 mL). 500 mL was then decanted to each of the triplicate flasks. 500 mL sterile media was poured directly into the control flask. The flasks were incubated under continuous light at RT

with continuous mixing at 120 rpm. 5-10 mL samples were taken aseptically every 2-4 days for OD, pH, and analytical measurements. Experiments lasted either 14 days, until stationary phase was achieved, or the control became visibly contaminated.

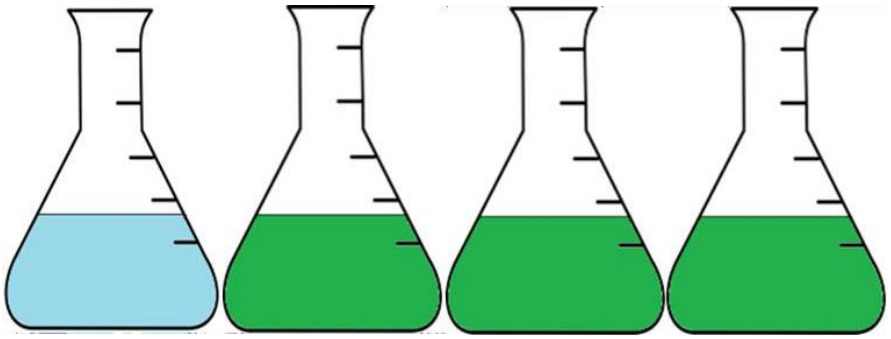

Figure 3.7: *Schematic representation of flasks set up in the no-aeration growth trials. Three experimental replicates (shown as green flasks in the image) were set up using the same algal-media mixture. The control (shown as a blue flask in the image) was media only (no algae) to confirm there are no changes in pollutant or CO_2 uptake without the algae present.*

3.5.2. *Chlorella* sp. Growth Under Varying CO_2 Concentrations

To obtain the optimal CO_2 concentration for *C. vulgaris* growth, cultures were grown under several different aeration regimes: air (0.04 % CO_2), 5 % CO_2, 10 % CO_2, 15 % CO_2. Experiments were ran in triplicate with a media only control as the fourth flask (Figure 3.8).

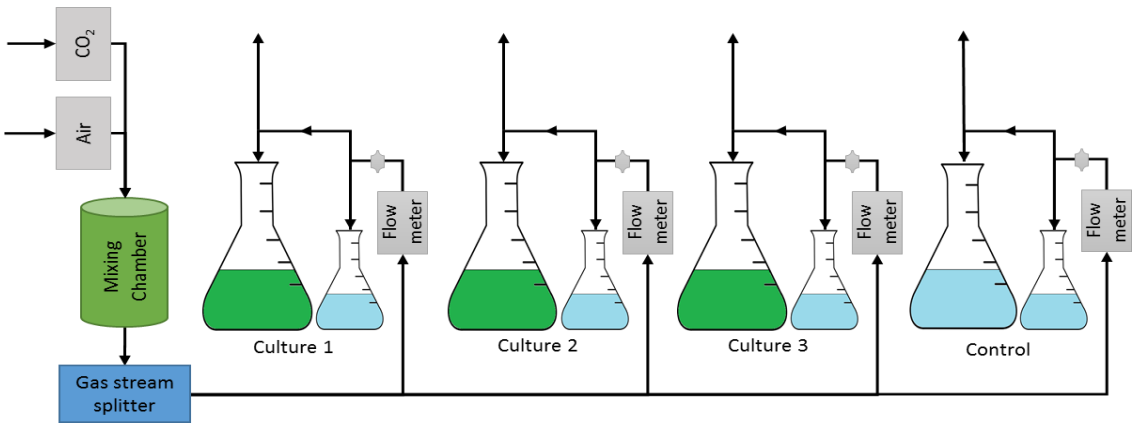

Figure 3.8: *Schematic representation of how flask cultures were set up for aerated growth trials.*

The CO_2 and air were supplied by gas taps to the laboratory and were mixed in a mixing chamber to achieve the desired CO_2 concentration as seen in Sophonthammaphat (2018). The concentration of CO_2 was determined by flow rate *i.e.,* the 5 % CO_2 concentration was achieved through mixing 0.5 lpm (litres per minute) CO_2 and 9.5 lpm air. The gas stream exiting the mixing chamber was then split using a gas splitter (RS Pro, USA) and each culture flask had a direct line. The flow of gas entering each culture was controlled and monitored individually by flow meter (FR2000 series, Key Instruments, USA) to ensure a consistent 1 L min^{-1} (2 vvm (volumes per volume minute)) to each flask throughout the experiment. Before entering the culture, the gas was passed through a 0.22 µm bacterial air-vent (Acro 37 TF, Pall) and hydrated by passing through a hydration flask (200 mL working volume) containing sterile dH_2O. It was found that the gas bubbling was sufficient to keep cells in suspension and therefore no additional mixing was required. Cultures were maintained under the same conditions presented in Section 5.2.1. Sampling was achieved without disturbing the gas flow using a sterile syringe on the sampling port of the silicon bungs. Sampling was conducted every 2-3 days and cultures were mixed by hand before each sample to ensure a homogenous and representative sample was taken.

3.6. Measuring CO_2 Reduction Efficiency

The optimal conditions for growth were then used in the same manner for the cultivation of one flask culture (Figure 3.9) connected to a non-dispersive infrared (NDIR) sensor (CO2meter.com, USA). The single flask experiment was conducted on three separate occasions. A fourth run with media only was conducted as a control. Before attaching the culture to the gas supply, the CO_2 and air mixture was measured with the sensor over a 0.5-

2.5-hour interval to ensure the correct concentration of CO_2 was flowing. The volume flow rates used to maintain the CO_2 concentration were then maintained throughout the experiment to achieve a uniform gas flow to the culture. The sensor took readings of CO_2 concentration (%), temperature (°C) and humidity (%) every minute throughout the entire growth period (14 days). The experimental set up was the same as stated previously with two exceptions: 1) the flow rate entering the culture was reduced to 0.5 L min^{-1} (1 vvm) to further reduce evaporative losses seen during the preliminary experimentation and 2) the addition of a shaker unit underneath (115 rpm) to keep the culture in suspension as settling of the biomass during the latter half of the experiment became a major concern during the previous experiments.

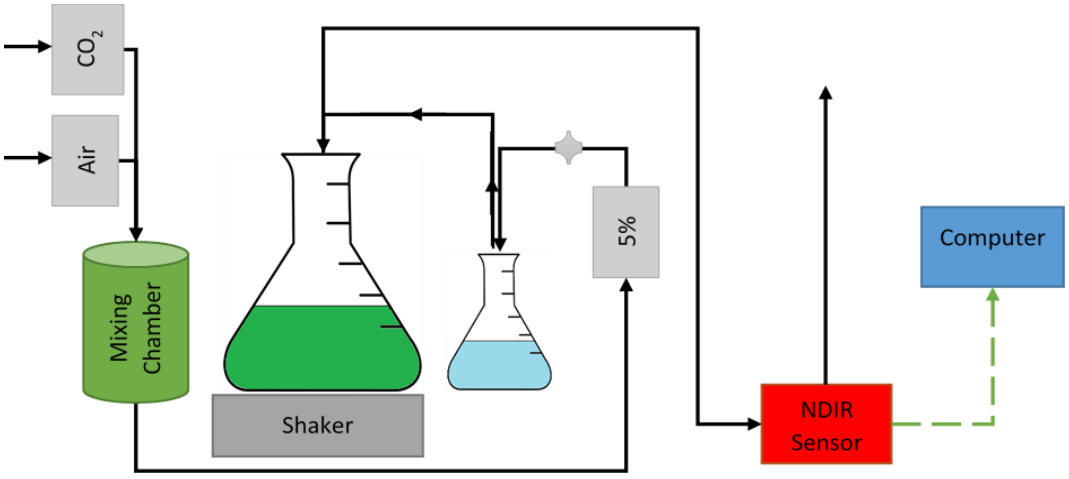

Figure 3.9: *Schematic representation of how individual flask cultures were aerated and monitored for CO_2 output.*

3.7. Consortia Growth with Leachate as a Nutrient Source

During the identification and adaption work performed with the consortia (Okurowska et al., 2021a), the optimal conditions for growth and nutrient removal were revealed as 10 % landfill leachate (LL) diluted in sterile dH_2O with additional key nutrients nitrogen and phosphorous

in the source of those found within BBM media (Okurowska et al., 2021a). The leachate dilution was supplemented with 10 mL L^{-1} of 25 gL^{-1} $NaNO_3$ and 10 mL L^{-1} of 7.5 gL^{-1} K_2HPO_4 and 17.5 gL^{-1} KH_2PO4, which were previously sterilised by autoclave.

3.8. Consortia Growth Under Varying Aeration Conditions with Leachate as a Nutrient Source

The same conditions as those used for *C. vulgaris*, described in Sections 3.5 were used for the consortium with different aeration regime work. The major change was that the OD_{695} was set to an initial value of 0.2 rather than 0.1 due to the inhibitory brown colour of the leachate preventing light penetration to the culture at lower densities. Sampling was conducted in the same manner, but a larger volume of 10 mL was taken every 3-4 days (5 samples total over the 14 day growth period) to allow for nutrient analysis. 6 mL of each sample was filtered through 0.22 µm syringe filters into three sterile 2 mL snap top Eppendorf tubes and frozen to store for ammonia, nitrogen, and phosphorous analysis. Of the remaining 4 mL, 1 mL was used for OD analysis and the rest for pH measurements and, if required, checking the culture under the microscope for contamination/anomalies.

3.9. Measuring CO_2 and Nutrient Removal Efficiency in Tandem

This process followed the same procedure as that described in Section 3.6 with a change in starting OD_{695} to 0.2 rather than 0.1 due to the inhibitory effects of the leachate colour as described previously. All experimental flasks were used to seed the next culture and the media (leachate dilution) control was conducted afterwards.

3.10. Statistical Analysis and Graphs

Where possible, results were obtained in triplicate and the average value used. All graphs were produced using Prism 9.0 (GraphPad, UK). All graphs show the mean point and standard error. Where no error bars are visible on a graph point, the error is smaller than the symbol used. Statistical analysis (one-way ANOVAs and un-paired t-tests) were performed using the pre-programmed methods within Prism 9.0. Results were accepted as statistically significant where the P value was < 0.05. Linear regressions were performed using the 'fit a line with simple linear regression' method within the analysis options.

4. Methods: Techno-Economic Analysis Based on Experimental Data

4.1. Introduction

Understanding the economics of a technology, particularly new and emerging technologies, is key to structuring research and development so as to improve economic viability and therefore increase uptake into the market. While Techno-Economic Assessments (TEAs) are a powerful tool which has been implemented plentifully within the open-cultivation and biofuels research area (Amer et al., 2011; Beal et al., 2015; Davis et al., 2011; Jonker and Faaij, 2013; Koutinas et al., 2014; Ou et al., 2015; Taylor et al., 2013; Xin et al., 2016), there is a lack of analyses considering closed-cultivation systems and ones which are not geared towards the sole purpose of producing biodiesel or bioethanol (Tredici et al., 2016).

This chapter details the methods used to construct the TEA model used in conjunction with experimental inputs. The methods chosen incorporates the underlying principles and assumptions of the current literature with a change in focus from biofuel production to treatment efficiency and cost, and crude biomass production and cost. The base model, used as a starting point for each different waste steam, is described here with alterations for specific wastes (CO_2 from flue gas or nitrogen and phosphorous from landfill leachate) described in the corresponding results chapters.

The aim of the TEA model is to indicate the annualised cost of treating a unit of waste and simultaneously producing a unit of wet algal biomass which can be sold on for downstream processing into a variety of products. The TEA is constructed in Microsoft Excel 2020 and can

be easily altered allowing different financial, operational, and capital-investment scenarios to be considered in tandem. Alongside the waste treatment and biomass production cost, the model highlights how key parameters such as batch operation time, growth rate of the algae, remediation efficiency affect the overall cost individually to highlight where research and development should be focused going forward.

4.2. Software

All analysis was conducted in Microsoft Excel (2020), utilising the various functions offered including data and pivot tables. Excel was chosen for this work due to its easy user interface and flexibility allowing changes to be made easily once the model was set up. The workbook was set up so that each sheet described a different aspect of the costs or benefits, i.e. capital expenditure, operational expenditure, experimental data input *etc.* and fed into the net cash flow (NCF) forecast where analysis such as rate of return and net present value (NPV) were also calculated. Each sheet was set up so that a user could enter new information and the NFC would automatically update based on the given information. For example, on the financial assumptions worksheet, the size of facility could be changed, altering the number of photobioreactor (PBR) units required and therefore facility capacity and production capability, allowing different scales of cultivation to be considered. This was done so that scenario-based analysis could be conducted where one or two key parameters were altered but the rest remained constant between scenarios.

All tables were formatted in Excel and graphs and diagrams in GraphPad Prism 9.0. Statistical analysis such as ANOVA and t-tests were also conducted within the GraphPad software.

4.3. Basic Principles: Facility Description and Scope of Analysis

The underlying principles and assumptions of the theoretical facility will be detailed in this section. Like many TEAs in algal biomass production the facility is theoretical (Asmare et al., 2013; Davis et al., 2013) and not based on an existing plant, therefore a variety of assumptions are required. Where possible these have been based on information available, for example, the land purchase price, water, and electricity rates are all reflective of those to the geographical location chosen. Where this is not possible, the information has been taken from the surrounding literature, either as exact figures or an average of the range used. Items in this section include the contingency and maintenance planning costs. Items such as labour will be site and production method specific and therefore are not well represented in the literature. These are estimated on the chosen facility size and real-life figures for the job titles used.

The TEA is based on a 1 ha facility, as seen in the literature (Tredici et al., 2016), which consists of a small laboratory and office space for inoculum preparation and sampling/analysis and a set of 300 L PhycoFlow™ PBRs (VariconAqua, UK), shown in Figure 4.1 . The number of units which could be feasibly allocated to the space was calculated using the spatial requirements of a single PBR and considering the requirement of an '*access perimeter*' between each unit for maintenance (1m from the PBR edge). With both factors considered, it was proposed that the 1 ha facility would house 738 individual PBR units, resulting in a total capacity of 221 m^3.

Figure 4.1: *300 L Phycoflow™ unit set up with and without the polycarbonate casing attached.*

4.3.1. Photobioreactor Information

The 300 L PBR is made of Duran borosilicate tubes with a 5 cm inner diameter. These are arranged horizontally in a serpentine fence design as discussed in the literature review. The system includes a 100 L non-transparent plastic tank from which the culture is pumped into the glass light-harvesting portion of the reactor. The PBR is encased in a Sunlite Multiwall polycarbonate unit which has a light transmission efficiency of 83 %, Figure 4.1. This was to protect the cultures from extreme temperature variations, particularly in the winter where the average temperature in Sheffield is 4 .4°C (Met Office, 2021).

While VariconAqua supply a similar, larger unit to the one mentioned above, the 300 L model has been purchased by the Pandhal research group and used for pilot scale leachate remediation work (Leflay et al., 2020), therefore detailed capital and operational figures can be used as input for the model. Alongside this, the location of the facility was also set as the North of England, with the water, electricity and land prices reflecting this. Data for operation in the winter months, when limited sunlight and low temperatures are inhibitive to growth, has already been procured, allowing these hinderances to be considered within the analysis.

4.3.2. Financial Assumptions

There are a variety of assumptions made in the base model, mostly relating to the capital expenditure and contingency planning. A detailed list of assumptions and references of their use in similar TEA and cost-benefit analyses for algae biomass production is shown in Table 4.1.

Table 4.1: *Assumptions made in the base model.*

Assumption	Value	Units	Reference
Project lifetime	20	years	(Davis et al., 2011; Doshi et al., 2017; Gallagher, 2011; Ventura et al., 2013; Wiesberg et al., 2016; Xin et al., 2016; Zamalloa et al., 2011; Zhang et al., 2017)
Depreciation of assets	Straight line, no salvage value		(Amer et al., 2011; Doshi et al., 2017; Tredici et al., 2016)
Maintenance budget	10 %	Of direct capital cost	(Davis et al., 2011, 2013; Doshi et al., 2017; Molina Grima et al., 2003; Tredici et al., 2016; Ventura et al., 2013)
Contingency allowance	15 %	Of direct capital costs	(Nagarajan et al., 2013; Ou et al., 2015)
Labour overheads	60 %	Of labour costs	(Brownbridge et al., 2014; Davis et al., 2011; Ventura et al., 2013)
Inflation	2.7 %		UK average from 2014-2017 (Bank of England, 2018)

The project lifetime considered is 20 years. This reflects the literature which varies from 15 – 30 years in length (Davis et al., 2011; Doshi et al., 2017; Gallagher, 2011; Ventura et al., 2013; Wiesberg et al., 2016; Xin et al., 2016; Zamalloa et al., 2011; Zhang et al., 2017). A construction period of 1.5 years is assumed at the beginning of the project for land and equipment acquisition, installation, and commissioning (Doshi et al., 2017). During this time, it is assumed that there is no treatment or biomass production during this time and therefore their rates are zero alongside operational costs such as chemicals and electrical demand. Depreciation of assets is considered to follow a straight line over the asset lifetime. The salvage value is

considered zero, as seen in the literature (Amer et al., 2011; Doshi et al., 2017; Tredici et al., 2016). A repurchasing schedule was made for assets with lifespans lower than that of the project lifetime, which require repurchase at a later date. This schedule included price adjustment for equipment based on the annual inflation rate chosen. Assets with longer lifespan than the project retain some value at the end of the project that is added back to the capital value. For example, the PhycoFlow™ unit is assumed to have a working life of 25 years, leaving 5 years of residual value at the end of the project which is accounted for in the depreciation calculations. The land value is an exception to the depreciation schedule and is assumed to retain its value over the project lifetime (AccountingTools.com, 2021). A detailed description of how the depreciation and repurchasing schedule was created can be found in Appendix C.

Maintenance costs are those linked to the continued commissioning and care of equipment, land, and buildings. Due to their on-going nature these costs form part of the annual budget and are incurred annually, with inflation applied accordingly. The amount set aside for a maintenance budget for algal biomass production varies throughout the literature. Due to the capital intensive PBR method chosen for cultivation, the maintenance has been assumed on the higher side at 10 %.

Inflation was considered in all models due to the time-length being considered. The average rate of inflation or the UK between 2014 and 2017 was 2.7 % and this was considered as the annual rate in all model variations. Costs incurred and benefits gained after the initial capital layout in year 0 have inflation added to their current value. Items that are purchased periodically and as replacement equipment (following the depreciation used) are all included in this.

The overhead costs associated with labour (janitorial, payroll, national insurance, and benefits) are assumed at a rate of 60 % of the total labour salary cost, in line with the literature (Brownbridge et al., 2014). The number of staff required was chosen for each model variation based on the experimental data collected. The salary information was based on the average UK salary for each described position according to leading recruitment websites *Reed.com* and *Indeed.co.uk*.

Due to the low TRL (technology readiness level) of the bioremediation processes described in later chapters, the contingency cost is relatively high at 15 % of total capital expense (Nagarajan et al., 2013; Ou et al., 2015). With further analysis, pilot scale demonstration and optimisation this will be reduced significantly, and this is considered in the scenario-based assessment method utilised, detailed in each result chapter.

4.4. Base Model: Capital Expenditure

Capital expenditure, CapEx, can be expressed as:

$$TCC = TDC + TIC \quad (4.1)$$

Where TCC is the total capital cost either over the entire project or on an annualised basis. This section will further describe the direct and indirect CapEx components of the above equation.

4.4.1. Direct CapEx

Direct costs are considered as those which result in acquisition of a product, piece of equipment or tangible asset with the majority cost being applied in year 0. Major equipment costs (MEC) such as the PBR units themselves, piping, harvesting boxes, aeration pumps *etc.* are included. The other major direct cost is the land and building procurement. While the

values for MEC are based on those for the PBR unit purchased by the research group and land on the average price for industrial land in England, the values used for buildings are estimated based on the values used in Tredici *et al.* (2016). This report is based on a real pilot plant, using similar PBR technology at the same scale, therefore the building costs are estimated from these values by conversion from 2016 Euros to 2018 GBP.

Direct costs are calculated as:

$$TDC = \sum MEC + Land + Buildings \qquad 4.2$$

Where TDC is the total direct cost and ΣMEC is all major equipment over the entire project lifetime.

4.4.2. MEC: Major Equipment Cost

Major equipment includes those listed in Table 4.2.

Table 4.2: *Major Equipment Required for 1 PBR Unit*

Equipment	Cost	Lifespan
Phycoflow™ with sunlite casing & installation	£31,107.60	25
Flow pumps	£802.80	20
Aeration pump	£50.00	20
Harvesting tank	£396.00	10
Fluidic oscillator	£100.00	20
Air diffusers	£210.00	10
Lights	£70.00	4
Thermoregulators	£38.40	20
Air compressors	£99.98	20
Heaters	£15.00	5
Inoculum vessel	£40.00	20
Inoculum aerator	£144.00	10

Due to the modular nature of the PBR units, the number of pieces of equipment per PBR is calculated then a final total based on the number of PBR units to be installed is calculated. This allows for the user to easily change the number of PBR units and modify facility setup. The number required is based on both practical operation and the expected lifespan of each piece of equipment dictating if it required repurchase during the 20-year project lifetime.

The land cost is based on the average industrial land value estimates, per hectare, from the Department of Communities and Local Government (Ministry of Housing, 2018) and is therefore taken as £241,000.00 for 1 ha. The buildings and facilities cost is based upon those used in Tredici *et al.* and €50,000.00 is converted to £43,625.00 in 2018. It is assumed this is adequate space for offices/laboratory and sampling and chemical preparation as stated in Tredici *et al.* 2016 (Tredici et al., 2016).

4.4.3. Indirect CapEx

The indirect capital costs are those which occur on an annual basis and support the tangible assets such as a maintenance and contingency budget. These are calculated as percentage values of the TDC, and their proportions are based on the TRL of the project and the scale. The 1 ha facility size was chosen as a large pilot or demonstration facility and this, alongside the low TRL of the described bioremediation methods lead to high maintenance and contingency budgets (Lauer, 2008).

$$TIC = 20\%\, TDC \qquad 4.3$$

Where TIC is the total indirect capital.

4.5. Base Model: Operational Expenditure

The operational expenditure, OpEx, was split into direct and indirect costs in a similar fashion to the CapEx.

$$OpEx = TDO + TIO \quad 4.4$$

Where TDO and TIO are total direct and indirect operational expenses, respectively.

4.5.1. Total Direct OpEx

The total direct OpEx consisted of two major annual costs: 1) cultivation costs for the facility and 2) labour and associated overhead costs:

$$TDO = FDCO + LO \quad 4.5$$

Where FDCO is the facility direct cultivation OpEx and LO is the labour OpEx. The cultivation costs were calculated using the experimental data input. The cost of chemicals for media preparation were taken from online bulk supplier values, the cost of water, electricity and heating were based on the experimental usage information and the local rates for each item.

4.5.1.1. Cultivation OpEx

To simplify the inputs required to the model, it was assumed that cultures were run in batch mode where the system is sterilised, a culture inoculated and allowed to grow for a given time before a complete harvest is done and the system sterilised ready for another inoculation, Figure 4.2. This was because the experimental data, on all scales, was conducted in batch mode. The total time for all four stages to be completed was taken as the total batch time required.

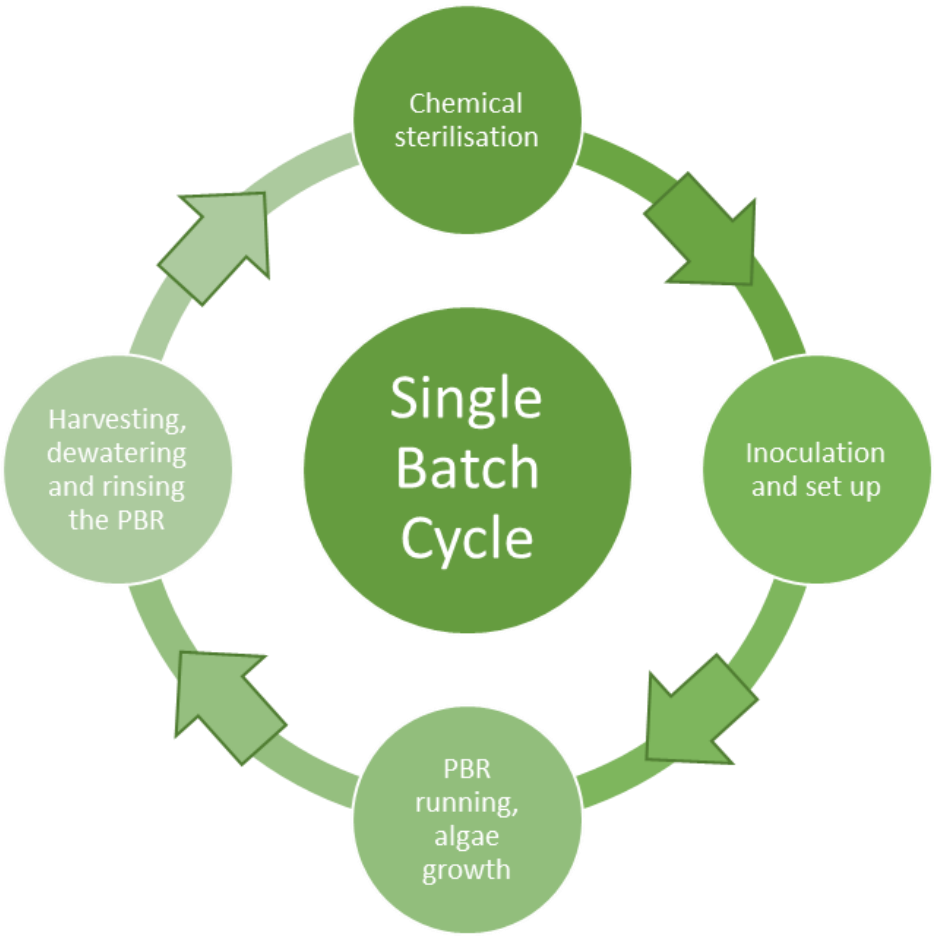

Figure 4.2: *Stages of a batch cultivation in a PBR unit*

The cost of a single batch culture for one PBR unit was calculated based on experimental growth data and operational data for the photobioreactor (energy and water demand) and then scaled to the number of achievable batches annually by using the floor function in Excel:

$$PBR\ Annual\ Batches = floor((batch\ time + sterilisation\ time)/360,1) \quad 4.6$$

This was then scaled to the number of PBR units within the facility to give the FDCO value:

$$FDCO = PBR\ Annual\ Batches \times Number\ of\ PBRs \quad 4.7$$

Table 4.3 highlights the cost elements included in the FDCO calculations with the base model example. All further information, such as energy demand calculations, nutrient media cost *etc.* can be found in APPENDIX D.

Table 4.3: *FDCO information*

Item	Cost	Amount Required	Total Cost
Sterilisation	£2.35	1	£2.35
Inoculum preparation	£0.52	1	£0.52
Chemicals for media	£0.58	1	£0.58
300 L water	£0.0016 / L	300 L	£0.50
Electricity - heating	£0.14 / kWh	115 kWh	£16.51
Electricity – non-heating	£0.14 / kWh	126 kWh	£18.11
Chitosan for harvesting	£9.56	0.3 kg	£2.97
Other consumables	10 % batch cost		£4.15
Total cost of one batch in one PBR unit:			**£45.68**

4.5.1.2. Labour OpEx

A large proportion of the literature for algal techno-economics either does not include or glosses over the inclusion of labour costs. The assumptions used differ dramatically between publications (Bhave et al., 2017; Brownbridge et al., 2014; Davis et al., 2011; Fasaei et al., 2018; Taylor et al., 2013; Ventura et al., 2013; Xin et al., 2016). In this work, the UK average salary for scientific technicians and laboratory supervisors are used with a 60 % overhead cost for additional services such as janitorial, payroll *etc.* as described in Brownbridge *et al.* (2016).

The labour OpEx (LO) is calculated as:

$$LO = (N_{staff} \times S_{staff}) \times (1 + 0.6) \quad 4.8$$

Where N_{staff} and S_{staff} are the number and salary of staff members, respectively and 0.6 represents the 60 % overhead cost for janitorial, payroll *etc*. (Brownbridge et al., 2014).

The number of staff required will vary based upon facility size and the process being undertaken, therefore is explained within each appropriate results chapter. On the other hand, the salary is standardised between models, with £22,000 the average annual income for a research/science technician (Reed.co.uk, 2018) being utilised in all cases.

4.5.2. Total Indirect OpEx

The indirect OpEx consists of annual charges which relate to the maintenance and insurance of the facility. These are calculated as percentages of the TDO in a similar fashion to the indirect costs involved with the capital expense. The TIO, total indirect OpEx is the sum of the cost of maintenance, $C_{maintenance}$, and cost of insurance, C_{insure}:

$$TIO = C_{maintenance} + C_{insure} \quad 4.9$$

4.5.3. Capacity Requirements

Unlike capital expenses, operational costs are directly related to the ongoing production in the facility and therefore are flexible and can change from year to year. To allow for this in the net cash flow calculations an operational load was added. If the operational load was 100%, as would be expected in most years, then the OpEx is calculated exactly as has been described. If there is no operational load for an entire year, the OpEx cost is 0. If operation is not at full capacity, for example, in the half year remaining after the 1.5 year installation and commissioning at the beginning of the process then the OpEx is calculated as a proportion of the usual cost, with the exception of labour costs, where the full amount is charged as staffing is less elastic.

$$OpEx = ((Operational\ Load\ (\%) \times FDCO) + LO) + TIO \quad 4.10$$

4.6. Revenue Streams and Benefits

As the scope of analysis does not include downstream processes and therefore products with known market values, no revenue streams are initially included. In some scenario cases, a monetary benefit may be applied. For example, a CO_2 capture incentive based on the amount of CO_2 captured by the system. Where these apply, the overall, annual, benefit is added to the annual costs to create a cost-benefit balance.

4.7. Cost of Treatment and Cost of Production

As there is no revenue stream the cost of both production of biomass and treatment of the waste in question are calculated. These are the minimum price required per unit for the facility to break even over the 20-year period. This includes the remaining value held in depreciable and facility/land assets. The cost of treatment and cost of biomass are independent of each other; however, a known price for either can be used to show the effect on the other by adding the sale price to the revenue streams. The values are worked out based on the annualised total costs and yields/treatment values to take into account the lack of operation in the first 1.5 years where the majority of capital expenses occur.

The cost of biomass production is calculated as:

$$Cost\ of\ Biomass\ Production\ (£\ kg^{-1}) = \frac{Annualised\ Total\ Cost}{Annualised\ Yield} \quad 4.11$$

And the cost of treatment is calculated as:

$$Cost\ of\ Treatment\ (£\ unit^{-1}) = \frac{Annualised\ Total\ Cost}{Annualised\ Treatment\ Units} \quad 4.12$$

Where the standard units are dependent on the waste stream(s) being assessed. For example, where CO_2 mitigation is the key, the units applied will be tonnes of CO_2.

4.8. Net Cash Flow

The NCF is projected over the project lifetime using the CapEx, OpEx and benefit figures obtained:

$$NCF = Benefits - (CapEx + OpEx) \quad 4.13$$

Each year is calculated individually in the NCF table (Appendix E) to allow for the construction/commissioning period at the beginning of the project and new purchases and inflation price increases throughout the project lifetime. The total and annualised costs are all calculated using the annual values for CapEx, OpEx and benefits, an example of the NCF is given in Table 4.4.

Table 4.4: *Example of how the net cash flow worksheet is laid out.*

Item	Years 1-20	Total Cost	Annualised Cost
Total liabilities	Value	Sum of values years 1-20	Total Cost/20 years
Total assets	Values		
Liquidity	Assets – Liabilities		
CapEx Items	Purchase costs (in applicable years)		
Total Direct Capital	Sum of CapEx costs		
Indirect CapEx	TDC x %		
Total CapEx	TDC + TIC		
Total CapEx (- dep)	TDC + TIC – Depreciable asset value in year		
Operational Load	% operation in year		
FDCO	FDCO value		
LO	LO value		
Total Direct OpEx	FDCO + LO		
Indirect OpEx	TDO x %		
Total OpEx	TDO + TIO		
Total Cost	Total CapEx (- dep) + Total OpEx		
Benefits	Value dependant on scenario		
NCF	Benefits – Total Cost		
Cumulative NCF	NCF + Previous years		

4.9. Conclusions

The basic spreadsheet layout and principles, which will be applied for all TEAs, has been described above. For each treatment method, additional items or changes to certain reagents/consumables/prices will be made but the backbone of the process will remain identical. This base spreadsheet is used to create multiple financial and operational scenarios by changing key parameters and highlighting the changes reported in the NCF. These scenarios are specific to each treatment and are detailed in their respective chapters. Alongside the scenarios, single and dual parameter sensitivities are conducted for key parameters such as batch cycles which can be completed, economies of scale for PBR and MEC purchases, increased efficiency of process *etc.* Sensitivity analysis is conducted either as a percentage change to the original value or using the data tables function in Excel (2020). The original data from the baseline scenario is used as inputs for the data table where 1 or 2 parameters are changed, and a new set of key outputs is calculated.

5. Investigation of Microalgal Carbon Capture

5.1. Introduction

Carbon capture and utilisation or storage (CCUS) is a required technology to reach global CO_2 emission reduction goals. While traditional capture and storage techniques remove carbon from the atmosphere and store it underground, utilisation offers a pathway to employ CO_2 emissions as a feedstock for the production of fuels, chemicals, and other high value products (HVPs) which can ultimately replace those from fossil fuels (Chauvy et al., 2019). The use of photosynthetic microorganisms, specifically microalgae, is believed to be one of the most promising techniques for the biofixation of carbon (Kassim and Meng, 2017).

Microalgae utilise CO_2 as their main carbon source during photosynthetic growth and do so at up to 100 times faster rates than terrestrial plants (Hariz et al., 2018; Lam et al., 2012; Li et al., 2011; Rogers et al., 2014), making them an attractive option for CCUS. Although the premise of algal-CCUS has been around for some time, the previous focus of algal research has been predominantly on biofuel production. Meaning there is a wealth of information regarding lipid and biomass productivity under the supplementation of CO_2 but little information on the potential carbon reduction efficiencies and economics of carbon-capture rather than biomass production.

Specifically, when it comes to the ability of algal cultures to remove CO_2 from the gas stream, the surrounding literature becomes unclear. Few studies have looked directly at the ability of algal cultures to remove CO_2 from a flow of gas (Chiu et al., 2008, 2011; Liu et al., 2011). Alongside this, the use of intermittent sparging can be seen throughout the literature (sparging for less than a minute an hour) (Assis et al., 2019; Chiu et al., 2011; Duarte et al.,

2016, 2017; Jiang et al., 2013; Yadav et al., 2015). However, the stop-start nature and low potential CO_2 usage of these systems, especially where it is being used to control pH, would not be suited to applications such as CCUS where there could be substantial amounts of CO_2 requiring usage/storage at any given time.

Alongside these examples, there have been many instances where research has focused on the CO_2 removal rate of cultures in terms of the grams CO_2 removed per litre of culture per day ($g\ L^{-1}\ d^{-1}$), as seen in Table 5.1. However, with the large variety of cultivation systems, aeration rates and CO_2 concentrations being applied to cultures this gives a poor basis for comparison between the different microalgal species. In many of the cases seen in Table 5.1, the fixation rate of carbon, R_{CO2} ($g_{CO2}\ L^{-1}\ d^{-1}$), is calculated using the equation presented in Chapter 2 (Eq. 2.7). Using those assumptions can give dramatically different results when compared to data produced from direct measurements of CO_2 in and out of the system. For example, Li *et al.* (2011) directly measured the CO_2 removal efficiency of *Scenedesmus obliquus* and a mutant WUST4, gaining a CO_2 removal of between 40 and 60 % of the CO_2. If the productivity of the culture and experimental conditions described in their publication are used with the assumption described above, a removal of just ~ 1 % (0.17 $g_{CO2}\ L^{-1}\ d^{-1}$) is seen instead of that measured.

Table 5.1: *Comparison of CO_2 biofixation rates within the literature and the methods used to calculate these values. * Denotes that an intermittent gas flow was used in these experiments rather than a constant flow of CO_2 to the cultures. Where the method is denoted as Eq 2.7. This represents the equation described previously with C_C assumed as 50 % unless otherwise stated.*

Genus and Species	CO_2 (%)	P ($g\ L^{-1}\ d^{-1}$)	R_{CO2} ($g\ L^{-1}\ d^{-1}$)	Method for determining CO_2 removal	Source
Botryococcus braunii	5	NA	0.497	Real time monitoring	(Sydney et al., 2010)
Chlorella fusca	10*	0.08	0.255	Eq 2.7.	(Duarte et al., 2017)
Chlorella kessleri	6*	0.087	0.163	Eq 2.7.	(de Morais and Costa, 2007b)
Chlorella minutissima	10	0.15	0.250	Eq 2.7.	(Freitas et al., 2017)
***Chlorella* sp.**	5	0.271	0.498	$P_x \times 0.5 \times \frac{M_{CO2}}{M_C}$	(Yadav et al., 2015)
***Chlorella* sp.**	25*	0.52	60%	Real time monitoring	(Chiu et al., 2011)
Chlorella pyrenoidosa	10	0.144	0.260	Eq 2.7.	(Tang et al., 2011)
Chlorella vulgaris	5	NA	0.251	Real time monitoring	(Sydney et al., 2010)
Chlorella vulgaris	5	0.11	0.15	Eq 2.7.	(Jain et al., 2018)
Dunaliella tertiolecta	3	0.17	0.313	Eq 2.7.	(Kishimoto et al., 1994)
Dunaliella tertiolecta	5	NA	0.272	Real time monitoring	(Sydney et al., 2010)
Scenedesmus obliquus	6	0.1	0.188	Eq 2.7.	(de Morais and Costa, 2007c)
Scenedesmus obliquus	10	0.155	0.288	Eq 2.7.	(Tang et al., 2011)
Scenedesmus obliquus	10	0.0653	40.2%	Real time monitoring	(Li et al., 2011)

***Scenedesmus obliquus* WUST4**	20	0.0971	59.8%	Real time monitoring	(Li et al., 2011)
***Spirulina* sp.**	6	0.2	0.376	Eq 2.7.	(de Morais and Costa, 2007c)
***Spirulina* sp.**	10*	0.04	0.120	Eq 2.7.	(Duarte et al., 2017)
Spirulina platensis	5	NA	0.318	Real time monitoring	(Sydney et al., 2010)

Whilst research has focused on the CO_2 removal rate of different algal systems and the potentially reduced environmental burdens over mature CCUS technologies (Beal et al., 2018; de Queiroz Fernandes Araújo et al., 2015; Wiesberg et al., 2017), the economics of the process have yet to be purposefully looked at with the focus being carbon capture.

In this chapter, *C. vulgaris* is grown under varying CO_2 concentrations to ascertain the optimal conditions for growth of this species. The CO_2 removal by the algal cultures, under these optimal conditions, is then monitored using a non-dispersive infrared (NDIR) sensor to calculate the real-time CO_2 removal efficiency of the system (Chapter 3.6). This is then compared to the literature and the theoretical CO_2 uptake rate calculated using Eq. 2.7 to establish any statistical difference between the two methods. Finally, a techno-economic assessment (TEA) was developed using the experimental conditions, as described in Chapter 4, and results to give a cost of capture (CoC, £ $tonne_{CO2}^{-1}$) value for the algal-CCUS process at pilot scale. Several different financial and socio-economic/political scenarios were considered for the TEA to highlight which parameters are most sensitive to change and reduce the CoC value. Key parameters were then put through single and double parameter sensitivity tests to understand how they affect the CoC and highlight where future research should be focused to reduce costs leading to an economically feasible process.

5.2. Results: Optimal CO_2 Concentration for *Chlorella vulgaris*

C. vulgaris was grown under five different CO_2 conditions to establish an optimal range for biomass production:

1. no aeration (0 %),
2. aeration with air (0.04 %),
3. 5 % CO_2 mixed in air,
4. 10 % CO_2 mixed in air and,
5. 15 % CO_2 mixed in air.

Experiments were performed as described in Chapter 3.4 using triplicate culture flasks and a negative control flask containing only media. The biomass concentration (g L^{-1}) over the 14 day growth period for each experiment is shown in Figure 5.1. Figure 5.2 compares the average growth for each experiment and shows the final biomass concentration for each experiment. The slowest growth was seen where no aeration was applied to the flasks. These cultures were allowed to grow for an extended period to confirm it was not simply a lag phase affecting the growth profile of the algae (Figure 5.1A). The highest growth was seen when 5 % CO_2 was added to the cultures, resulting in a final biomass concentration of 3.2 g L^{-1}. Increasing the CO_2 concentration further to 10 and 15 % CO_2 did not further improve the growth of *Chlorella* sp., actually resulting in a decrease in final biomass concentration at the end of the experiment. The cultures grown with 10 % and 15 % CO_2 reached a maximum biomass concentration of 2.122 and 2.174 g L^{-1}, respectively. When the final biomass concentrations and average culture productivities were compared against the non-aerated control cultures, all aerated cultures grew significantly faster ($P<0.05$). There was a statistical difference between all conditions except for 10 % and 15 % CO_2.

Although giving a lower final total biomass yield than 5 % CO_2, conditions of both 10 and 15 % CO_2 gave a higher biomass concentration than aeration with air alone, suggesting the cultures are carbon limited when supplied with air only. This is further supported as the only independent variable within the experiments was the aeration carbon dioxide concentration. Therefore, it can be assumed that carbon was the limiting factor when no air or air alone was supplied and that around 5 % CO_2 is optimal for this *C. vulgaris* growth under these conditions.

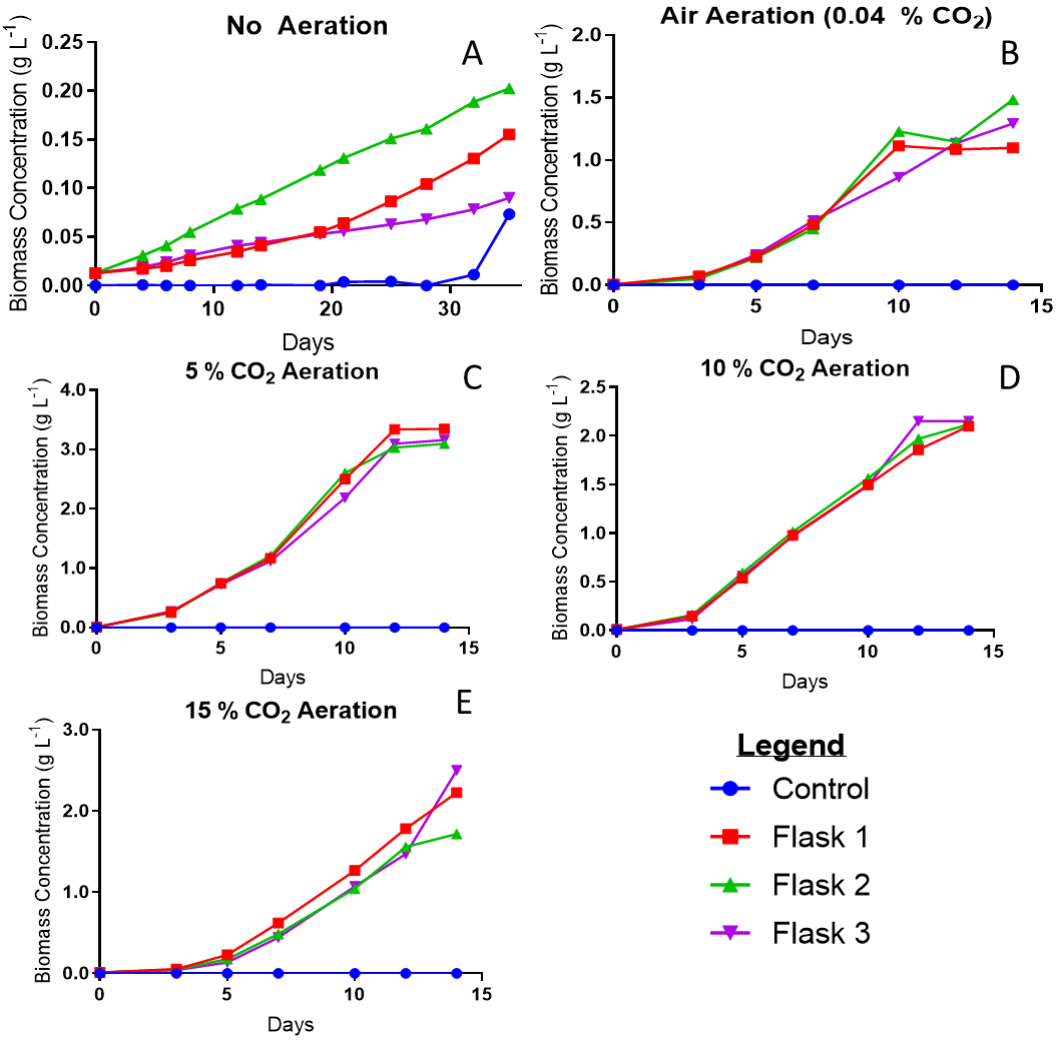

Figure 5.1: *Growth of Chlorella vulgaris under different CO_2 concentrations. For each time point three measurements were taken, the average is plotted with standard error.*

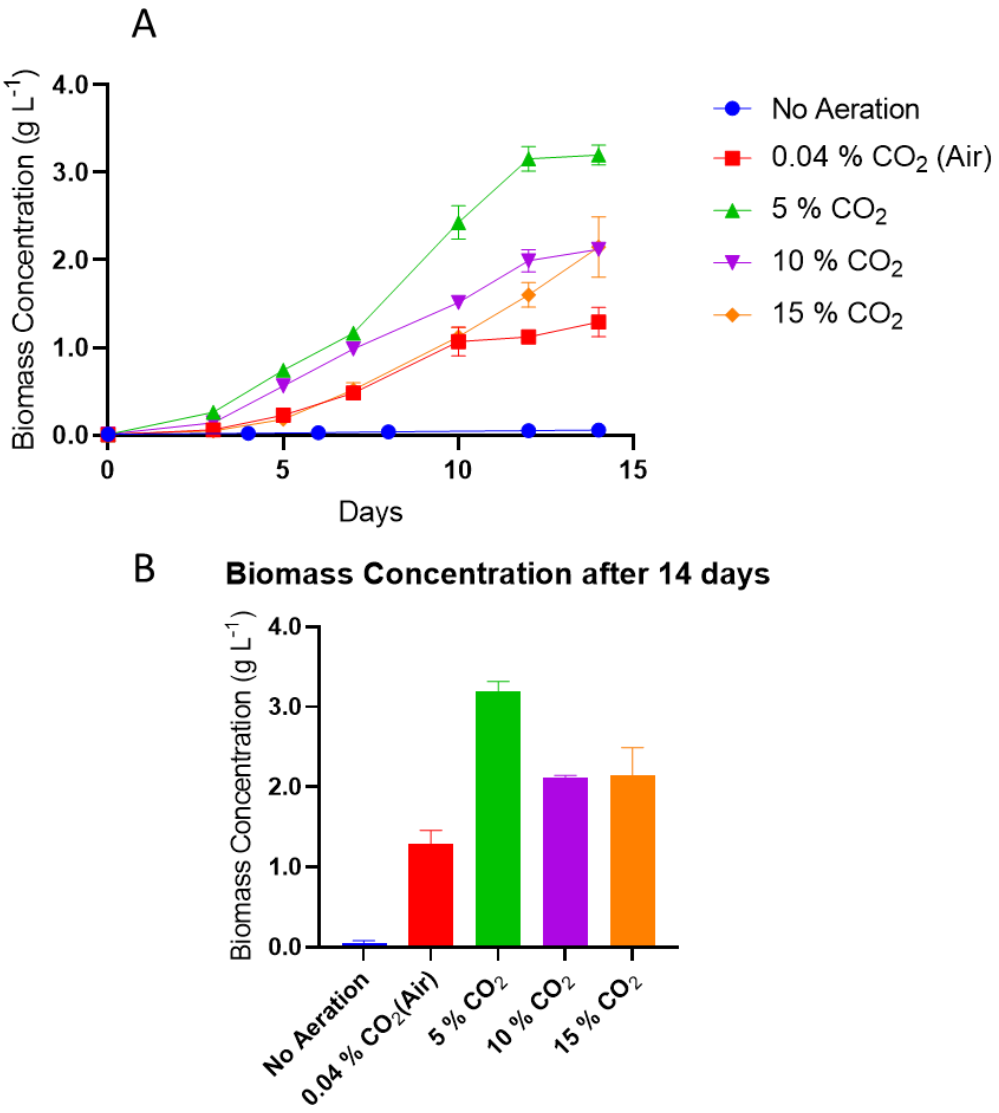

Figure 5.2: *Growth profiles for C. vulgaris grown under different aeration conditions (top) and the final biomass concentration for each experiment after 14 days growth (bottom).*

5.3. Carbon Removal of *C. vulgaris* Cultures Aerated With 5 % CO_2

The previous experiments highlighted that, of the five concentrations tested, 5 % CO_2 resulted in the highest growth rate and biomass production for *C. vulgaris*. This was, therefore, the CO_2 concentration used in the next phase of experiments. *C. vulgaris* was grown under the conditions described in Chapter 3.6. As only one NDIR sensor was used for this work, the

experiment was conducted over a two-month period with triplicate culture runs of two weeks each and one control in which only media was used.

Figure 5.3 shows the average culture growth and CO_2 removal efficiency of the cultures over the 14-day experimental period. Figure 5.4 shows the results for the control, highlighting that there is no contamination within the experimental set up (no growth) and no abiotic CO_2 capture by the system itself due to the chemical makeup of the media or conditions used. Figure 5.4b shows that during the first 5 minutes of gas flow through the media there is a removal of CO_2 from the gas stream. This then quickly tails off until the CO_2 concentration stabilises again at the pre-set 5 %. This initial removal of CO_2 is due to some of the CO_2 dissolving in the media, reducing the pH. The media is quickly saturated with carbon, resulting in no more CO_2 being removed from the incoming gas stream.

The CO_2 removal efficiency by the culture is based on the difference in concentration entering and exiting the system as measured by the NDIR sensor (Chapter 3.6). The growth of the cultures shows a similar pattern to the 5 % CO_2 cultures for the previous experiments. Notably there is a larger error on each time point compared to the previous experiment and this is due to the fact each replicate was not conducted in tandem but one after another. After two weeks, the final average biomass for the cultures was 2.11 ±0.009 g L^{-1}. The CO_2 removal efficiency of the culture changes over the cultivation time. At the beginning of the experiment where the biomass concentration is very low the CO_2 removal efficiency is very low, reaching only 10 % after 24 hours of growth. As the biomass concentration increases the CO_2 removal efficiency does as well, until a plateau at ~ 20 % efficiency is reached in the latter half of the experiment, during the linear growth phase. The average CO_2 removal efficiency over the entire growth period was equal to 17.5 % CO_2 removed.

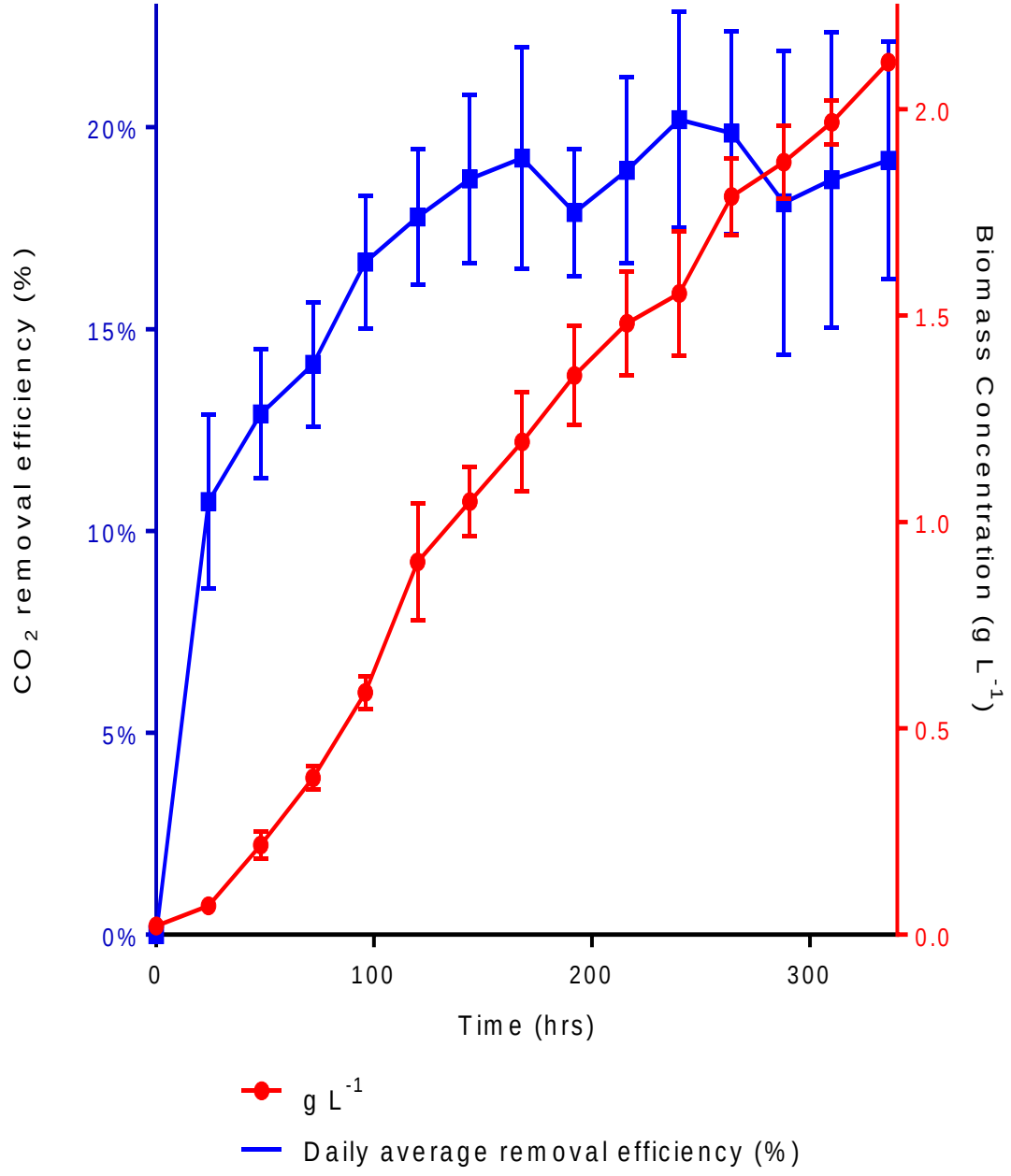

Figure 5.3: *The growth of Chlorella sp. grown under continuous aeration with 5 % CO_2 (red) and the CO_2 removal efficiency of the culture measured by real-time CO_2 measurements of the off-gas (blue). The results are averages from three replicate cultures.*

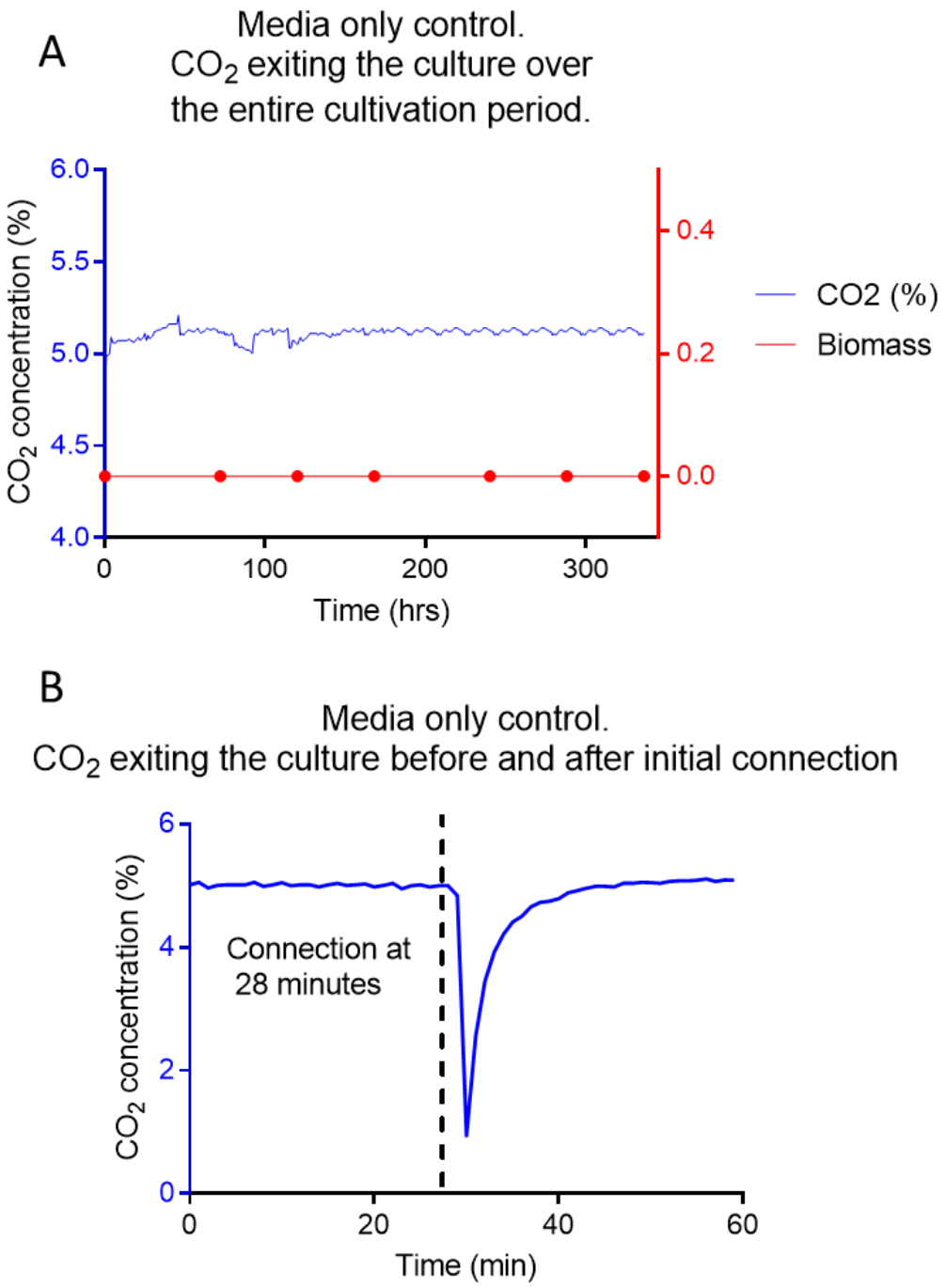

Figure 5.4: *Media only control results. A shows the CO_2 concentration exiting the culture throughout the experiment (left y-axis) and the biomass concentration in g L^{-1} based on OD_{695} readings (right y-axis). B shows in closer detail the CO_2 concentration when the culture is first connected to the gas stream. The culture was attached to the gas flow at 28 minutes, where a sharp decrease in CO_2 concentration can be seen due to the gas dissolving in the media. Once the liquid phase is saturated the CO_2 concentration returns to the previous concentration.*

5.3.1. Comparison of CO_2 Removal Efficiency to the Literature

To allow comparison with information available within the literature (Table 5.1), the average culture productivities for each experiment were used to calculate the R_{CO2} as described by Eq. 2.7. The average productivity of the triplicate cultures for each condition was used along with the assumption that *C. vulgaris* has a carbon content of 50 %, based on the approximate molecular formula for microalgal biomass proposed by Chisti (2007). Figure 5.5 shows the R_{CO2} values for each experimental condition. The highest R_{CO2} was seen for 5 % CO_2 during the first experiment, where the final biomass concentration was also the highest. This R_{CO2} value of 0.31 $gCO_2\ L^{-1}\ d^{-1}$ is higher than the average of those presented in Table 5.1 (0.279 $gCO_2\ L^{-1}\ d^{-1}$).

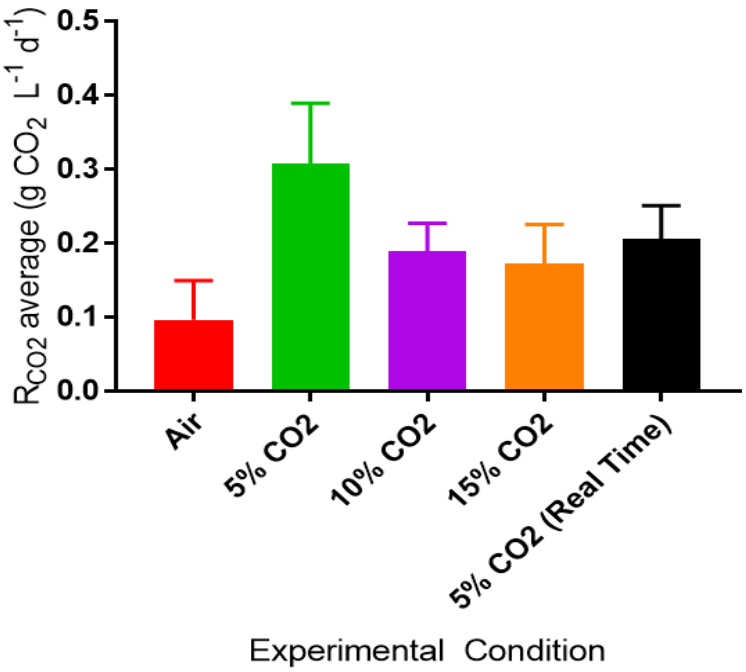

Figure 5.5: *The average R_{CO2} for Chlorella sp. grown under different CO_2 concentrations over a two-week period. The R_{CO2} value is calculated using Eq. 2.7 from the literature to allow for comparison with the surrounding literature. The 5 % CO_2 Real Time (final column) denotes the second experiment where CO_2 removal was measured in real-time also.*

Although the R_{CO2} values mentioned above are higher than seen elsewhere in the literature, these values do not tell us how much CO_2 was removed from the inflowing gas stream. For aeration with air, 5 %, 10 % and 15 % CO_2 in air, the maximum Estimated RE (when using Eq. 1) are 7.58 %, 0.14 %, 0.04 % and 0.03 %, respectively. Although the biomass production has been visibly improved by increasing CO_2 concentration, the CO_2 availability now supersedes the difference in growth meaning a lower percentage of that available is actually used, when comparing the air and 5 % CO_2 experiments.

Table 5.2 shows the comparison of these results to the real-time monitored CO_2 removal efficiency from the second experiment. As can be seen, there is a large difference in the CO_2 removal efficiency, with the real-time monitoring showing a much larger CO_2 removal efficiency than that predicted by Eq. 2.7. One reason for this may be the fact that the equation assumes that the carbon content of the biomass remains as a fixed value. The carbon content of the biomass may fluctuate over time leading to a higher or lower CO_2 consumption at any given time point (Safi et al., 2014), missed by the assumptions made in the equation. Another reason would be the production of excreted products (Chen et al., 2020) or storage of dissolved inorganic carbon in vacuoles within the cells (Ma et al., 2019). Neither of these would be seen in the measurement of biomass productivity, which the equation relies on. The media only control for this experiment highlights that there is an initial capture of CO_2 by the media in the first 30 minutes of bubbling but after this the carbon balance is maintained and the media does not take up any additional CO_2 from the gas inlet over the entire 14-day period.

Table 5.2: *Productivities, RCO_2 and CO_2 removal efficiency for Chlorella sp. grown under different CO_2 conditions.*

Experimental conditions	**Average Productivity ($g\ L^{-1}\ d^{-1}$)**	**RCO_2 based on Eq. 2.7 ($g\ CO_2\ L^{-1}\ d^{-1}$)**	**RCO_2 based on NDIR sensor ($g\ CO_2\ L^{-1}\ d^{-1}$)**	**Estimated RE**
5 % CO_2, 1 vvm, Real time monitoring	0.11	0.21	11.53	17.27% Monitored RE 0.08% Estimated RE
Air, 2 vvm	0.05	0.10		7.58%
5 % CO_2, 2 vvm	0.17	0.31		0.14%
10 % CO_2, 2 vvm	0.10	0.19		0.04%
15 % CO_2, 2 vvm	0.09	0.17		0.03%

The difference between the R_{CO2} and sensor CO_2 removal values is statistically significant ($P<0.0001$) in all time periods. Therefore, it can be deduced that Eq. 2.7 under-estimates the CO_2 capture potential of microalgal cultures. This is further corroborated by the information shown in Table 5.2. Where real-time monitoring has been used (Kuo et al., 2016; Li et al., 2011) there are significantly higher CO_2 removal capacities by the cultures even though the species and experimental conditions are similar to those presented in the rest of the literature.

5.4. Techno-Economic Analysis Results

The experimental results reported in the previous section were then extrapolated to pilot scale for use in a techno-economic assessment (TEA) based on a theoretical facility located in Sheffield, UK. Details of how the model was created and the financial assumptions and capital inputs are presented in Chapter 4.

A 'baseline' model was created using the experimental results from the previous section and operational information for the 300 L photobioreactor (PBR), Phycoflow™ (VariconAqua) shown in Figure 4.1. The experimental inputs are detailed in Table 5.3. The operational information is based on data obtained by Kasia Emery (2021) and the basis for the TEA model in Chapter 6.

Table 5.3: *Biological and operational inputs for the baseline model*

Item	Value	Units
Inoculum volume	20	L PBR^{-1} $Batch^{-1}$
Inoculum growth time	28	days
Inoculum cost	0.52	£ $Batch^{-1}$
Starting biomass concentration	0.1	g L^{-1} d^{-1}
Growth rate	0.143	g L^{-1} d^{-1}
Final biomass concentration	2.11	g L^{-1}
Batch length	14	days
Downtime (harvesting/cleaning)	2	days
Harvesting method	Flocculation/Floatation	
Harvesting efficiency	90	%
Harvested biomass	1.90	g L^{-1}
Moisture content	80	%
Wet biomass weight	3.14	g L^{-1}
Gas feed time	24	Hours day^{-1}
Flow rate	1	vvm
CO_2 concentration (in)	5.12	% average
CO_2 concentration (out)	4.13	% average
CO_2 removal efficiency	20	%

5.4.1. Baseline Model Results

The baseline scenario was produced based on the extrapolated laboratory data and costs typically associated with pilot scale/new technology contingency planning. A cost breakdown for each major section was produced, Figure 5.6 and Table 5.4, as well as the overall cost of CO_2 capture (CoC, £ $tonne_{CO2}^{-1}$), £1,527.89 $tonne_{CO2}^{-1}$. As can be seen from the figure, the largest incurred cost is associated with the major equipment purchases at 47.84 %. Of this, the Phycoflow™ contributes over 90 % of the cost, highlighting that modular, highly

controllable and glass systems like this are often not practical for low value applications such as bioremediation. The electricity demand of the system is the second largest cost at just under 20 %. This is evenly split between general electricity demand (lighting, flow pumps, aeration) and energy required for heating the PBR, showing that the heat requirement is another area which could be exploited to reduce costs further. As flue gases leaving the stack can be around 150 °C and will require cooling before treatment/feeding to algal cultures, the waste heat could be utilised for the PBRs rather than using a small area heater as used in the pilot reactor set up.

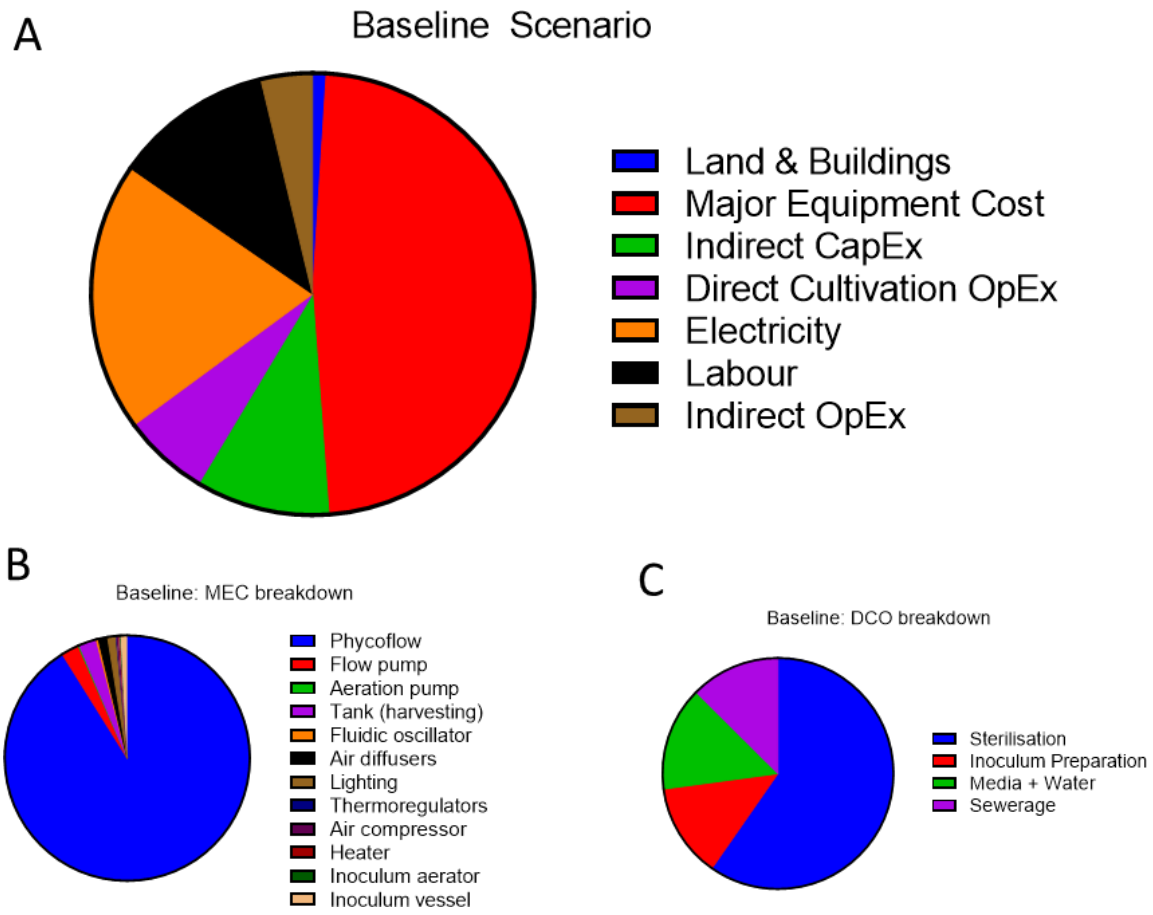

Figure 5.6: *Baseline scenario cost breakdown. A) Total cost breakdown of the process. B) Major Equipment Cost (MEC) cost breakdown into constituent parts. C) Direct Cultivation OpEx (DCO) breakdown into constituent parts.*

Table 5.4: *Baseline scenario cost breakdown values. Italicised values refer to cost breakdown of the larger component.*

Item	% Cost Contribution	
Land and Buildings	0.99	
Major Equipment Cost	47.84	
Phycoflow (with installation)		*91.09*
Flow pump		*2.35*
Aeration pump		*0.15*
Harvesting tank		*2.32*
Fluidic oscillators		*0.29*
Air diffusers		*1.23*
Lighting		*1.02*
Thermoregulators		*0.11*
Air compressors		*0.29*
Heaters		*0.18*
Inoculum aerator		*0.12*
Inoculum vessel & piping		*0.84*
Indirect CapEx	9.77	
Direct Cultivation OpEx	6.31	
Sterilisation		*59.68*
Inoculum preparation		*13.18*
Media & Water		*14.58*
Sewerage		*12.56*
Electricity	19.74	
Labour	11.60	
Indirect OpEx	3.76	

5.4.2. Scenario Based Analysis

A variety of different financial and operational scenarios were put through the model to help determine which parameters would produce the highest cost-reduction and are therefore where optimisation should be focused. This scenario based analysis is seen throughout the literature as a way of showing how different financial, political and technological situations can either increase or decrease the economic viability of algal based remediation and biofuel production (Acien Fernandez et al., 2012; Amer et al., 2011; Beal et al., 2018; Davis et al., 2011; Doshi et al., 2017; Hoffman et al., 2017; Holtermann and Madlener, 2011; Norsker et

al., 2011; Rezvani et al., 2016; Richardson et al., 2012; Rogers et al., 2014; Slade and Bauen, 2013; Thomassen et al., 2016; Ventura et al., 2013).

Six scenarios (seven including the baseline) were input to the model using a variety of different financial and operational changes. Each scenario is described in Table 5.5, the input changes for each scenario can be found in Appendix F.

Table 5.5: *Scenario descriptions*

Scenario	Description
1	Sub optimal, there are issues with scaling up the experimental results and therefore the biomass productivity and CO_2 capture efficiency are halved compared to baseline.
2	Baseline, the original information used to create the TEA.
3	Reduction in CapEx, due to the high volume of equipment being purchased a bulk-order discount of 25 % is applied to all major equipment purchasing, including the PBR units.
4	Reduction in OpEx, assuming there is no change in the biomass productivity or CO_2 capture efficiency, the cost of operational expenditures such as lighting, media nutrients and heating are no longer required and omitted.
5	Combination of both CapEx and OpEx reduction, both scenarios 3 and 4 combined.
6	CO_2 credits, again assuming there is no change to the biomass productivity or CO_2 capture efficiency, there is an introduction of a 'carbon credit' where a revenue of £50/tonne captured CO_2 is applied.
7	Improvements in efficiency, without the biomass productivity changing, the efficiency of the capture process is doubled.

Figure 5.7 shows the cost breakdown and CoC values for each of the scenarios studied. From the graph, it can be seen that Scenarios 3-7 all lead to a reduction in CoC values compared to the baseline (Scenario 2) and Scenario 7 gives the largest decrease in value of 52 % to £769.05 $tonne_{CO2}^{-1}$. The next lowest CoC value was obtained for Scenario 5 where both CapEx and

OpEx are reduced significantly. A combination of species optimisation for increased efficiency and reduced capital and operational expenditure are therefore key areas for cost reduction.

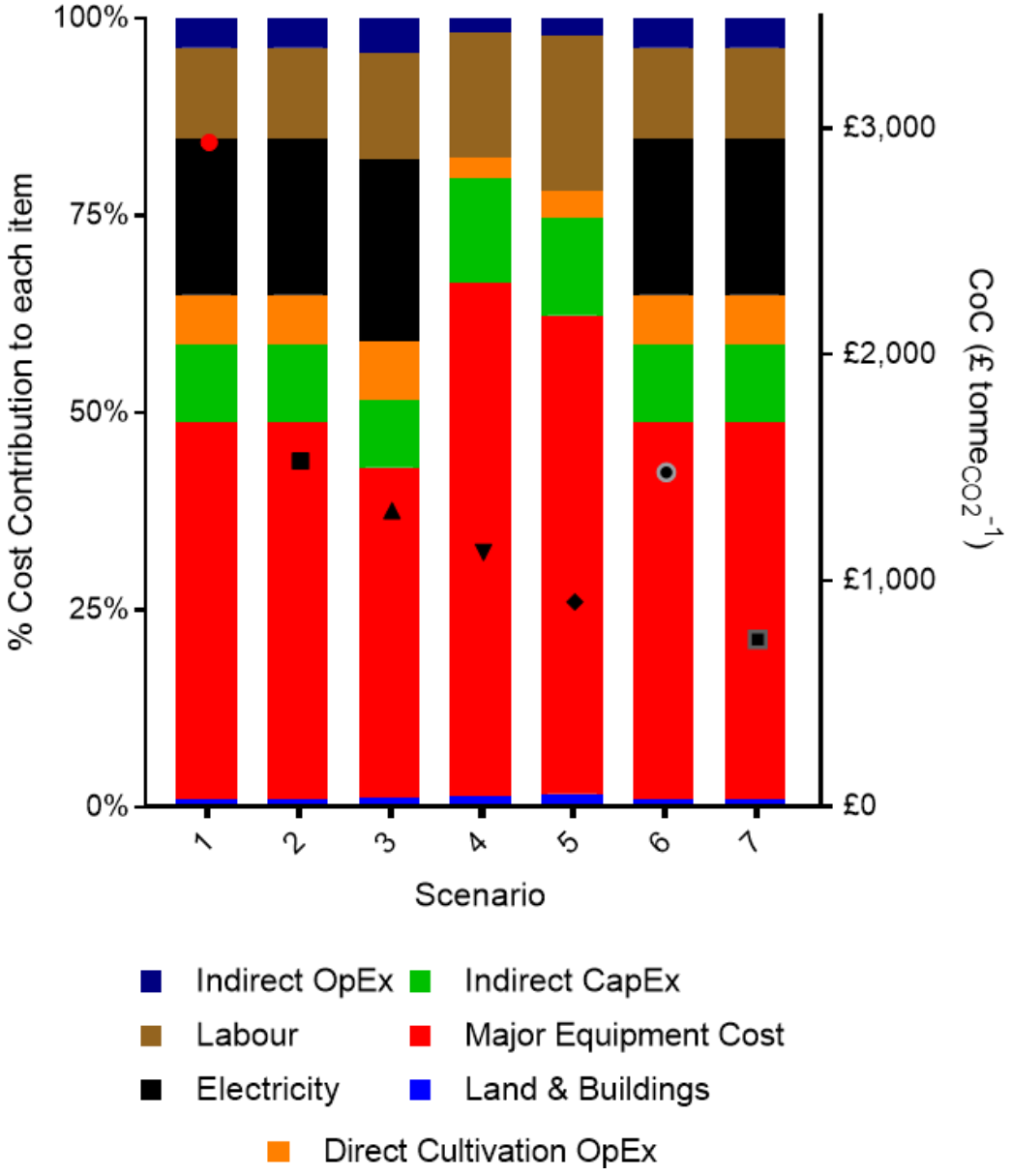

Figure 5.7: *Results for the scenario analysis. The bars represent the cost breakdown for each scenario (left y-axis). The black points in each bar represent where the cost of capture (CoC) lies for each scenario (right y-axis).*

The cost-breakdown for each scenario shows that major equipment cost (PBR, pumps, harvesting tanks, heating *etc.*) is the largest expense in all cases, contributing ~ 50 % of the cost in all scenarios. Labour is the next most expensive parameter for all cases, followed by electricity demand and indirect CapEx. The indirect CapEx includes 15 % of the direct CapEx for contingency, a large value used for new and developing technologies, with advancements in the field this is likely to drop alongside the direct CapEx costs. Land and buildings contribute the least to cost, and this is partially due to the lack of depreciation applied to these items. It is assumed in the model that land does not lose any value over the project lifetime and can be sold at the end of the project lifetime for the purchase value. This being said, industrial land value in the UK has increased over 30 % between 2014 and 2017 (Ministry of Housing, 2018) and therefore it can be assumed that if land appreciation is taken into account CoC can be further reduced.

5.4.3. Single Parameter Analysis: Process Efficiency

As the scenario with strain development and optimisation for improved CO_2 capture efficiency gave the most dramatic cost reductions, a sensitivity analysis of all scenarios to this parameter was conducted. Each scenario was tested with different CO_2 capture efficiencies from 0 – 100 %, shown in Figure 5.8. In each scenario, the CoC value drops with increasing capture efficiency with a minimum value of £ 176.58 $tonne_{CO2}^{-1}$ obtained for Scenario 5. The graph also highlights where the baseline TEA and experimental data currently sits. This single-parameter analysis only considers the improvement of CO_2 uptake by the cultures and not the increased biomass production which would accompany it. Sales of the additional biomass for HVPs, feed or fertiliser with higher sale prices than energy and fuel biomass would further reduce the overall CoC value, making the algal CCUS more competitive with mature CCS

technologies such as amine scrubbing (€ 55-77 $tonne_{CO2}^{-1}$ (Grande et al., 2017; Romeo et al., 2008)).

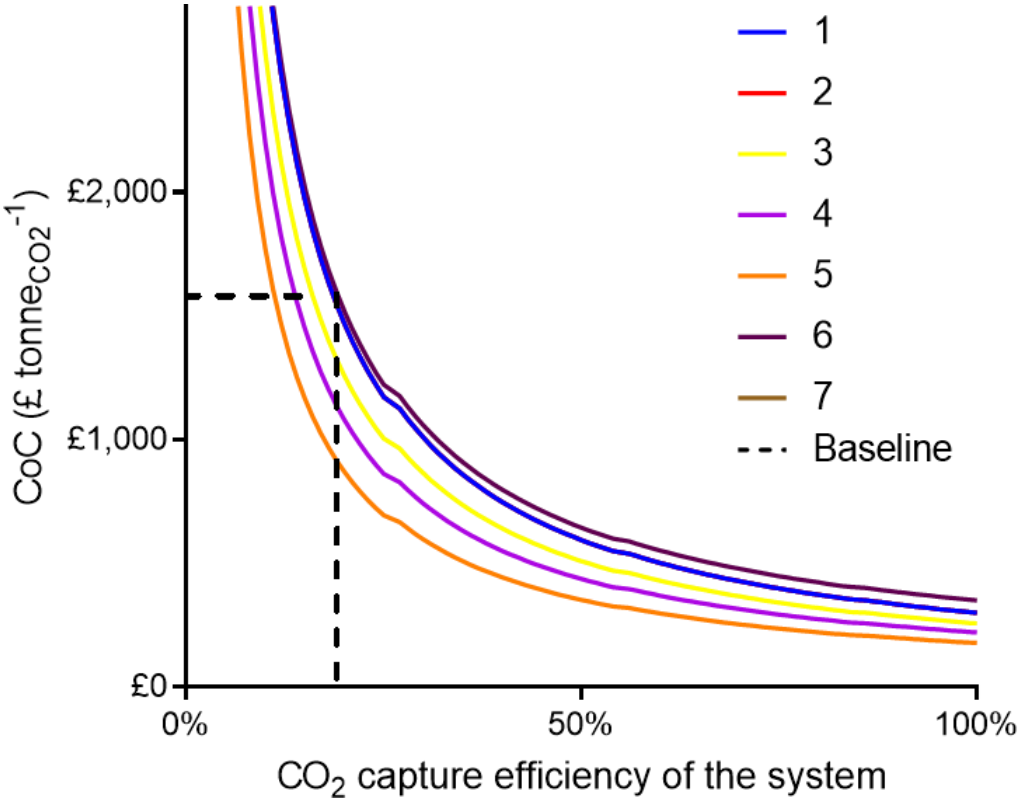

Figure 5.8: *Single parameter analysis of capture efficiency by the culture against each scenario tested*

5.4.4. Dual Parameter Analysis: Process Efficiency and Carbon Credit Price

Beal *et al.* (2018) stated in their algae bioenergy CCS (ABECCS) TEA that as the system is specifically designed to take up CO_2 it would be "*unrealistic to consider scenarios without a significant carbon credit*". Therefore, as this process is designed for the same purpose, a further analysis based on both the CO_2 removal efficiency and an increasing CO_2 credit was conducted. The baseline scenario was used for this and the CO_2 credit was varied from £0 – £300 $tonne_{CO2}^{-1}$ and the efficiency from baseline (17 %) to 100 %, shown in Figure 5.9. The heat map shows how the CoC value changes with the two parameters. To achieve an overall negative CoC the efficiency needs to be above 98 % and the credit around £300 $tonne_{CO2}^{-1}$.

This is not realistic, especially in the near-to-medium term and is why research focus on key parameters such as bioreactor design and cost and efficiency are to improving economic viability of algal-CCUS.

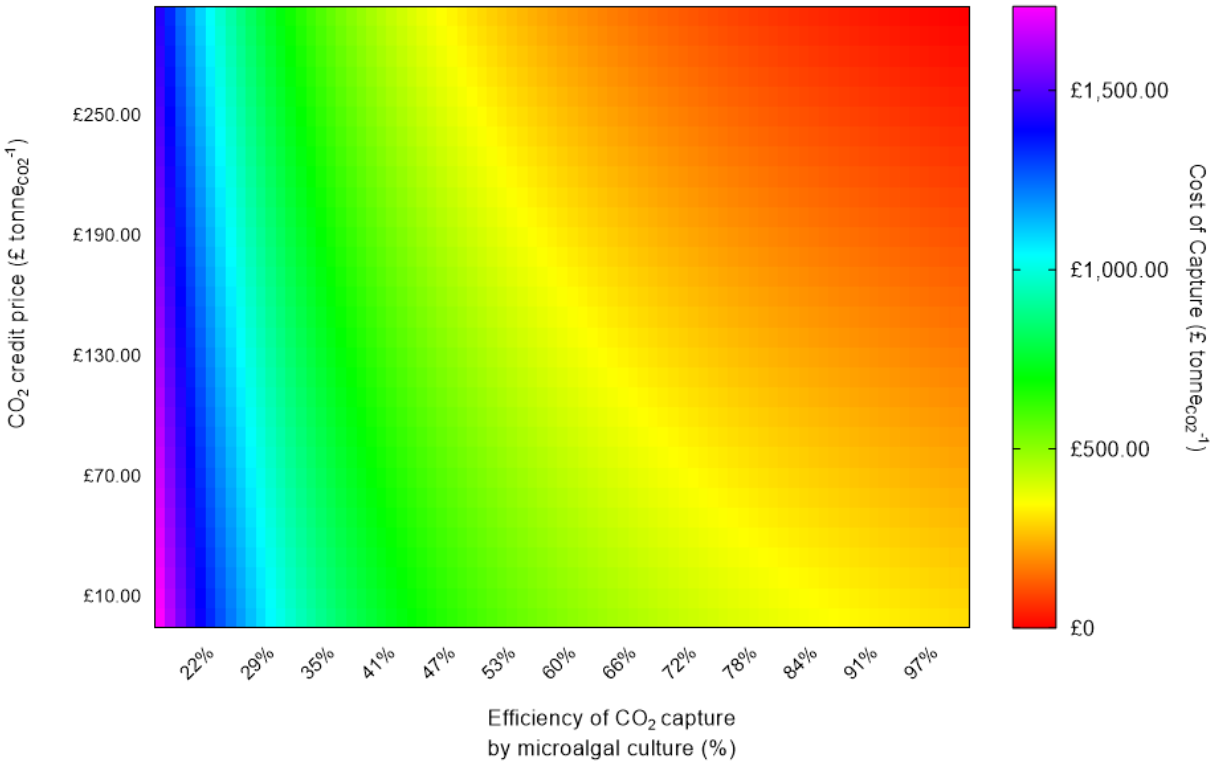

Figure 5.9: *Dual parameter analysis (based on scenario 2) of changing CO_2 credit price and capture efficiency.*

5.5. Discussion

5.5.1. Optimal CO_2 Concentrations for *Chlorella vulgaris* Growth

In this work, *C. vulgaris* grew the best under conditions of 5 % CO_2, similar to the concentrations found in closed-cycle gas turbine (CCGT) power plants (Farrelly et al., 2013; Molitor et al., 2019), the second largest power generation type in the UK after petroleum oil (National Statistics UK, 2017a). These results show similar trends in microalgal growth under elevated CO_2 concentrations to that seen within the literature. Yang *et al.* found that *Desmodesmus* sp. and *Scenedesmus* sp. both grew best at 5 – 10 % CO_2 but that increasing the concentration to 15 % CO_2 caused a negative effect on the cultures (Yang et al., 2020a). The likely reasoning for inhibited growth at higher concentrations is not due to carbon limitation but in fact due to the dissolved CO_2 within the media causing the pH to drop below the optimal for this species.

On the other hand, Chiu *et al.* (2009) found that *Nannochloropsis oculata* was extremely sensitive to elevated CO_2 concentrations above 2 %. At all conditions above 2 % the algae did not grow, while 2 % vastly improved the growth rate compared to aeration with only air (Chiu et al., 2009a). This highlights that CO_2 sensitivity is extremely species specific and will be a key consideration when looking to move into industrial applications.

This work was conducted using a 24-hour light cycle, as many other publications have also done (Chiu et al., 2009b; Li et al., 2013). The change in CO_2 capture efficiency when different light cycles, including pulsed, flashing, and traditional 12-hour light: dark, are used should be considered in future analyses and experimental work. The difference between the CO_2 uptake of cultures and the CO_2 emissions from providing artificial lighting during night hours will be an important ratio for the consideration of algae as a CCUS option.

5.5.2. Measuring the CO_2 Removal Versus the Theoretical Estimation

The experimental results expressed in Table 5.2 for CO_2 removal based on real-time monitoring and based on the assumption used vastly within the literature, compared to the figures presented in Table 5.1, highlights the key issues with current research techniques. The microalgal cultures can capture a larger proportion of the carbon presented to them than is estimated in Equation 2.7. Jacob-Lopez *et al.* (2008), Neves *et al.* (2018), and Gonzales Lopez *et al.* (2009) all give examples of exopolysaccharides and additional metabolites which are produced by microalgae which will contribute to the carbon capture but not be evaluated when only the cell density/productivity is assumed to be capable (González López et al., 2009; Jacob-Lopes et al., 2008; Neves et al., 2018). Continued development with real-time monitoring of CO_2 in flow and out flow from bioreactors will allow for a much better understanding of how the cells utilise the resource when it is not a limiting factor. Much research has been published on how cells adapt to carbon limited environments with the carbon concentrating mechanism, but few have considered how to adapt cells to higher concentrations and allow for a better capture rate.

Alongside the requirement for more direct measurements of CO_2 uptake, optimisation of the PBR for CO_2 capture should be addressed. The low CO_2 capture (10-20 %) seen throughout the literature and this work (de Morais and Costa, 2007c; Yang et al., 2020a) highlights that this is a key area for improvement. Yang *et al.* 2020 showed that the use of sequential reactors can improve the CO_2 capture efficiency of *Chlorella pyrenoidosa* cultures from 10 % up to 90 % at a CO_2 concentration of 10 % CO_2 (Yang et al., 2020b).

It is important to note that the process used in this work was not optimised and therefore improvements could readily be made including the use of sequential reactors and reduced

flow rates (Li et al., 2011; de Morais and Costa, 2007c; Yang et al., 2020a). Adaptive evolution of the species, increasing inoculation concentration of the microalgae and optimised nutrient and light feeding could also further increase the capture efficiency and are where future work will be focused. Alongside this, efforts should be made towards using real and simulated flue gases from various applications such as Kao *et al.* (2014) and Doucha *et al.* (2005) to highlight how other components will affect the growth of the microalgae.

5.5.3. Techno-Economic Assessments

As this is an initial assessment, based on laboratory data and a theoretical 'first of its kind' facility there are limitations to the results gained. All the scenarios tested are realistic but changes as singular as those shown are unlikely. For example, improvement of the capture efficiency in Scenario 7 does not consider that, as a result, more biomass will be produced. This could be sold on to increase revenue, but also requires more nutrients feeding into the PBRs, which will incur additional charges. It is also important to note that while the analysis highlighted that the introduction of a government policy for carbon credits could aid the feasibility of this process, these credits will likely not exist for the entire project lifetime (20 years) and their value may fluctuate overtime. Improvements in the scenario management and analyses based on government policy for previous, similar, technologies (first and second-generation biofuel production) would help further improve the accuracy of this assessment.

As stated in the methodology, downstream processing of biomass and sale of by-products is not included within the analysis. Analysis of the biomass content for ash (unusable percentage) and the presence of high value products would allow for the inclusion of these which could have favourably affected the results.

5.6. Conclusions

In this chapter, *C. vulgaris* was grown under different CO_2 concentrations to ascertain an optimal condition for growth. The CO_2 removal efficiency of the cultures was then measured using a NDIR sensor connected to the gas flow directly leaving the culture. This real-time monitoring showed that the microalgae could remove ~20 % of the CO_2 from the incoming gas stream. This was then compared to the literature to highlight that the CO_2 uptake is vastly underestimated when using the theoretical assumptions that 1 kg of biomass fixes 1.88 kg of CO_2 (Chisti, 2007).

The experimental data was then extrapolated and used as an input to model the cost of CO_2 capture and which parameters are key in reducing this cost. The TEA highlights that improvements in the efficiency of capture by the microalgae and cost reduction in both the capital and operational aspects of the process would greatly benefit the economics of the proposed facility. The conclusions drawn from it can be used to direct further research to focus in these key areas. Combining the carbon capture process with other waste treatment technologies (*e.g.* leachate and wastewater bioremediation) should also be considered for improving the feasibility of the process.

6. Leachate Bioremediation Using Microalgal-Bacterial Consortia

In the previous chapter, the bioremediation of gaseous waste streams using microalgae was evaluated. In this chapter, the bioremediation of liquid waste streams, in particular landfill leachate, using a microalgal-bacterial consortium will be investigated. This waste stream consists of different pollutants to remediate and different essential nutrients for algal growth. As each waste stream offers a different set of nutrients their bioremediation could be coupled, which is evaluated in the next chapter.

6.1. Introduction

Although the rate of recycling in the UK has been growing over the past decade, 14 Mtonnes of municipal waste was still produced and sent to landfill in 2017 (DEFRA, 2016). This landfilled waste is contained but not treated or eliminated, which can lead to potential environmental hazards and loss of valuable resources. Percolation of rainwater through the solid waste and decomposition of components within the waste result in the production of a toxic liquid termed leachate. This liquid effluent is a major environmental concern due to its high chemical oxygen demand (COD), ammoniacal-nitrogen (NH_3-N) and heavy metal concentration (Ozturk et al., 2003; Pacheco et al., 2015). When released into the environment, nutrients within the untreated liquid can cause eutrophication of nearby water sources (Eze et al., 2018). Furthermore, heavy metals within the effluent, such as arsenic and mercury, can bioaccumulate within the ecosystems, affecting flora, fauna, and human health(Pacheco et al., 2015; Suresh Kumar et al., 2015).

Biological, chemical, and physical methods can be used to treat leachate (Wiszniowski et al., 2006), although there is no 'most appropriate treatment' available (Renou et al., 2008).

Current treatment methods come with both advantages and disadvantages (summarised in Chapter 2) meaning they are often used in combination. Problems with current technologies include relatively high expense, energetically demanding, environmentally unsustainable processes and efficiency issues as the characteristics of leachate change (Suresh Kumar et al., 2015). For example, air stripping followed by reverse osmosis (Renou et al., 2008) produces a more concentrated toxic waste product: retentate.

The use of photosynthetic microalgae for nutrient and pollutant removal from leachate offers an alternative method of treatment where a useable by-product in the form of biomass is produced. The concept of simultaneous wastewater treatment and algal production was proposed by Oswald *et al.* in 1957 (Leong et al., 2018; Oswald et al., 1957). Since then, an increasing number of studies utilising a diverse collection of microalgal strains have demonstrated that microalgae can remove COD, NH_3-N, orthophosphate, and heavy metals, from these wastewaters with varying degrees of success. As each leachate has its own unique composition and each species or strain react differently to each component there is not one-optimal strain using algal-based treatment of leachate. However, the ability to generate algal biomass through the treatment process is advantageous both from an environmental and economic standpoint. The resulting algal biomass can be converted to a variety of products, including plastics, fuels, fertiliser or animal feed (Bhalamurugan et al., 2018; Khan et al., 2018), improving the economics of the process and producing a closed-loop of nutrient usage where the waste is reutilised rather than disposed of. Integrating bacteria into the process in the form of an algal-bacterial consortium can also aid the treatment process by targeting the bio-degradation of more recalcitrant compounds and hence reducing COD within the leachate (Cuellar-Bermudez et al., 2017). Moreover, the algae can capture CO_2 generated by the biological oxidation of organic compounds. The symbiotic relationship has been shown to improve nutrient removal and can make the process more robust to changes in nutrient flux

(Cuellar-Bermudez et al., 2017).

There are currently several limitations to algal-bacterial leachate treatment including issues with the dark colour, sub-optimal phosphate levels, toxic organics, and very high ammonia levels. The dark colour often associated with leachate affects the photosynthetic potential of algae, adversely impacting the biomass productivity (Lin et al., 2007). Similarly, the presence of toxic organics and heavy metals can adversely affect productivity. Algae require both a nitrogen and phosphorous source to grow and leachates can often offer too high concentration of NH_3-N and too little phosphorous. Consequently, leachate is often diluted to 10 % (v/v) to increase transparency and reduce the NH_3-N concentration, together with supplementation with a phosphorous source if necessary (Eze et al., 2018; Lee et al., 2016; Lin et al., 2007).

Another major limitation of algal leachate bioremediation currently is the economic viability of the process. Without any comprehensive techno-economic analyses there is little insight into the economic competitiveness with conventional treatments. Most studies to date state that the use of "wastewater" as a nutrient source will benefit the economics of microalgal production (Asmare et al., 2013; Cruce and Quinn, 2019; Mousavi et al., 2018b) but predominantly from the viewpoint of generating lipids for conversion to fuels. Furthermore, where economics are considered, biomass production is the sole focus, not the treatment of wastewater and potential for nutrient recovery.

The aim of this chapter is to evaluate the economic potential of a microalgal-bacterial consortium for the treatment of landfill leachate based on pilot scale experimental data. The experimental data was used as a basis to assess the potential treatment cost (£) per M^3 leachate in a 1-ha facility located within the UK. In a similar style to the previous chapter, a cost breakdown for the baseline scenario is produced then a variety of financial and

operational scenarios are input to the model to highlight where research should be focused to reduce the cost. The key cost parameters were then subjected to a ±20 % change to explore the cost sensitivity.

6.2. Experimental Methods and Results

The experimental data used as an input to the model was produced by Kasia Emery using the research groups pilot scale PBR located outside the Arthur Willis Environmental Centre (University of Sheffield). Here, a brief description of the experimental set up and results are described. Information on the initial consortium collection and the adaption over 24 months prior to this work is published in Bioresource Technology (Okurowska et al., 2021a).

6.2.1. Phycoflow™ Pilot Scale Experiment for Leachate Treatment

The 300 L PhycoFlow™ PBR (Varicon Aqua, Figure 4.1) was used for the pilot scale treatment of leachate by the adapted algal-bacterial consortia. The experiment was conducted between September and November 2017 in Sheffield, UK and lasted a total of 42 days. The PBR was located outside and utilised natural sunlight as the only lighting source. The PBR was chemically sterilised prior to inoculation with the consortia using sodium hypochlorite (2 %) and then sodium thiosulfate (5 %) to neutralise the chlorine. The working volume of the PBR was 311 L. The media for algal growth consisted of 10 % landfill leachate diluted in non-sterile tap water with additional key nutrients added (0.25 g L^{-1} sodium nitrate, 0.075 g L^{-1} dipotassium phosphate, 0.26 g L^{-1} monopotassium phosphate). The inoculum (20 L working volume) was grown under the laboratory conditions described in Chapter 3 to an OD_{695} of 3 before transferring to the PBR.

During cultivation, the optical density, cell count, total nitrogen, ammoniacal-nitrogen, and dissolved inorganic phosphate (DIP) were measured periodically. Removal efficiencies of key nutrients were calculated using the following equations:

$$RE = \frac{X_0 - X_t}{X_0} \times 100 \quad 6.1$$

$$RR = \frac{X_0 - X_f}{t_f} \quad 6.2$$

Where RE is the removal efficiency (%), RR is the average removal rate (mg L^{-1} d^{-1}), X_0, X_t are the concentrations at the beginning and end of the experiment, respectively, and t_f is the total time of the experiment (in days).

6.2.2. Phycoflow™ Results

A pilot scale, batch experiment for the treatment of landfill leachate and simultaneous algal biomass production was conducted using the 300 L Phycoflow™ PBR. The growth rate and reduction of key nutrients was followed through the experiment and key results are presented in Table 6.1 and Figure 6.1.

Table 6.1: *Experimental results for the pilot scale experiment*

Parameter	Value	Units
Time of the batch run	42	Days
Culture productivity (average)	0.124	g L^{-1} day^{-1}
Final biomass concentration	2.4	g L^{-1}
Wet biomass harvested	0.7215	kg PBR^{-1}
Harvesting efficiency	95%	% removal
Moisture content of harvested biomass	80%	%
Leachate ammonia removal efficiency	86%	%
Leachate DIP removal efficiency	100%	%

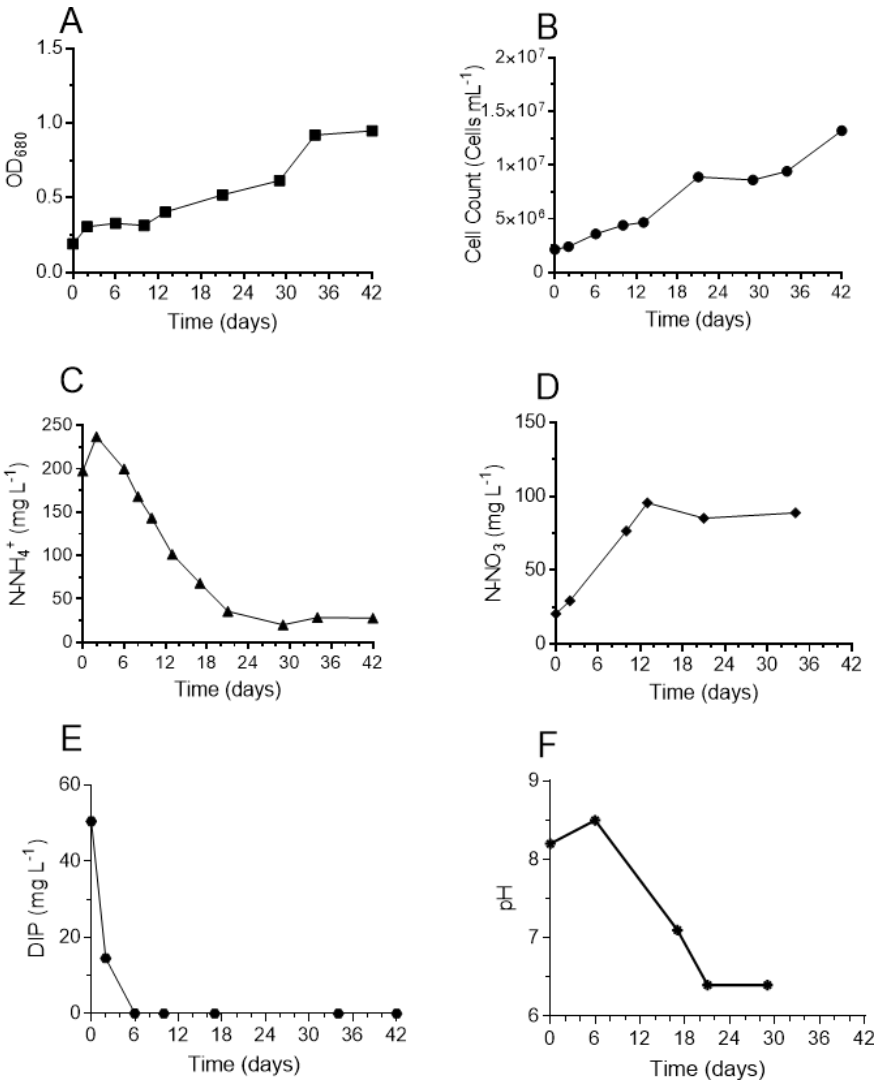

Figure 6.1: *Experimental results for the PBR batch experiment. A and B show the consortia growth over the 42-day period by optical density (A) and cell count (B). C shows the ammoniacal-nitrogen removal, D) the nitrate/nitrite removal, E) the dissolved inorganic phosphate (DIP) removal and F the pH of the culture.*

The microalgal cell numbers increased steadily over the course of the experiment, although there were fluctuations in the rate of growth, particularly noticeable through an acceleration between days 13 and 21 and between days 34 and 42 (Figure 6.1.B). There were periods where OD_{680} increased, but not at the same time points as microalgal cells (Figure 6.1.A and 6.1.B). This is not entirely unexpected as peaks in heterotrophic bacterial activity have previously been shown to follow peaks in primary production in algal-bacterial cultures (Russo et al., 2016), a phenomenon potentially occurring within the PBR in three different stages. The temperature fluctuated in the reactor from 19 °C to 26 °C. The warmest period was between days 13 and 21 which coincides with an increase in growth rates (based on cell counts and OD_{680}).

Although phototrophic microalgal growth and/or excretion of basic metabolites from biodegradation of organic matter often increases the pH of the media (Delgado-Baquerizo et al., 2016), there was an overall decrease in pH from 8.2 on Day 0 to 6.4 in the PBR over the course of the experiment (Figure 6.1.F). The complexity of the leachate composition as well as the microbial consortium within the PBR means there are many factors which could impact on pH changes, including microbial activity generating CO_2 and volatile fatty acids.

6.2.2.1. Nitrogen Removal

The ammoniacal-nitrogen (NH_3-N) concentration at Day 0 was 197 mg L^{-1}. There was an initial increase in concentration to 237 mg L^{-1} in the first two days, before the concentration reduced steadily to below 20 mg L^{-1} at day 29 (Figure 6.1.C). The initial increase was likely due to bacterial ammonification of other nitrogen sources within the complex leachate. This is evidenced by an OD_{680} increase in this period, which was not followed by microalgal cell count, implying bacterial growth.

The dissociation constant, pKa, of the ammonia/ammonium reaction is approximately 9, depending on a reaction conditions (temperature, salinity etc.). This pKa value and the low pH of the diluted leachate (<8) mean that ammonium ions (NH_4^+) were dominant over ammonia (NH_3) within the PBR. Ammonium ions have lower toxicity and volatilisation rates in comparison with ammonia, allowing for greater overall removal by the microalgae. 86 % of the ammoniacal-nitrogen was removed from the PBR over the 42-day cultivation period, with a relatively high average and maximum removal rate of 7.7 and 14.0 mg L^{-1} day^{-1}, respectively. Current understanding of using microalgae for ammonia removal from leachate varies depending on the species, cultivation vessel design, aeration, mixing as well as the pH, temperature, and photoperiod (Cheah et al., 2016; Fernandes et al., 2013; Martins et al., 2013). A previous study by Martins *et al.* reported 75 – 99 % removal of the ammonia from landfill leachate using stabilization ponds (Martins et al., 2013). Interestingly, a nitrogen balance revealed that under the conditions of the continuous treatment system tested, 64 – 79 % was contained within dead or inert settled algal cells, whereas 1 – 6 % was assimilated into live algae (*Chlamydomonas* genera), with 12 – 27 % of removal by volatilization. The ammonia volatilization rate was not measured during this experiment, however considering the design of the PBR (closed system), pH, reduced flow rate, short aeration period (4 hours per day) and temperature range (19 – 26 °C) during the experiment, the volatilization rate is expected to be lower than in an open pond (Martins et al., 2013). There was evidence that bacterial based nitrification had taken place during the first 10 days of cultivation as levels of nitrate and nitrite increased (Figure 6.1.D).

6.2.2.2. Dissolved Inorganic Phosphorous (DIP) Removal

Concentrations of bioavailable phosphate in landfill leachates are generally quite low, and as expected, the DIP concentration in the 10 % dilution of leachate was only 0.061 mg L^{-1}. Like

other landfill leachate treatment studies using microalgae (Dogaris et al., 2019), P-supplementation was undertaken to avoid P-limitation. After supplementation, 50.5 mg L^{-1} DIP was measured on day 0 of the experiment. Within 6 days, DIP was almost below detection limits in the leachate (Figure 6.1.E), with an average removal rate of 8.4 mg L^{-1} d^{-1}.

Although the majority was assumed to be consumed by microalgae, most of the growth took place after 12 days: implying the use of luxury-P or alternative sources of P after this time. It is known that phosphate can precipitate in microalgal cultures where the pH is higher than 8 and it should be considered that some may have precipitated during the first few days of cultivation when the pH was recorded above 8. Our results do indicate that although algal growth is possible when DIP levels are close to zero, additional provision of this essential element would likely increase biomass accumulation within the PBR.

6.3. Techno-Economic Analysis

6.3.1. Baseline Model

A techno-economic model was based on the experimental results presented above and the capital and operational information for the modular PBR system (Information regarding these can be found in Chapter 4). The analysis assumes the production/treatment is scaled up to 1 ha, similar to that seen within the literature for small scale algal cultivation (Tredici et al., 2016; Valdovinos-García et al., 2020). Each modular PBR unit requires 6 m^2 of floor space. With the allocation of room for a laboratory/office for sampling and inoculum preparation (Tredici et al., 2016) and space between each unit for maintenance access, it is assumed 738 units (230 m^3 culture volume) can be achieved. Due to the modular nature of the PBRs, no

economies of scale are achieved with the purchase of this item so the purchase price from the manufacturer is used in all calculations.

As the experimental data comes from the UK during late autumn – early winter, it is assumed that the algal growth and leachate treatment can be achieved all year round and therefore the facility is operational 360 days of the year. To maintain coherence with the experimental data the same operational procedure of batch culture was chosen. With the 42-day treatment time used in the experiment this equates to a full 8 batches being produced annually, with additional time being used for cleaning and maintaining the facility.

The biomass productivity and nutrient / HM removal results from the experiment are used to determine the outputs from the system in the form of: a) wet biomass which can be sold on for further downstream modification and b) the remediation of leachate, allowing water to be discharged. No downstream processes are included within the scope of this assessment, as shown in Figure 6.2. The financial assumptions and calculations for capital and operational expenditure can be found in Chapter 4.

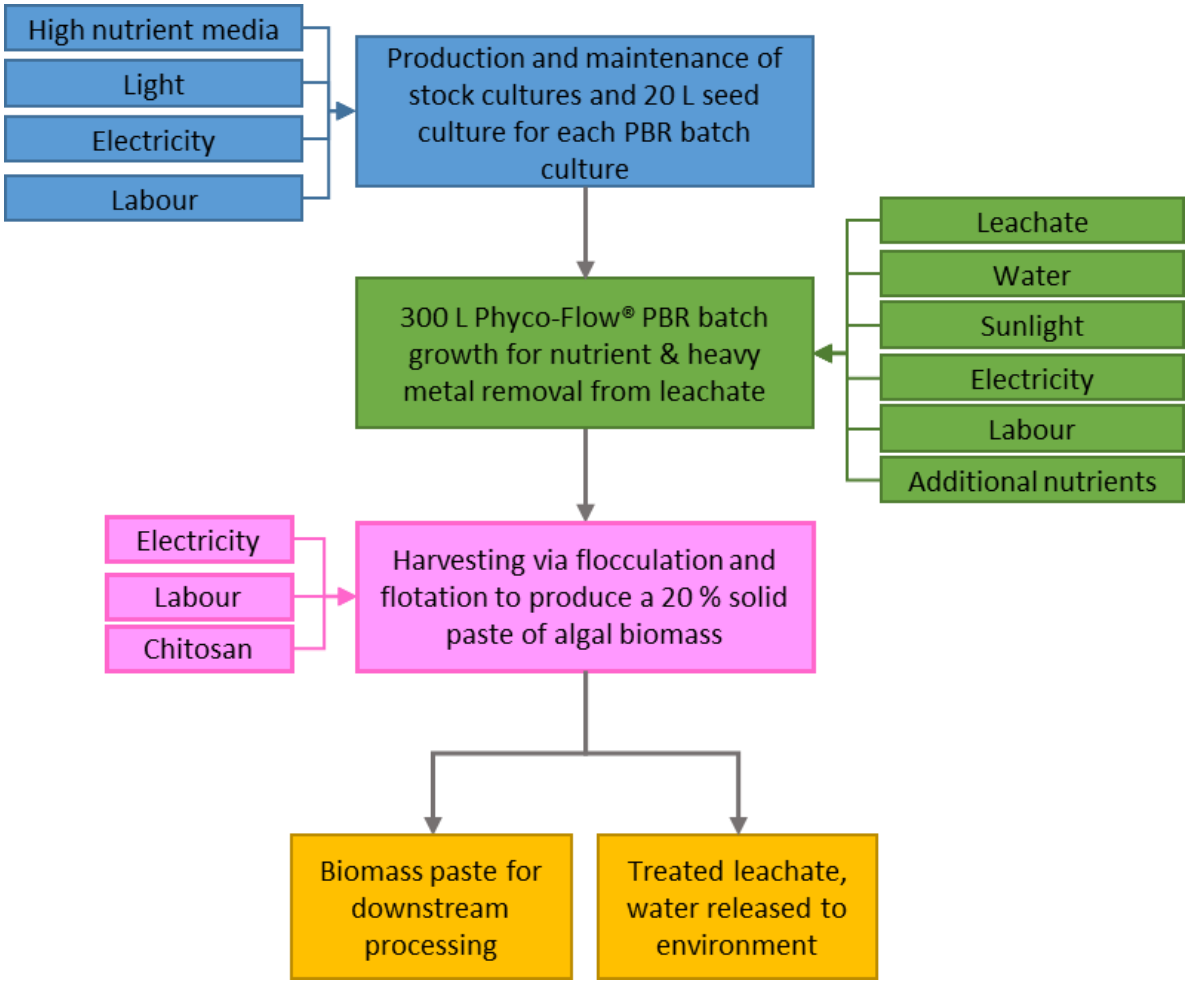

Figure 6.2: *Flow diagram of the processes included within the assessment. The final steps associated with downstream processing of the biomass are not included in the scope.*

The cost breakdown of the process is shown in Figure 6.3. The major equipment cost (MEC) contributes the largest portion of the overall cost at 48.86 %. Within this (Figure 6.3.B), the PBR unit contributes the largest proportion at 91.38 % of the MEC. In a similar fashion to the previous chapter, this highlights that the use of a modular, glass system may not be ideal for low value applications such as remediation. To avoid the high-capital investment associated with such PBRs, alternatives made of plastic could be utilised (Tredici et al., 2016). These units however have a much shorter lifespan to the glass counterparts which may reduce the cost-reduction potential. Of course, this baseline assessment does not consider the potential for

wholesale bulk trading discounts to the capital price of the PBR due to a large order of units, this is therefore addressed in future scenarios.

The second largest cost was the direct cultivation OpEx (DCO), contributing 22.67 %, followed by indirect CapEx at 13 %. Within the DCO value, electricity demand is the largest contributing factor. The requirement for heating the units over autumn and winter months, where average temperatures in Sheffield is 5.5 °C (Climate-Data.org, 2019), is a major factor here (Figure 6.3.C). The sourcing of process heat from elsewhere would be highly advisable to reduce these costs. The electricity requirements for the pump operations also contributes significantly to the DCO, which is typical of PBRs with similar designs (Choi et al., 2019). In this base case scenario, it is clear that optimisation of the energy demand and capital input are key for economic viability.

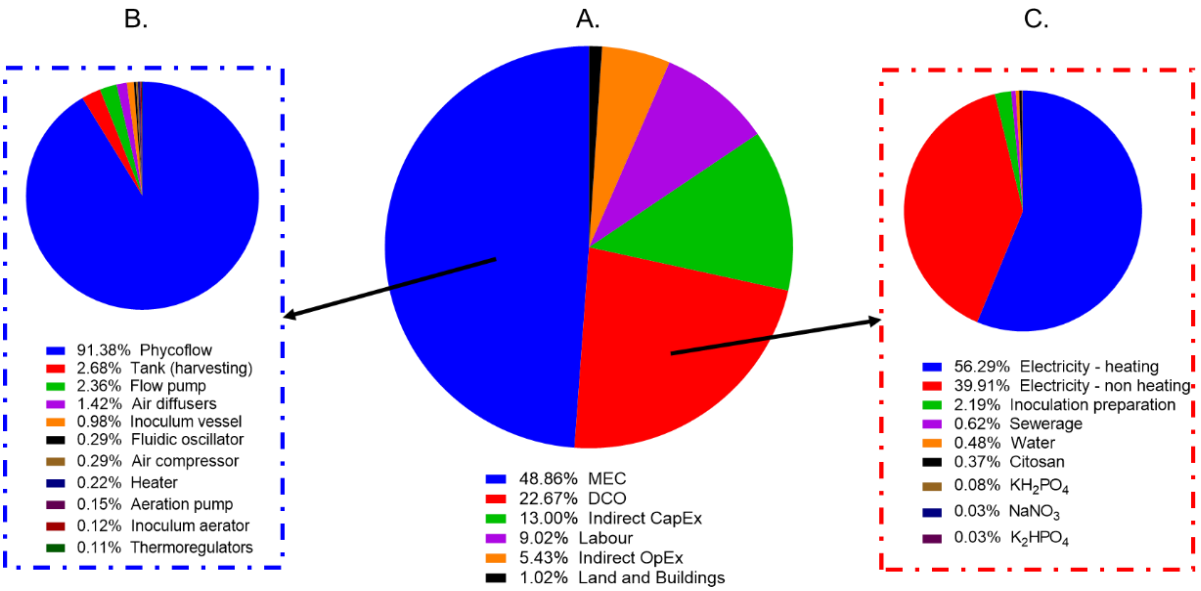

Figure 6.3: *Baseline model cost breakdown. A) overall cost breakdown of the facility. B) Cost breakdown of the constituent parts of the major equipment cost (MEC). C) Cost breakdown of the constituent parts of the direct cultivation OpEx (DCO).*

6.3.2. Scenario Based Analysis

Five different financial and operational scenarios were input to the model to highlight the cost of treatment sensitivity to key parameters. A description of each scenario is given in Table 6.2, information on variable input changes can be found in APPENDIX G.

Table 6.2: *Description of scenarios tested.*

Scenario	Description
Baseline	No changes made, based on the experimental data provided
1	Reduced capital expenditure for major equipment using economies of scale with an average exponent factor of 0.6.
2	Reduced operational expenditure – no heating cost, higher efficiency pumps and aerators, no additional nutrients required.
3	Combined effects from scenarios 1 and 2.
4	Improved biological efficiency. Based on the premise of adaptive evolution through laboratory work leading to the batch time requirement being halved.
5	'Best case' based on combining scenarios 3 and 4.

A cost breakdown and change in treatment cost (% change against the baseline value) are presented for each scenario and the baseline in Figure 6.4. In Scenario 1, the cost of major equipment such as the PBR and aeration equipment were reduced through economies of scale, based on communications with the manufacturer. This change reduced the overall costs so that the cost of treatment dropped by approximately 53 %. The MEC contribution to the cost was reduced from 49 % to 8 % and as a result the DCO's contribution to cost rose from 23 % to 53 %, becoming the largest contributing factor.

Scenario 2, where the cost of operational parameters such as nutrient input and electricity demand were reduced, shows treatment was reduced by approximately 18 %. The contribution to costs of the DCO was reduced by ~ 50 % from 23 % contribution to 11 %, also causing the indirect OpEx to be reduced and the capital proportion (MEC etc.) to increase.

In Scenario 3 both the effects of reducing capital and operational costs were assessed together. This reduced the overall treatment cost by 70 %, with DCO being the primary contributor to the overall cost at 36 % with labour expenses as the second largest cost at 33 %.

In Scenario 4, improvements in the consortia treatment and growth were considered, assuming the same biomass concentration and treatment quality can be achieved in half the time currently used in the base model. This resulted in the largest reduction to the treatment cost thus far with a 51 % decrease from the baseline value. Due to the increased capacity seen in this scenario and therefore the larger requirement for reagents, the DCO increased by 9 %, while the MEC remained the largest contributing factor overall.

In the final, best-case, Scenario 5, the contribution of all reductions / operational adjustments resulted in the treatment cost dropping to 15 % of the original baseline value. This was achieved with basic operational and capital modifications. Further research and development into both the experimental methodology and expenditure, both in capital and operational sense, could further reduce these values causing the process to become economically feasible.

To further understand the costs associated with algal leachate treatment, the cost of each scenario is broken down into capital and operational expenditure and these values are shown in Table 6.3. As mentioned previously, the main proportion of costs (for all scenarios) is attributed to either the capital investment required or the DCO. When the capital investment is removed (as seen in the literature (Cruce and Quinn, 2019; Al Ketife et al., 2019; Thomassen et al., 2016)) and the operational costs are presented, the cost of each batch culture is £170 for the baseline and £60 in the best case scenario.

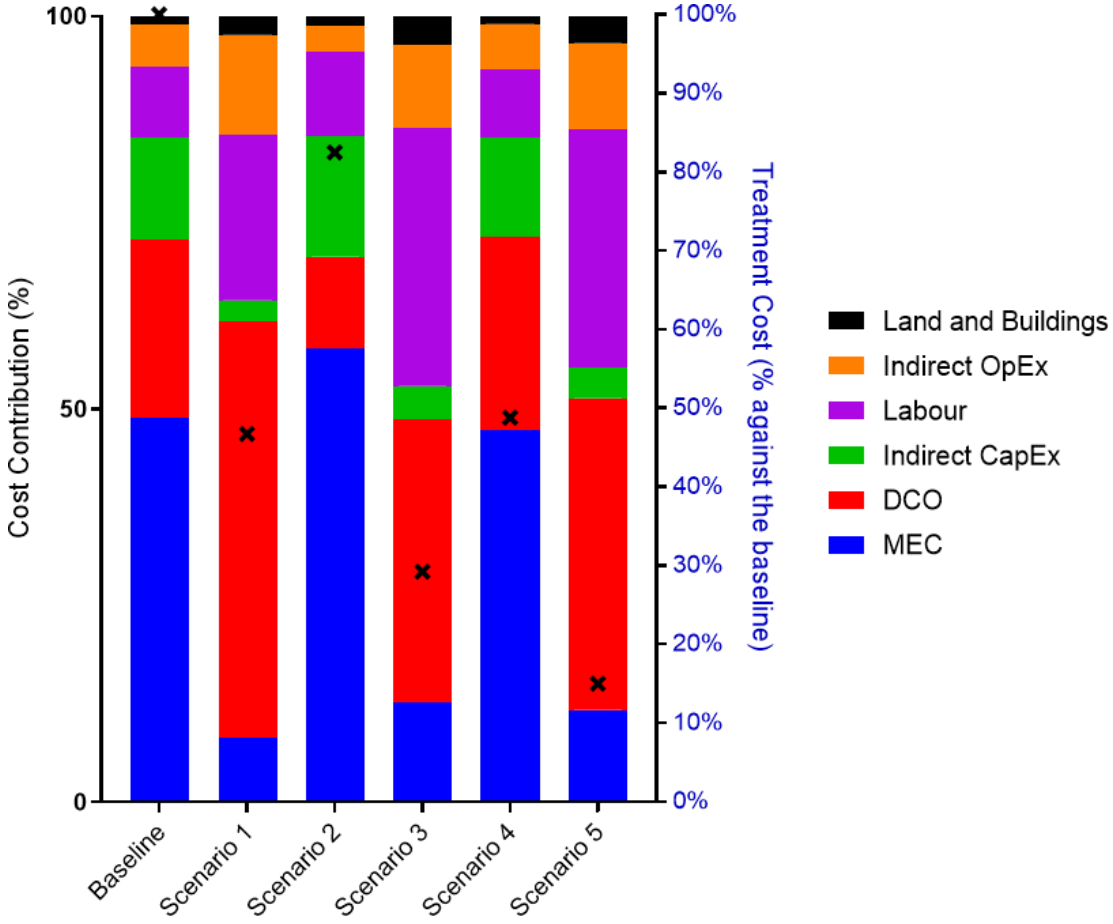

Figure 6.4: *Cost breakdown (left y-axis, columns) and change in treatment cost (%) (right y-axis, crosses) for each scenario tested and the baseline model.*

Table 6.3: *Split CapEx and OpEx results for the cost of producing biomass (COPB), a single batch operation and leachate treatment for each scenario.*

	Baseline	**Scenario 1**	**Scenario 2**	**Scenario 3**	**Scenario 4**	**Scenario 5**
CapEx (% of cost)	63 %	13 %	74 %	21 %	61 %	19 %
OpEx (% of cost)	37 %	87 %	26 %	79 %	39 %	81 %
Total COPB (£ kg^{-1})	530	250	440	160	260	80
CapEx contribution to COPB (£ kg^{-1})	310	20	310	20	140	10
OpEx contribution to COPB (£ kg^{-1})	220	230	130	140	120	70
Total Cost per Batch Operation (£ $batch^{-1}$)	410	190	340	120	210	70
CapEx per Batch Operation (£ $batch^{-1}$)	240	20	240	20	110	10
OpEx per Batch Operation (£ $batch^{-1}$)	170	170	100	100	100	60
Total Cost of Leachate treatment (£ m^{-3})	12,280	5,740	10,140	3,590	6,000	1,840
CapEx contribution to Leachate treatment (£ m^{-3})	7,090	540	7,090	540	3,340	260
OpEx contribution to Leachate treatment (£ m^{-3})	5,190	5,200	3,050	3,051	2,660	1,580

6.3.3. Parameter Sensitivity Analysis

Further to the scenario analysis, key parameters were taken individually and altered by ± 20 % of their original value to highlight how sensitive the treatment cost is to each parameter. Figure 6.5 shows that the number of batch cultures/treatments which can be achieved annually has the most profound effect on the overall treatment price. Reducing the residence time required for nutrient removal and biomass growth will increase the number of batches each PBR can produce annually, ultimately reducing the cost of the leachate treatment significantly. In this example, the residence time has been reduced from 42 days to 33, allowing 10 batches to be completed annually by each unit rather than 8 (20 % increase in the number of batches performed). This small increase in productivity allows the treatment cost to be reduced to 80 % of the cost in the original assessment. This suggests that improvements in treatment efficiency and/or changing to a semi-continuous method may be advantageous when trying to optimise against costs.

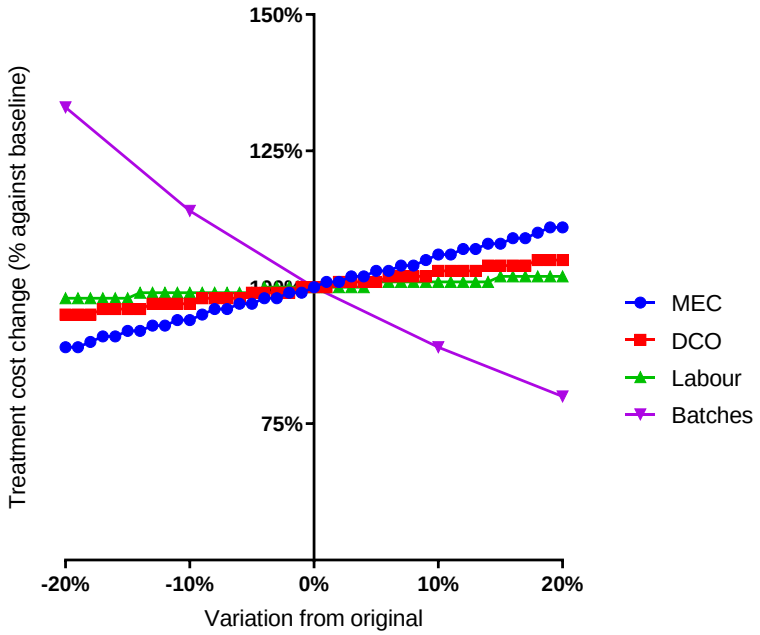

Figure 6.5: *Sensitivity of key parameters: MEC (major equipment cost), DCO (direct cultivation OpEx), labour and batches performed to a ±20 % variation.*

The MEC cost was the next parameter to cause the most significant change from the baseline. Reducing MEC by 20 % allowed the cost to be reduced by 10 % to 90 % of the original baseline value. As previously mentioned, the usage of a lower capital-intensive reactor would help lower these costs further. The DCO and labour both affect the treatment cost in a similar manner to one another, with little change either side of the original value, ± 4.7 and 3.4 %, respectively.

6.4. Discussion

As highlighted in Section 5.5.3 of the previous chapter, initial assessments based on a theoretical 'first of its kind' facility like this has limitations related to the results obtained. While all the scenarios tested are realistic, changes as singular as those shown are unlikely to occur. For example, improving efficiency of the consortia to remediate the leachate in a shorter time frame would not just affect the number of batch cultures performed, but also the direct cultivation costs as more biomass requires harvesting and more chemicals for cleaning & sterilisation are required.

Alongside this, the experimental results, while produced at scale, were not gained under optimal conditions. The weather throughout the growth period was cloudy and cold, giving a good simulation of how the treatment and cultivation processes occur over winter months but not during the summer where conditions are better for algal growth due to the longer day-light hours and higher temperatures. To gain a better idea on how the monthly variations in temperature and sunlight exposure affect the culture's ability to remove key pollutants future work should aim to operate the pilot scale PBR for a whole year so more accurate experimental data can be used for economic analyses of the process.

6.5. Conclusions

In this chapter, pilot scale experimental data for algal-bacterial leachate treatment was used to perform a TEA of the bioremediation process. The initial results showed that operational costs for each batch culture/treatment is approximately £170 when no optimisation or cost reduction strategies are put in place. This would need to be reduced to improve economic viability of the process. Useful product(s) can be derived from algal biomass and intensive research is currently being undertaken to broaden this to different markets including food, plastic alternatives, fertilisers, fish and aquaculture feed and biofuel (both biodiesel or direct burning), reutilising components which are otherwise lost in landfill. Other treatment methods, such as reverse osmosis, do not currently offer this advantage and still lead to the production of a toxic retentate waste.

The scenario-based analysis highlighted that reductions in both CapEx and OpEx are key to make algal-bacterial leachate remediation feasible. Applying economies of scale to PBR purchases in line with manufacturer quotations and reducing the reliance on fresh water and bulk chemicals for supplementation can reduce the overall cost by 85 % against the baseline.

The sensitivity analysis highlighted that increasing the number of batch treatments that can be achieved annually by either increasing algal-bacterial growth rates or moving to a continuous treatment method can reduce the retention times required and would yield the greatest reduction in overall costs. While this analysis is theoretical in nature it provides key insight to where research should be focused to achieve a more financially feasible algal bioremediation technology.

7. Simultaneous Remediation of Two Waste Streams: Is There an Effect on The Efficiencies of Treatment

In the previous two chapters, the bioremediation of a gaseous waste stream (flue gas) and a liquid waste stream (landfill leachate) using microalgae were evaluated. In this chapter the proposal by many publications of treating both streams simultaneously will be investigated (Eze et al., 2018; Gentili, 2014; Ji et al., 2015).

7.1. Introduction

Resource recovery is an essential part of a green circular economy (Vieira de Mendonça et al., 2021); microalgae can be employed for this purpose. It has already been shown in the previous chapters (Chapters 5 and 6) of this thesis that microalgae can utilise nutrients from both gaseous and liquid waste streams, recovering key resources (nitrogen, phosphorous and carbon) as biomass. As the treatment of these waste sources has been demonstrated in isolation of one another, the ability of these microalgae to remediate both waste streams concurrently require investigation.

Most of the literature focusing on the bioremediation of both gaseous and liquid waste streams with microalgae was published in the 2010s, with the majority being published in the last few years up until 2021. While the focus has broadly moved towards bioremediation, many publications still highlight lipid productivities and narrate towards the end use of third generation biofuels.

In 2011, Jiang *et al.* stated that the use of wastewater as a culture medium and CO_2 (from flue gases) were required in order to reduce the cost of producing microalgae for biofuels. This is a stance many publications have taken over the years with the justification of these resources being significantly cheaper than alternatives or completely free (Chisti, 2007; Christenson and Sims, 2011; Ferreira et al., 2019; Norsker et al., 2011). These publications refer to these algal cultivation systems as remediation with little evidence that key pollutants are removed (Ferreira et al., 2019; Kothari et al., 2019; Song et al., 2020; Yang et al., 2020a).

Alongside this, numerous publications consider concurrent remediation but only focus on the nutrient (nitrogen and phosphate) removal capacity and ignore the CO_2 abatement potential of the system (Bolatkhan et al., 2020; Chang et al., 2020; Cohen et al., 2020; Mat Aron et al., 2020; Neves et al., 2018). Many papers ignore the CO_2 removal efficiency altogether, simply utilising the carbon to improve nutrient removal and biomass productivity (Bolatkhan et al., 2020; Cutshaw et al., 2020; Hu et al., 2012; Ji et al., 2015). Others rely on the equation for carbon fixation presented in Chapter 5.1 (Equation 2.7), rather than directly measuring the CO_2 consumption of the system (Hu et al., 2020; Nayak et al., 2016). Issues with using this methodology have been described previously, nonetheless, additional concerns are raised when the systems contain multiple carbon sources (such as organics within the wastewater). Equation 2.7 does not consider mixotrophic or heterotrophic growth regimes, by which many microalgal species can grow (Khanra et al., 2020; Zhou et al., 2017). Leachate and other wastewaters contain various organic carbon sources which could be preferentially consumed by the microalgae and used for growth (Ji et al., 2015; Nayak et al., 2016).

As neither waste stream is an *'ideal*' growth condition for microalgae, the capability of cells to grow and remediate all key pollutants needs to be investigated to discover if there is a

negative effect of concurrent remediation. Some research has focused on this, for example, Hu *et al.* (2012) found that while adding CO_2 to their *Auxenochlorella prothecoides* cultures did not affect ammoniacal-nitrogen and COD (Chemical Oxygen Demand) remediation, the phosphorous uptake was negatively affected due to acidification of the medium. Aerating cultures with 1 % CO_2 (compared to no aeration) caused the remediation rate to drop from 75.05 % to 26.90 %. Alongside this, the COD remediation was not affected by the addition of CO_2, remaining at 80 %. This suggests that the algae are growing mixotrophically and therefore will directly affect the CO_2 removal efficiency, which is not considered in this work. Other considerations include nitrogen stripping, as highlighted by Gentili *et al.* (2014). In their research with *Scenedesmus dimorphus* and *Scenedesmus minutum*, significant nitrogen stripping was seen when flue gas containing 10 % CO_2 was bubbled through their cultures.

While many publications focus on nutrient removal over CO_2, Yang *et al.* (2020) took the alternative approach. This work focused on the real time monitoring of CO_2, similarly to research presented in Chapter 5, and failed to report on the nitrogen and phosphorous removal efficiencies alongside.

The aim of this chapter is to investigate how key nutrients (nitrogen, ammonia, phosphorous and carbon dioxide) are removed when attempting to remediate both landfill leachate and CO_2 gases concurrently. The adapted consortium, originally isolated from a leachate pond, utilised in Chapter 6 is utilised in this work. This consortium has already been shown to effectively remove nitrate/nitrite, ammonia, and phosphate sources from 10 % and 20 % leachate dilutions while withstanding the higher pH and other toxic contaminants present. As the dominant algal species within this consortium is *Chlorella vulgaris*, the microalgal species used in Chapter 5, the culture can adapt to elevated CO_2 conditions and grow autotrophically,

removing CO_2. Experiments are carried out using the same methods as described in Chapter 3, with samples being taken every 3-4 days throughout the growth period for nutrient analysis.

7.2. Growth Under Different CO_2 Conditions

The adapted algal bacterial consortium was grown under three different aeration conditions: no aeration, air aeration and 5 % CO_2 aeration as described in Chapter 3, Sections 3.7 and 3.8. The consortium growth under these different aerations is shown in Figure 7.1.

When the cultures are not aerated, the consortium begins to die after 7 days of cultivation (Figure 7.1A). This is due to the rising pH of the culture medium. The pH of the leachate-based media is already at the upper end of optimal conditions (pH 8.5), and as the cells proliferate it rises, becoming unfavourable. After day 7, the pH of the culture had risen to above pH 9.5, ultimately inhibiting the algal growth, causing the downward turn in biomass concentration.

Aeration allows for pH stabilisation and therefore, allows the cells to continue growing over the entire experimental period, as seen in the (air) aerated cultures (Figure 7.1.B). The final biomass concentration for this condition was 0.565 ± 0.069 g L^{-1}, significantly less than values seen in Chapter 5. This could be attributed to the move from a pre-defined optimal growth media to 10 % leachate, or that the dominant *C. vulgaris* within the consortium is a different strain with a slower growth rate. The consortium growth at scale in Chapter 6 highlights that the consortium does grow at a slower rate than the axenic *C. vulgaris* utilised in Chapter 5.

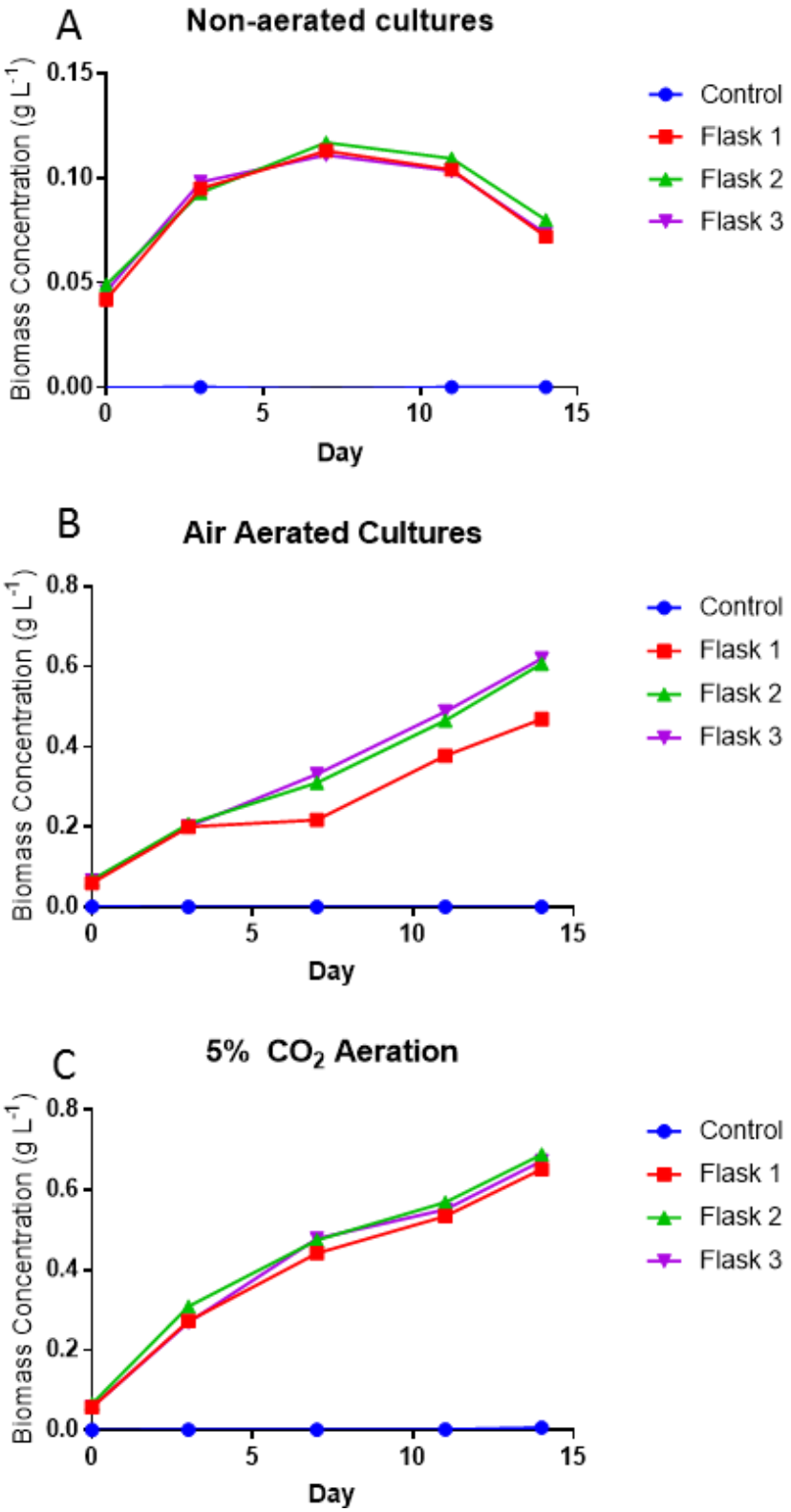

Figure 7.1: *Growth curves of the adapted algal-bacterial consortium growing in 10 % leachate media under different aerations. A) growth when no aeration is applied, B) growth with air aeration (0.04 % CO_2), C) growth with 5 % CO_2 aeration.*

When 5 % CO_2 is bubbled through the cultures (the optimal concentration for the axenic *C. vulgaris* cultures), the final biomass reached just above that of aeration with air only, at 0.671 ± 0.015 g L^{-1} (Figure 7.1.C). This highlights that while elevated CO_2 does not hinder the algal consortium's growth, it does not increase the culture productivity as it had with the previous culture. The final biomass concentrations in the air aerated and 5 % CO_2 aerated cultures were not significantly different from each other, but were both significantly different from the non-aerated cultures, confirming that aeration improves the biomass productivity. In general, the adapted consortium was seen to have a slower growth than axenic *C. vulgaris.*

7.3. Ammonia Removal Under Different Aeration Conditions

The ammonia (NH_4^+) concentration in each experimental flask was monitored using the modified Nessler method (Jeong et al., 2013). The 10 % leachate media contained approximately 250 mg L^{-1} of NH_4^+ at the beginning of each experiment. Ammonia concentrations throughout each experiment are shown in Figure 7.2. There is minimal ammonia removal in the non-aerated cultures (Figure 7.2A) which corresponds to the lack of consortium growth seen in the previous section. When cultures are aerated with air, major ammonia stripping of the media occurs (Figure 7.2B), this is seen as the control (media only, no consortium) concentration also drops at the same rate as the experimental flasks. Negligible concentrations of ammonia were left by the final day of the experiment under these conditions.

On the other hand, no air stripping was seen when 5 % CO_2 was used to aerate cultures (Figure7.2C). There is no change in concentration in the control over the two-week period, but all experimental flasks show a gradual decline in ammonia concentration, ending atapproximately 119 ± 3.45 mg L^{-1} on day 14.

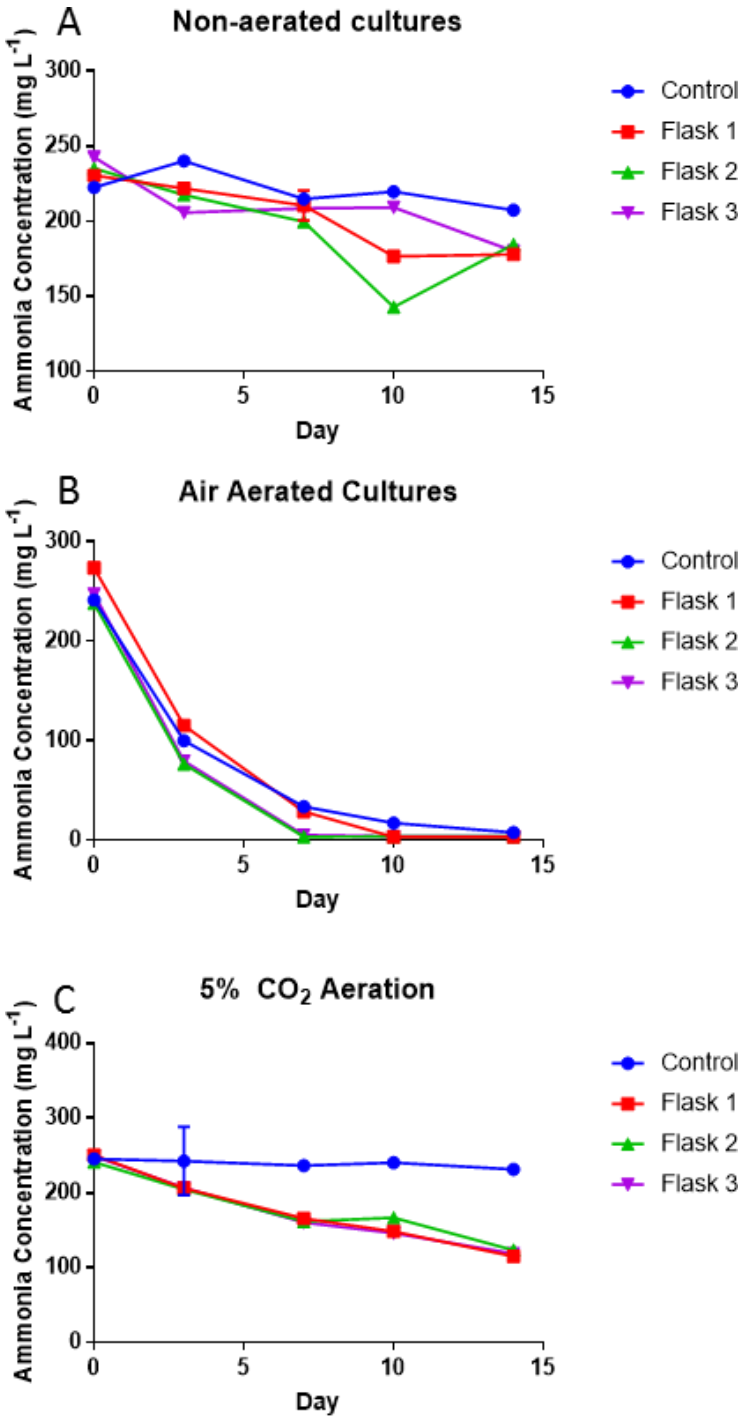

Figure 7.2: *Ammonia concentration in cultures with different aeration conditions over time. A) non-aerated cultures, B) cultures aerated with air (0.04 % CO_2), C) cultures aerated with 5 % CO_2 mixed in air*

The removal efficiency (RE) for each condition is presented in Figure 7.3. While the RE for the air aeration condition is the highest, this does not relate to algal uptake as air stripping removed a large proportion of the ammonia present in the control. As can be seen from Figure 7.3, aeration with 5 % CO_2 does increase ammonia compared to when no aeration is applied to the culture.

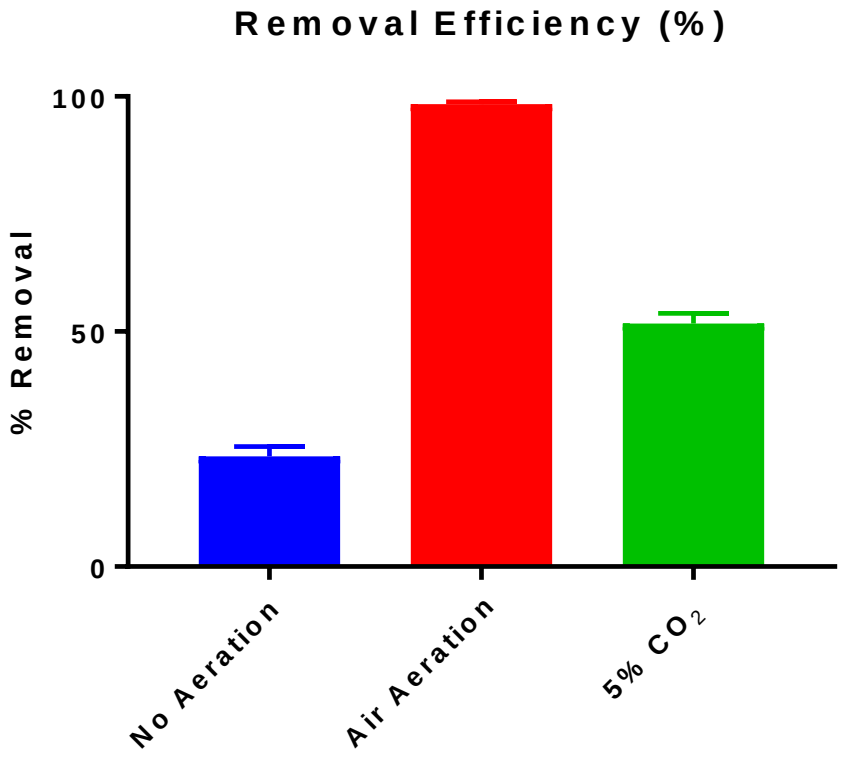

Figure 7.3: *Ammonia removal efficiency of consortium under different aeration conditions.*

7.4. Nitrate and Nitrite Removal Under Different Aeration Conditions

The removal of both nitrate and nitrite sources was jointly measured using the two stage vanadium (III) chloride Griess method as proposed by Miranda *et al.* (2001). In this assay, vanadium (III) chloride reduces nitrate to nitrite and then the total nitrite concentration is determined using the Griess method. The nitrite concentrations for each condition over the experimental period are shown in Figure 7.4.

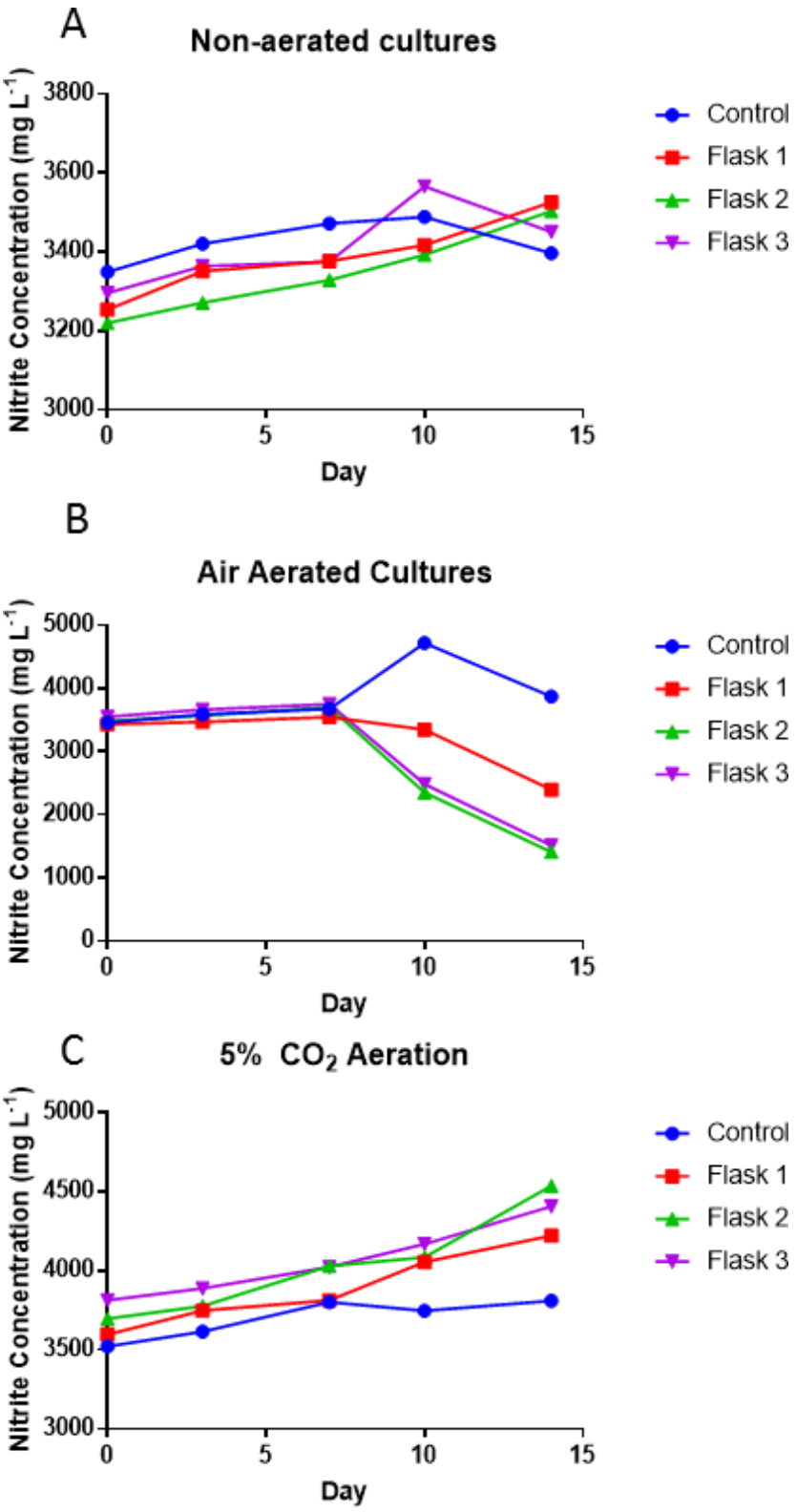

Figure 7.4: *Nitrite concentrations in 10 % leachate media in different aeration conditions. A) cultures are not aerated, B) cultures are aerated with air (0.04 % CO_2), C) cultures are aerated with 5 % CO_2 mixed in air.*

In cultures where no aeration is utilised, the nitrite concentration slightly increases over the 14-day period from 3,300 mg L^{-1} to 3,500 mg L^{-1} (Figure 7.4A). As the cells do not grow well under these conditions, it is expected that little to no nutrients are removed from the media. Oxidation of ammonia to nitrite may be responsible for this slow increase in nitrite concentration over time.

Alongside this, the concentration of ammonia reduces under these conditions, suggesting that the consortium is preferentially utilising ammonia as a nitrogen source over the others present (nitrate and nitrite). Preferential utilisation of ammonia over nitrate and nitrite has been shown in microalgae before (Lachmann et al., 2019) and is often closely related to the carbon and phosphorous levels present. This suggests that the additional $NaNO_3$ added to the 10 % leachate media may not be a necessary addition (and expense) for improving algal growth.

When the cultures are aerated with air, about 40 % of the total nitrate/nitrite is removed from the media. As significant ammonia stripping was seen under this particular condition (Figure 7.2B), this may likely be due to a lack of other nitrogen sources being available to the algae. This highlights that the consortium can utilise a wide variety of nitrogen sources to sustain their growth, but preferentially utilise the ammonia present within the leachate. As less than half of the additional nitrate was utilised, this suggests that further optimisation could be done to reduce wastage of nitrogen sources being added to cultures.

When 5 % CO_2 was bubbled through the cultures, the nitrate/nitrite concentrations followed a similar pattern to that shown with no aeration, increasing in concentration (about 15 %). This is likely attributed to the conversion of ammonia to nitrite by ammonia oxidising bacteria, present within the consortium.

The removal efficiency of nitrate and nitrite under each aeration condition can be seen in Figure 7.5. For non-ammoniacal nitrogen removal, air aeration is the optimal condition when utilising this consortium, with 49.00 % of the nitrate and nitrite within the media being removed after 14 days. The other conditions tested both lead to an increase in nitrate/nitrite concentration within the media, suggesting that the consortium can preferentially utilise other nitrogen sources present, and that nitrate supplementation is not necessarily required.

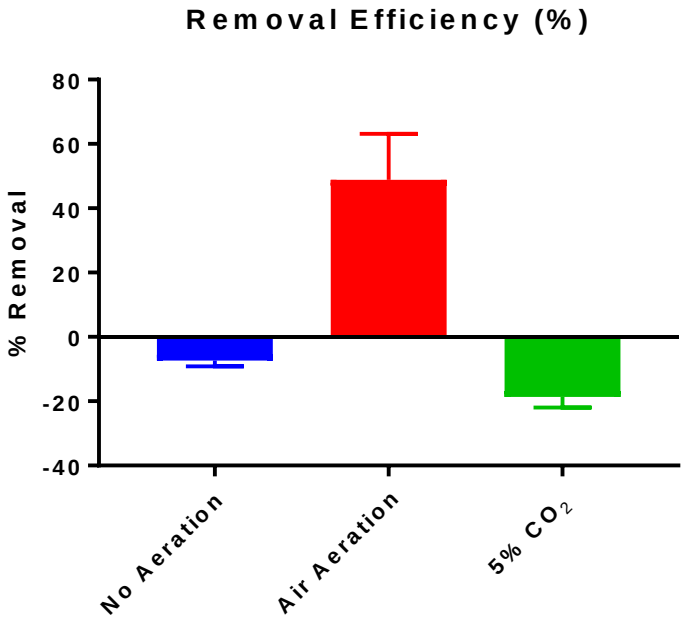

Figure 7.5: *Average removal efficiency (RE) of nitrite from each aeration condition after 14 days.*

7.5. Dissolved Inorganic Phosphate Removal

The dissolved inorganic phosphate (DIP) concentration was measured using the molybdate blue colorimetric assay as proposed by Murphy and Riley (1962). The results for each aeration condition can be found in Figure 7.6. When no aeration is applied and the cells do not proliferate well and die after 7 days of cultivation, the DIP concentration remains relatively stable, only dropping slightly at the beginning of the experiment (Figure 7.6A).

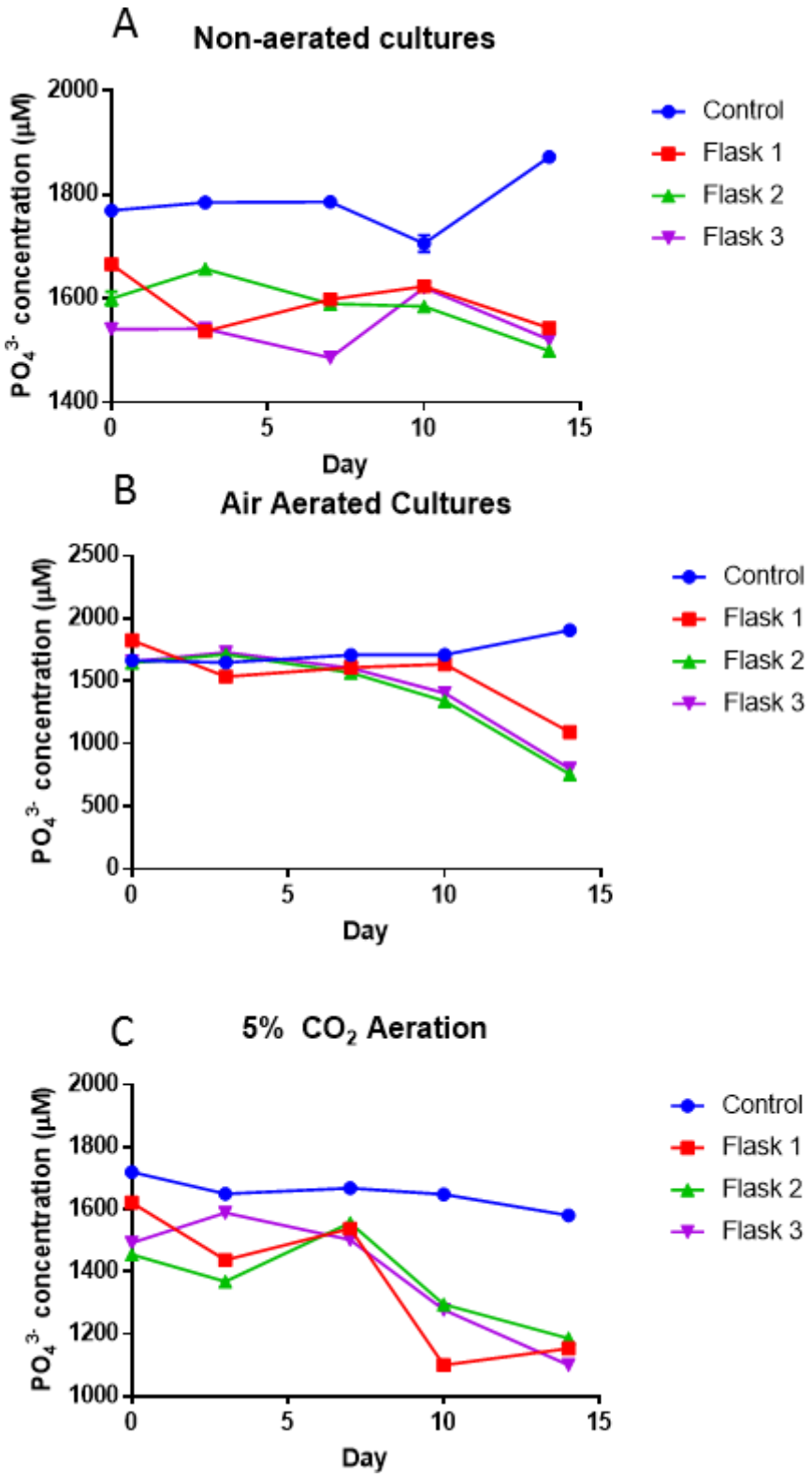

Figure 7.6: *DIP concentrations in 10 % leachate media in different aeration conditions. A) cultures are not aerated, B) cultures are aerated with air (0.04 % CO_2), C) cultures are aerated with 5 % CO_2 mixed in air.*

When the culture is aerated with air, the DIP concentration remains stable for the first 6 days of cultivation and then drops steadily during the second half of the cultivation time (Figure 7.6B). Although a key nutrient for algal growth, the concentration of phosphorous required is much smaller than nitrogen and carbon. In the Redfield ratio, only one unit of phosphorous is required for 106 of carbon and 16 of nitrogen. This may be one of the reasons why, while it is reduced, the DIP is not completely used up within the media. A similar, reduction in DIP concentration is seen when cultures are aerated with 5 % CO_2 (Figure 7.6C).

The removal efficiency of each condition after 14 days is shown in Figure 7.7. As with the nitrate/nitrite removal, the optimal condition for DIP removal efficiency is aeration with air. When adding CO_2 to the culture, the removal efficiency drops from 48.67 % (air aeration) to 24.33 % (5% CO_2 aeration), highlighting that the addition of CO_2 to cultures may benefit biomass production but have a negative impact on nutrient removal.

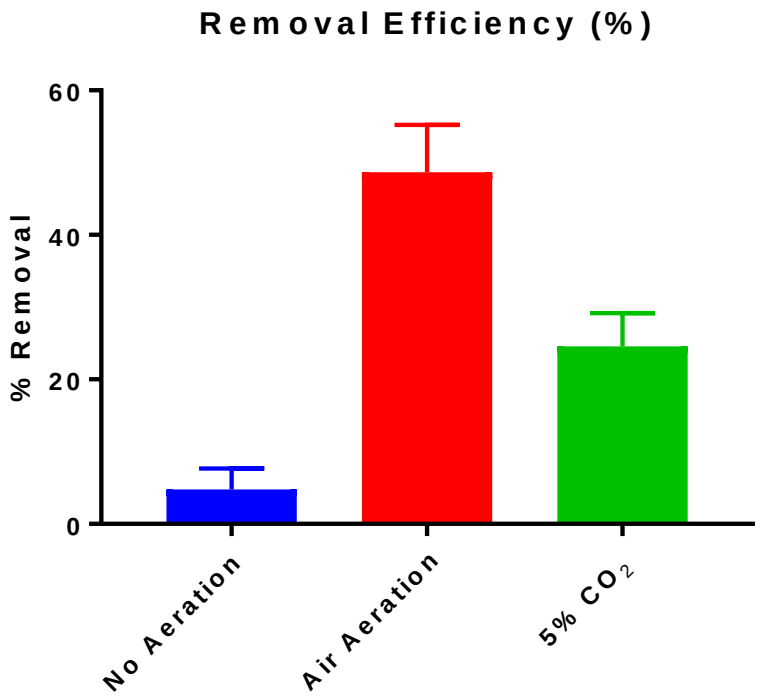

Figure 7.7: *Average removal efficiency of DIP from each aeration condition after 14 days.*

7.6. Measuring CO_2 and Key Nutrient Removal at 5 % CO_2

Although introduction of 5 % CO_2 to cultures only marginally improved the biomass productivity of the consortium, this condition was studied further to address whether the consortium could remove CO_2 from the gas stream alongside the key nutrients within the leachate. The experiment was conducted using the same conditions as previously mentioned with the notable exception of each replicate flask being studied on separate occasions, one after another. This was to allow for direct and constant measurement of CO_2 flow out of the cultures (Section 3.9). The biomass from the previous experiment was washed thoroughly and used to seed the next. This can influence biomass productivity, as the consortium adapts to the new conditions, highlighted in Figure 7.8. The figure shows that the consortium has adapted to the new growth conditions over the two month experiment, with the final replicate growing at a significantly faster rate than the first and second, despite all conditions being controlled. This improved culture productivity, after only a few months, highlights that adaption, especially when considering complex consortium of algae and bacteria, can be key to improving processes.

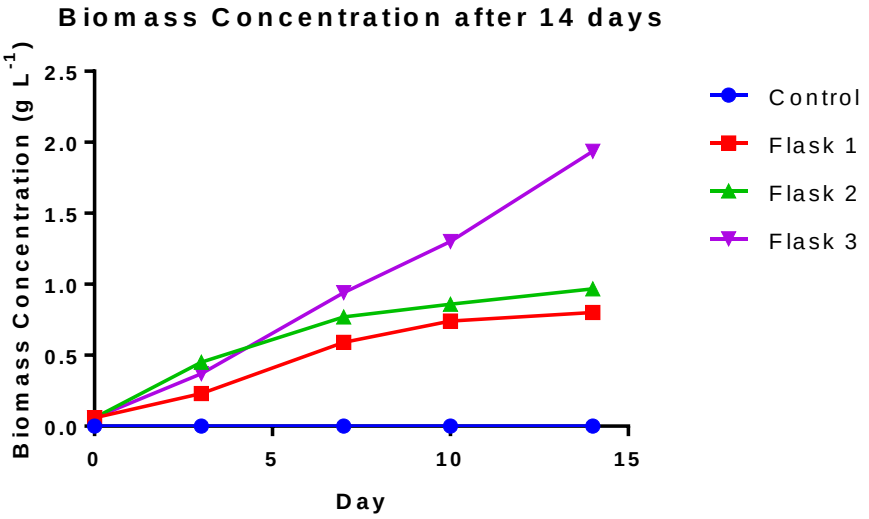

Figure 7.8: *Growth of the leachate consortium over 14 days when grown in 10 % leachate media with 5 % CO_2 aeration.*

This being said, the nutrient removal rates for the third replicate are not dissimilar to those for the other cultures. The ammonia, nitrite, and DIP concentrations in all replicates are shown in Figure 7.9, Figure 7.10, and Figure 7.11, respectively. The ammonia concentration drops during the cultivation time in a similar manner to the 5 % CO_2 condition in the previous experiment, with the RE being slightly higher (averaging 65 %) this time round. This could be attributed to the higher biomass growth in all flasks seen during this experiment. The nitrite concentration increases, giving a negative RE value in the same manner as before, solidifying that the consortium, under these conditions, will preferentially utilise ammonia as their primary nitrogen source. There is a much larger reducing in DIP concentration in this experiment with the average RE reaching 57 % rather than 20 % as seen previously. There is no abiotic removal; therefore, it can be assumed that the additional biomass productivity is affecting this.

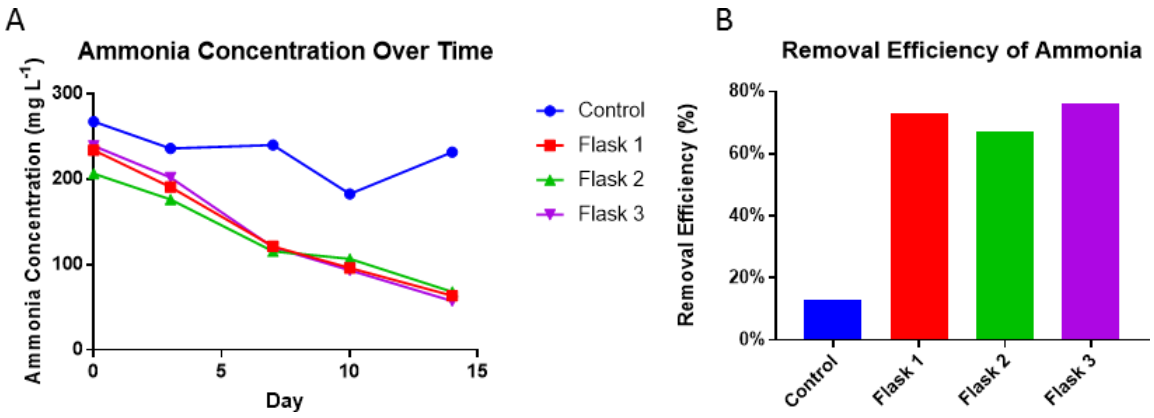

Figure 7.9: *Ammonia concentration (A) and removal efficiency (B) in leachate consortium cultures aerated with 5 % CO_2.*

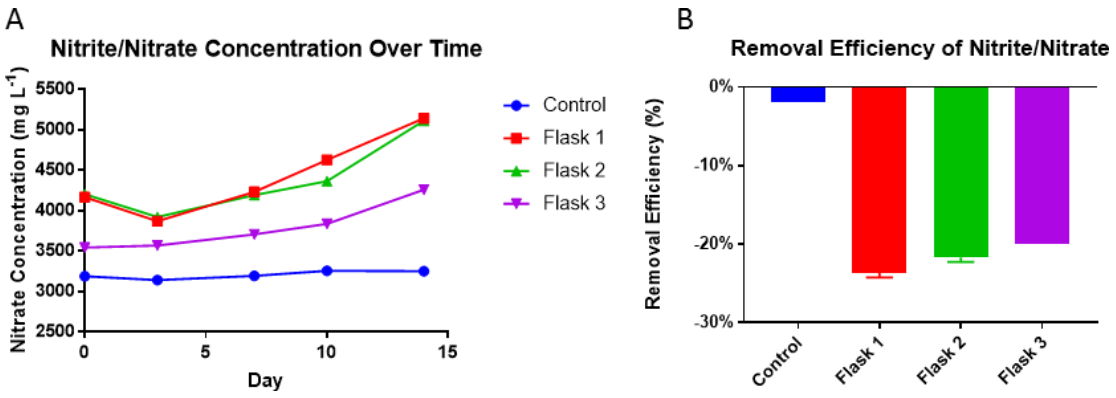

Figure 7.10: *Nitrite / Nitrate concentration (A) and removal efficiency (B) in leachate consortium cultures aerated with 5 % CO_2.*

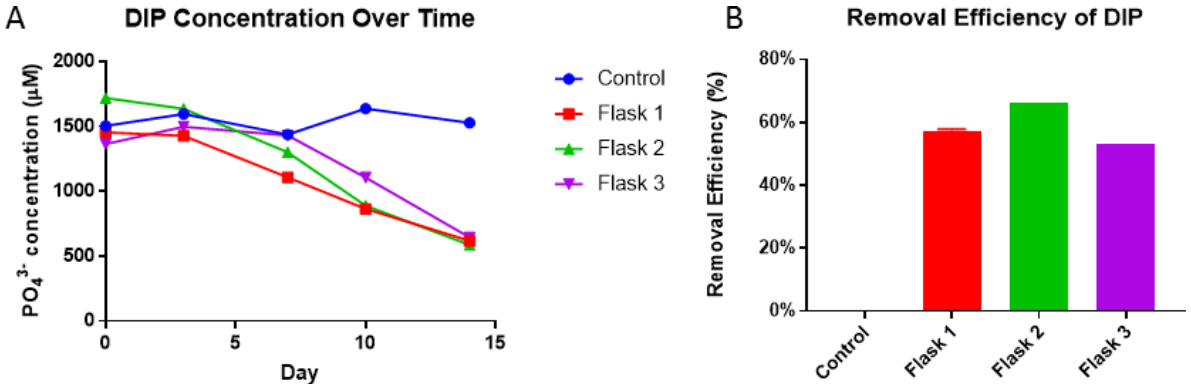

Figure 7.11: *DIP concentration (A) and removal efficiency (B) in leachate consortium cultures aerated with 5 % CO_2.*

As can be seen from Figure 7.12, the CO_2 removal efficiency varied dramatically over both time and replicate. This can be attributed to a number of causes. Firstly, as the control shows (Figure 7.12A), the CO_2 concentration entering the culture was not completely stable during the experimental timeframe. Small falls and increases in CO_2 concentration can affect the RE of the cultures. Alongside this, the consortium mix, which includes both algae and bacteria, will have an important effect on the CO_2 removal. As bacteria and algae can both reproduce heterotrophically, it is not uncommon to see increases in CO_2 concentration (negative RE) in systems containing organic carbon sources.

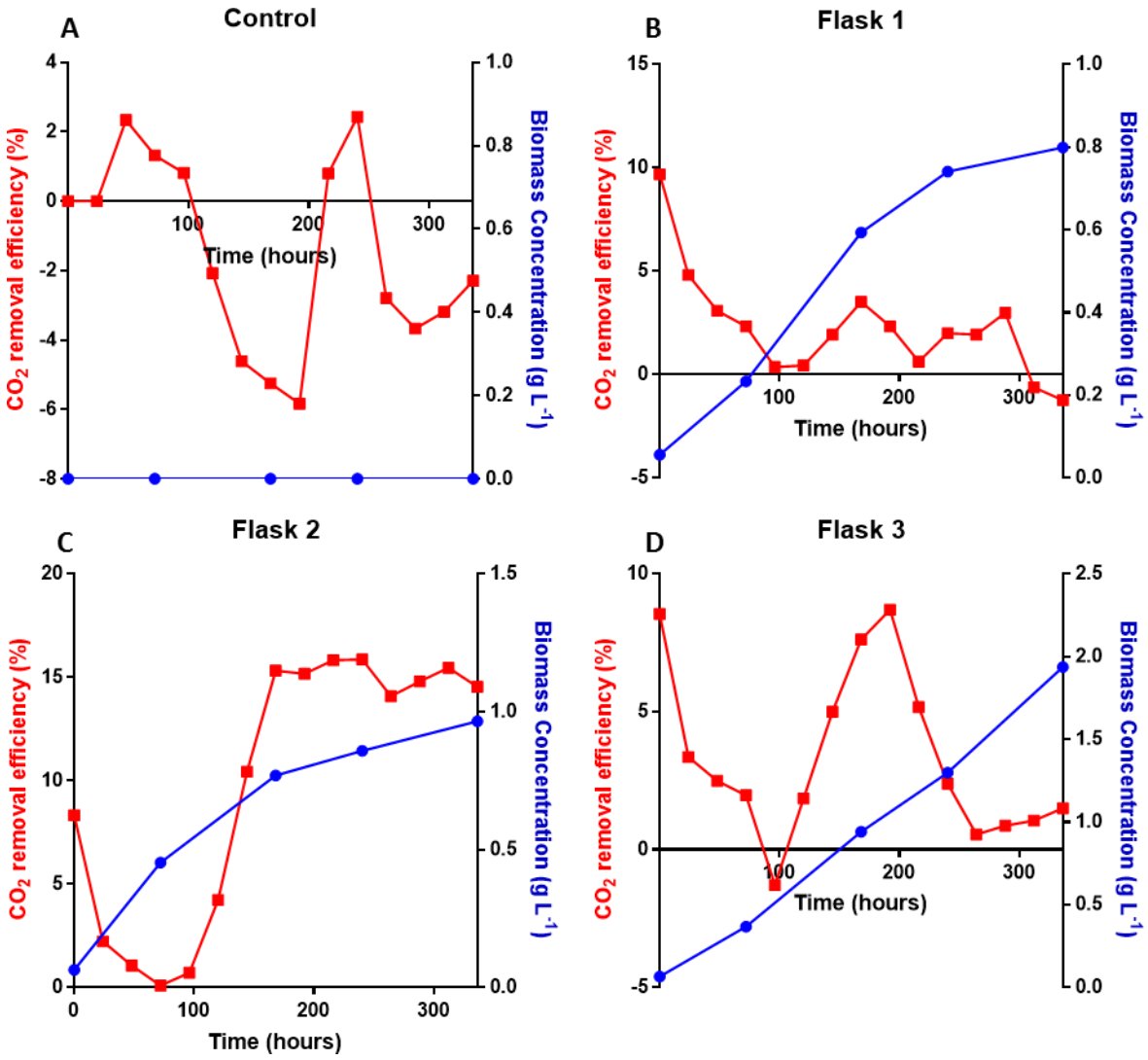

Figure 7.12: *CO_2 removal efficiency (left y-axis on all graphs and in red) and biomass concentration (right y-axis on all graphs and in blue) for the three replicate flasks (B-D) and the media only control (A).*

Notably, Hu *et al.* (2020) employed a very similar experimental set up, utilising simulated flue gas with CO_2 concentrations of both 5 and 10 % with soybean processing wastewater. Their gas chromatography results show similar fluctuations in CO_2 removal over the experimental period (increasing then decreasing and giving a negative value). The authors explain that the "*growth and proliferation of Chlorella in the logarithmic period needs to use a large number of carbon sources to synthesis organic matter*" (Hu et al., 2020) so the CO_2 fixation rate

fluctuates greatly. The authors acknowledge that CO_2 removal efficiencies are much higher elsewhere in the literature (de Morais and Costa, 2007c) and the key reasoning for this is that the CO_2 is the only carbon source present within the other systems within the literature. This is true for the results presented here also. The *C. vulgaris* studied in Chapter 5 had a much higher average CO_2 RE of 20 % and this likely contributed to both the higher growth rate and the requirement for CO_2 utilisation, as there are no other carbon sources presented within the system.

7.7. Discussion

The results presented in this chapter show that treating two waste streams concurrently with microalgae might not be as simple as some of the literature suggests (Chisti, 2007). While the adapted consortium could effectively remove large concentrations of ammonia and DIP from the leachate under conditions of aeration, addition of gaseous CO_2 (5 %) reduced the nutrient uptake rates and gave a poor CO_2 utilisation. Alongside this, the addition of CO_2 did not significantly improve the biomass productivity of the cultures.

7.7.1. Reasons Additional CO_2 May Not Increase Culture Productivity

The addition of 5 % CO_2 to the algal-bacterial consortium did not improve the culture productivity in comparison to aeration with air, as it did for *C. vulgaris*. This could be caused by many factors. For example, the literature shows that optimal CO_2 concentrations vary widely across different algal species and even between strains. Many different strains of *C. vulgaris* exist within the literature all having different optimal conditions (Adamczyk et al., 2016; Clément-Larosière et al., 2014; Lakshmikandan et al., 2019; Lam and Lee, 2013; Liu et al., 2020; de Morais and Costa, 2007a). While *C. vulgaris* is the dominant algae within this

consortium, it is also important to note that it is not the only one species and or strain present. The larger number of species interacting in a system makes defining an optimal condition harder. What may be optimal for *C. vulgaris* may not be for other algae (and bacteria) within the consortium, leading to changes in proportions under new conditions. This particular consortium has been adapted to survive and remediate landfill leachate over a 24-month period (Okurowska et al., 2021b), an adaption process of similar length and structure should be conducted for adaption to elevated CO_2 concentrations as well.

Another important factor is the presence of organic carbon sources within the leachate. Many algal species can grow using both inorganic and organic carbon sources, often switching easily between the different methods (mixotrophic growth). Preferential use of the dissolved organic carbon may mean the addition of carbon as CO_2 through aeration will not aid growth, as it is not utilised. This being said, aeration (in both forms tested) was invaluable to these cultures as a method of pH control, if nothing else. A full analysis of carbon sources within the leachate and detection throughout the experimental period should be conducted in future work to examine which compounds are preferentially utilised by the consortium.

A final, important factor could be the bacterial components of the consortium. Bacteria grow heterotrophically, producing CO_2 gas through respiration and this may already provide adequate dissolved CO_2 for algal photosynthesis when the cells are growing autotrophically, making the additional CO_2 '*extra*' which goes unused. This component was not present for the *C. vulgaris* cultures in Chapter 6, which may be a factor leading to those cultures benefiting from the additional CO_2 input.

7.7.2. Ammonia and Other Nitrogen Sources

When the cultures were aerated with only air, a large proportion of the ammonia was stripped out of the media. While this leads to a high RE value, ammonia volatilisation is a major problem. Ammonia gas cannot be released directly to the environment, as it is a corrosive and irritant, which can cause issues to human health. This would therefore require the placement of a scrubber at the end of the algal system, increasing costs associated with the process. Ammonia gas loss also limits the use of algal ponds for this process, as these are open systems.

As the ammonia was lost during aeration with air, the nitrate and nitrite compounds present within the leachate media were utilised for growth and therefore reduced. However, when 5 % CO_2 was applied to the cultures and the ammonia was not stripped, the nitrite concentrations within the media rose. Sniffen *et al.* (2017) saw a similar accumulation of nitrite and nitrate within their leachate treatment system. Ammonia oxidising bacteria, present within the algal-bacterial consortium, are likely responsible for this increase in nitrite concentration, especially when the ammonia concentrations are high as they were in this system (Sniffen et al., 2017). In future work, a detection method than can distinguish between nitrate and nitrite would be important to more closely see how the ammonia oxidising bacteria are altering the nitrogen balance within the system. Alongside this, further optimisation of supplementary nitrogen (nitrate) would be necessary to avoid waste nitrogen within the media. It is clear from the results that under the chosen conditions, the supplementary nitrate is seldom required.

7.7.3. Phosphate Uptake

Phosphate uptake by microalgae in waste treatment can be influenced by numerous factors and is highly variable (Powell et al., 2008). Luxury uptake of phosphate is well known to occur in microalgae. This could be a reasoning for the lack of DIP removal seen in the results. The cells are washed by centrifugation before inoculation; however, this would not remove any phosphorous they have stored within the cells as polyphosphate. The temperature, pH and other nutrient concentrations also effect the rate at which microalgae uptake phosphate and which sources. Temperature and light have been shown to affect the fraction of polyphosphate within algal biomass and the uptake efficiency from wastewater (Powell et al., 2008). While phosphorous is a key nutrient, it is also important to remember that in the Redfield ratio (the mass balance of key nutrients required for algal growth) phosphate plays a very small part. The additional phosphate supplied in this experiment may simply be over supplementation, as seen in many defined media recipes.

7.8. Conclusions

Throughout the literature, the addition of CO_2 to algal systems is seen as a positive, improving biomass productivities and lipid concentrations. The use of flue gases is boasted as an opportunity to add CO_2 to cultures at little expense while simultaneously cleaning a difficult waste stream. In reality, the interactions between the algae, the flue gas (or pure CO_2) and the media (wastewater or otherwise) are more intricate than this and the addition of CO_2 may not be advisable in every situation. The results presented in this chapter show that the treatment of landfill leachate using a microalgal-bacterial consortium might be one of those situations where the addition of CO_2 is not fruitful.

The consortium growth did not benefit from the additional carbon accessible from aerating with 5 % CO_2 over just air. As this is a complex mixture of both microalgae and bacteria, the respiration by bacteria producing CO_2 and mixotrophic growth of algae using organic carbon sources may contribute to this. The additional CO_2 also negatively affected the nutrient removal efficiencies for ammoniacal-nitrogen, non-ammoniacal-nitrogen, and DIP. The efficiency of removal for all three nutrient sources was reduced by at least half when cultures were aerated with CO_2 rather than air. Alongside this, it was shown that the CO_2 removal from the gas stream was unreliable and varied widely between replicates and over the growth period.

8. Conclusions

This chapter will outline the motivation of this work, the main conclusions drawn from the research presented within this thesis and then suggest possible avenues for future work based on these conclusions.

8.1. Motivation

The production of waste (solid, liquid and gas) is a global societal problem, which requires new and improved treatment methods. These methods should be integrated into a green, circular economy where the use of fossil fuels and other non-renewable sources is extremely limited. Microalgal cultivation offers a pathway to treat several different waste types and produce biomass, which can be utilised to replace fossil fuel created products. This being said, there are still many limitations to algal bioremediation technologies.

Much of the literature surrounding algal carbon capture rely on theoretical estimations of carbon utilisation based on the culture productivity rather than directly measuring the CO_2 removal. The theoretical estimations do not take into consideration the production of exopolysaccharides and other released compounds, which will have contributed towards the carbon utilisation of cultures. Nor do the removal rates, $g_{CO2}\ L^{-1}\ day^{-1}$, presented within the literature, detail if the introduction of elevated CO_2 to systems increases the CO_2 removal efficiency.

Alongside this, while the bioremediation of wastewater and leachates have been considered plentifully within the literature, many publications state that the treatment of multiple waste

streams (CO_2 from flue gases and wastewater or landfill leachates) can be achieved simultaneously and at little cost. These publications often refer to research which focuses on the treatment of key nutrients from one waste stream and not the other, or that solely focus on biomass productivity and not reducing the toxic effects of the waste. Very little research has been conducted which evaluates the bioremediation of both waste streams concurrently.

Furthermore, economic analysis of algal bioremediation is infrequently seen within the body of literature. Many techno-economic analyses (TEAs) have been performed within the literature; however, they all focus on the production of biomass and algal lipids for conversion to third generation biofuels.

In this research, we investigated how different microalgal species and communities could remediate two key waste streams alone and simultaneously. We used the experimental data produced to model the economic viability of each bioremediation process. Scenario based and parameter sensitivity analyses were performed in an effort to understand where future research and optimisation efforts should be focused.

8.2. Main Findings

8.2.1. The Potential of Algae to Capture CO_2

The research presented in Chapter 5 focused on evaluating how *Chlorella vulgaris* grew under different CO_2 concentrations and how effectively – under optimal growth conditions – the CO_2 could be removed from the gas stream. The results showed that *C. vulgaris* grew significantly faster when aerated with 5 % CO_2, compared to the other conditions tested. Under these optimal aeration conditions, the CO_2 removal efficiency (RE %) of the culture was calculated by monitoring the CO_2 exiting the system using an NDIR sensor. The results

demonstrated that *C. vulgaris* could remove up to 20 % of the CO_2 from the gas stream. This equates to a significantly higher CO_2 concentration than is calculated using the standard assumptions used throughout the literature (Table 5.2). This highlights that the continued development and utilisation of real-time monitoring for CO_2 removal is essential to understanding how effective microalgae can remediate gas waste streams. The low RE results presented also make it clear that further optimisation of algal CO_2 capture is required if it is to compete with mature technologies based on efficiency.

8.2.2. Algal Carbon Capture: Improving the Economic Viability

The experimental results presented in Chapter 5 were used to model the economic viability of algal carbon capture at pilot/demonstration scale. The results were based on a 'first of its kind' facility with high contingency costs and a low technology readiness level (TRL). Scenario based analyses, surrounding improvements in efficiency, capital expense and changing political landscapes highlighted that the cost of major equipment (the photobioreactor specifically) and efficiency of the algal culture are key parameters. Improvements in these two areas will have the largest effect on reducing the carbon capture cost and should be where future research and development are focused.

8.2.3. Improving the Economic Viability of Algal Leachate Remediation

Chapter 6 presents the techno-economic analysis of landfill leachate bioremediation using an algal-bacterial consortium. An experiment at pilot scale (300 L bioreactor) was conducted focusing on the biomass productivity, ammoniacal-nitrogen, total nitrogen and phosphorous removal efficiency of the consortium. These results were used to create a TEA model for the process at demonstration scale, utilising the same system. Initial results demonstrated that

without improvements, the operational treatment costs are £170 per batch treatment, extremely high in comparison with mature, physico-chemical technologies.

Scenario based analysis highlighted that utilising a system which can benefit from economies of scale, reduced reliance on freshwater, and additional bulk chemical purchasing have the highest impact on cost. Introduction of these changes can reduce the treatment cost by over 85 % (against the original). A sensitivity analysis also demonstrated that improving the consortiums treatment efficiency could lead to significant cost reductions. Therefore, further lab and pilot-scale based optimisation of the system should be a focus in future research.

8.2.4. Can Algae Efficiently Treat Both CO_2 and Leachate?

As mentioned in Section 8.1, many publications assume that multiple waste streams can be treated in tandem without any effect on the efficiency of treatment. The research presented in Chapter 7 intended to evaluate this claim by measuring nutrient removal of the algal-bacterial consortium presented in Chapter 6, while also measuring the CO_2 removal efficiency when aerated with 5 % CO_2 (the optimal condition found in Chapter 5). The results demonstrated that while the culture was not hindered by the additional CO_2 inflow to the culture, it did not improve the growth rate significantly, as it had done for *C. vulgaris* cultivation. This initially suggests that the CO_2 RE will not be as high as presented in Chapter 5. Landfill leachates contain a high concentration of organic carbon, which is not present in algal defined media, therefore mixotrophic growth utilising these other carbon sources could reduce the CO_2 RE. Alongside this, the ammoniacal-nitrogen and phosphorous REs were reduced when cultures were aerated with 5 % CO_2 rather than air. This exhibits that concurrent treatment of flue gases and landfill leachates is not as straightforward as suggested in the literature, and the inhibitory effects of each on the other can lead to a

reduction in treatment efficiency. For this reason, a TEA was not carried out, as further optimisation of the dual treatment for increased efficiency should be considered first.

Without this further optimisation and analysis, it can be concluded that simultaneous treatment of CO_2 and leachate waste streams will not be economically viable, and that research efforts should focus on treating each waste stream independently.

8.3. Areas for Future Work

In this thesis, we have presented a comprehensive study on how algal cultures can be used for bioremediation and how to improve the economic viability of these processes. Throughout, we have discussed the advantages and limitations associated with the experimental set up, analytical methods, and the economic analysis methods utilised. Although we have shown that algae are capable of treating multiple waste streams independently and that concurrent treatment is not as straightforward as suggested within the literature, many more ideas were generated from this work for future investigation to understand the biological systems better and further optimise the treatments. While there are countless parameters which could be individually evaluated under multiple scenarios to ascertain optimal treatment conditions, this section presents three main ideas for future work based on the results of this thesis.

8.3.1. Analysis of How Algal Cells Withstand Elevated CO_2 Conditions

The results presented in Chapter 5 demonstrated that aeration with 5 % CO_2 could improve biomass productivity when compared to aeration with air alone, but that concentrations of CO_2 higher than this can detriment the algal cultures. Many researchers have looked, in-depth at how algae can adapt to conditions of little CO_2, using the carbon concentrating mechanism (CCM) but far fewer publications focus on how the cells adapt to conditions of

high CO_2 (Miyachi et al., 2003). Omics technologies have rapidly advanced over the last decade. They are now readily being used with model microalgal organisms (Guarnieri and Pienkos, 2015). An in-depth proteomic, genomic, transcriptomic and/or metabolomic analysis of microalgae under different CO_2 concentrations would help establish pathways involved in the adaption process. Allowing for further optimisation of algal cells as microbial factories and the ability to selectively improve their bioremediation potential (Guarnieri and Pienkos, 2015; Jamers et al., 2009).

8.3.2. Optimisation Under 'Real Life Conditions' to Aid in Better Economic Analysis

The TEA conducted in Chapter 5 relied on the extrapolation of laboratory scale data to pilot scale data. While this is not uncommon within the literature (Gouveia et al., 2016; Molino et al., 2019), it is not ideal for gaining accurate economic forecasts. Therefore, we suggest one avenue for future work would be to scale up the previous experimental work to the pilot scale photobioreactor (PBR) and optimise under these conditions. Using the PBR will allow for accurate pumping, lighting, and heating demand evaluations alongside real CO_2 utilisation efficiencies and biomass productivities. Conducting experiments throughout the calendar year would also be an important part of this work, highlighting how seasonal changes in daylight and temperature will affect both the algal productivity and the associated operational costs (heating, additional lighting).

Alongside this, extending the scope of the experimental analysis to include analysis of the biomass product should be considered. The protein, fat, and ash content of the biomass produced should be examined throughout the experiments, allowing a more accurate representation of by-product sales revenue to be included within the economic analysis.

8.3.3. Measuring Micro-pollutants and Other Key Contaminants

The literature, and this research, focus heavily on key pollutants found in the two waste

steams: CO_2 in flue gases and ammonia, nitrate/nitrite and dissolved inorganic phosphorous (DIP) in leachates. However, it is important to remember that these are not the only contaminants within these waste streams. Another opportunity for future work would be to consider other pollutants within the landfill leachate, not only the compounds favourable to the algal growth. Microalgae have been shown to remove a variety of micro-pollutants including paracetamol, aspirin, heavy metals, and even pathogenic bacteria (Escapa et al., 2017; Kumar et al., 2021; Liu et al., 2021). However, most of the research focuses on each pollutant in isolation. An analysis of algal bioremediation which focuses on all-encompassing remediation may well demonstrate how efficiently algal systems could lead to clean, fresh water.

Furthermore, analysis of the biomass composition under these conditions would give further insight into whether the biomass can be processed into high value products, for example food for human consumption, or low value products (biofuel). The production of by-products can drastically affect the economics of a process and therefore the composition of the biomass should be considered.

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10. Appendices

A. Leachate and Wastewater Compositions Available from the Literature

Summary of the different leachate and wastewater compositions available within the current body of literature. All sources are based on biological treatment (algal or bacterial). The major contaminants, COD, Ammoniacal-Nitrogen, Nitrate, Ortho-P and a variety of heavy metals are shown. Some sources may have presented information for other compounds which do not appear in this table. If a cell is left blank it denotes that values for this compound were not given. All components (other than pH) are given in mg L^{-1} concentrations.

*Junk Bay, °Gin Drinkers Bay, LF = landfill, WW = Wastewater, M = municipal, TP = treatment plant, CGW = contaminated ground water, R = raw, RC = recirculated. For Talalaj *et al.* the leachates differ by age (a < 5 years old, b 5-10 years old and c is stabilised at more than 10 years old).

Reference	Source	pH	COD	Ammonia	Nitrate	Ortho-P	Al	As	Ba	Ca	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
(Ho et al., 1974)	LF	5.9 – 6.1	10650											330				
(Talalaj et al., 2019)	LF a	<6.5	>15000	< 400														
	LF b	6.5 – 7.5	4 – 15000															
	LF c	>7.5	< 4000	> 400														
(Lin et al., 2007)	LF	7.6	1280	1345	68.4	5.13												
(Eze et al., 2018)	WW TP	8 - 8.5	280	102.66	1.8	25.6												
(Martin and Johnson, 1995)	LF			405	0.2	2.3								351	2.00			
(Kjeldsen et al., 2002)	LF										0.006	0.08	0.07			0.13	0.07	0.67
	LF										0.005	0.28	0.065			0.17	0.09	0.6
	RC										0.006	0.01	0.04			0.05	0.02	2.2
	LF										0.0002	0.003	0.002			0.028	<0.005	0.2
	LF										0.0004	0.016	0.007			0.084	<0.005	0.36
	LF										0.0003	0	0.034			0.054	0.053	0.085

(Cruce and Quinn, 2019)	LF									0.0036	0.002	0.002			0.062	0.188	5.31
	CGW									0.002 - 0.008	0.033 – 0.085				0.01 - 0.08	0.016 – 0.067	0.003 – 0.011
	LF									0.0002 -0.018	0.005 – 1.62	0.004 – 0.27			0.0036 - 0.348	0.005 – 0.019	0.05 – 9
	LF									<0.1 - <0.04	<0.01 – 0.05	<0.02 - 0.17			<0.01 – 0.1	<0.04 – 0.13	<0.01 – 0.47
(Paskuliakova et al., 2018b)	LF	6.9	145	152		0.5	0.14		111		<0.1	0.4	4.0	1.3	<0.1	<0.001	0.1
	LF	6.3	1505	98		13.8	0.71		445		0.3	<0.1	5.1	0.5	0.16	<0.001	0.3
	LF	7.8	2455	1480		16.5	1.8		295		0.45	<0.1	8.6	0.6	0.19	<0.001	0.2
	LF	8.4	5030	2510		14.8	3.6		413		1.1	<0.1	2.6	0.3	0.5	<0.001	0.2
	LF	7.4	97	122		<0.05	0.2		98		<0.1	>4.0	5.3	1.0	<0.1	0.39	1.7
	LF	7.7	526	506		1.7	0.28		194		<0.1	0.2	3.9	>2	<0.1	<0.001	<0.1
(Kang et al., 2015)	WW		81.45	29.1									0.18	0.118			0.008
(Lee et al., 2015)	M	7.2	295.5	32.5		40.6 ±1.3	7.7 ±0.2										

(Ozturk et al., 2003)	LF	5.6 - 7	35000	2020	2370	5 - 6										
(Cheung et al., 1993)	LF*	7.2	595	724		2.87			<0.01	0.08	<0.01	0.67	0.04	0.07	0.03	0.74
	LF°	7.2	140	147		0.34			<0.01	0.04	0.01	0.88	0.64	0.03	<0.01	0.33
(Richards and Mullins, 2013)	M	8.44 – 8.6	1008				33.56	0.175				15.37	0.27			
(Vedrenne et al., 2012)	LF	8	14680	381		<1	0.233		0.433	<0.1		65	<0.1	<0.1	19.59	0.33

B. Experimental Conditions Used in the Literature

Summary of the experimental conditions used throughout the literature, where algal cultures are aerated with additional CO_2. Specific strains of species have not been recorded here for simplicity. The term PBR (Photobioreactor) refers to culture volumes of up to 1 L (bench scale), unless stated otherwise in the additional notes. "Pure CO_2 mixed in air" denotes the use of compressed air and CO_2 mixed to specific concentrations before feeding to the culture. All experiments, apart from Zheng et al. 2016 and 2017, are aerating cultures by bubbling gas through the culture. Zheng et al. (2016, 2017) are using membrane separated loaded-solvent and cultures to transfer the CO_2 to the culture.

Genus and Species	Reference	System used	Light regime	CO_2 (%)	CO_2 source	Additional notes
Anabaena PCC 7937	(García-Cubero et al., 2017)	Bubble PBR	Artificial – 12 hours illumination d^{-1}	12	Synthetic flue gas	Cyanobacteria Intermittent CO_2 injection
Anabaena sp.	(González López et al., 2009)	Bubble PBR	Artificial – Mimicking natural cycles	0.04	Air	Cyanobacteria
Aphanothece microscopica Nageli	(Jacob-Lopes et al., 2008)	Bubble PBR	Artificial – 12 hours illumination d^{-1}	15	Pure CO_2 mixed in air	Cyanobacteria
	(Jacob-Lopes et al., 2009)	Bubble PBR	Artificial – Independent variable	15	Pure CO_2 mixed in air	
Botryococcus braunii	(Yoo et al., 2010)	N/A	Artificial – 24 hour illumination	10	Flue gas	Petroleum flue gas
Chlorella emersonii	(Borkenstein et al., 2011)	Air-Lift PBR	Artificial – 16 hours illumination d^{-1}	15	Flue gas	Cement production site
	(Scragg et al., 2002)	Tubular PBR	Artificial – 16 hours illumination d^{-1}	0.04	Air	
Chlorella fusca	(Duarte et al., 2016)	PBR	Artificial – 12 hours illumination d^{-1}	10	Synthetic flue gas	
Chlorella kessleri	(de Morais and Costa, 2007c)	PBR	Artificial – 24 hours illumination d^{-1}	0 – 18	Pure CO_2 mixed in air	

	(de Morais and Costa, 2007a)	PBR	Artificial – 12 hours illumination d^{-1}	18	Pure CO_2 mixed in air	
Chlorella sorokiniana	(Kumar et al., 2014)	Air-Lift PBR	Artificial – 24 hour illumination	0.04	Air	
Chlorella sp.	(Adamczyk et al., 2016)	PBR	Natural (day) & Artificial (night)	4 – 8	Pure CO_2 mixed in air	
	(Chiu et al., 2008)	PBR	Artificial – 24 hour illumination	2 – 15	Pure CO_2 mixed in air	
	(Chiu et al., 2009b)	PBR	Artificial – 24 hour illumination	5	Pure CO_2 mixed in air	
	(Doucha et al., 2005)	Open PBR	Natural	7	Flue gas	
	(Kao et al., 2014)	PBR	Artificial – 24 hour illumination	23 – 25	Flue gas	Included large scale outdoor operation trial
	(Maeda et al., 1995)	PBR	Artificial	13	Flue gas	
	(Ryu et al., 2009)	Tubular PBR	Artificial – 24 hour illumination	0.5 – 5	Pure CO_2 mixec in air	
	(Sung et al., 1999a)	PBR	Artificial	10 or 70	Pure CO_2 mixec in air	
	(Sung et al., 1999b)	PBR	Artificial	10	Pure CO_2 mixec in air	
	(Yue and Chen, 2005)	PBR	Artificial –	10	Pure CO_2 mixec in air	

			12 hours illumination d^{-1}			
	(Zheng et al., 2016)	Membrane PBR	Artificial – 24 hour illumination	20-70% loaded solvent	Solvent loaded with CO_2	Membrane separated culture and loaded CO_2-solvent
	(Zheng et al., 2017)	Membrane PBR	Artificial – 24 hour illumination	0.4 – 2.5 mol L^{-1} solvent	Solvent loaded with CO_2	Membrane separated culture and loaded CO_2-solvent
Chlorella vulgaris	(Anjos et al., 2013)	Bubble PBR	Artificial – 24 hour illumination	2 – 10	Pure CO_2 mixed in air	Assessing different aeration rates
	(Clément-Larosière et al., 2014)	PBR	Artificial – 24 hour illumination	2 – 13	Pure CO_2 mixed in air	
	(de Morais and Costa, 2007a)	PBR	Artificial – 12 hour illumination d^{-1}	6	Pure CO_2 mixed in air	
	(Douskova et al., 2009)	Bubble PBR	Artificial – 24 hour illumination	11	Flue gas	Municipal Waste Incinerator flue gas
	(Jin et al., 2006)	Bubble PBR	Artificial	15	Pure CO_2 mixed in air	
	(Naderi et al., 2015)	PBR	Artificial	2	Pure CO_2 mixed in air	
	(Yoo et al., 2010)	NA	Artificial – 24 hour illumination	10	Flue gas	Petroleum flue gas
Chlorococcum littorale	(Hu et al., 1998)	Flat Plate PBR	Artificial – 10 hour illumination d^{-1}	5	Pure CO_2 mixed in air	

	(Kurano et al., 1995)	PBR	Artificial – 24 hour illumination	10 or 20	Flue gas	Oil fired boiler flue gas
	(Ota et al., 2009)	PBR	Artificial – 24 hour illumination	50	Pure CO_2 mixed in N_2	
Chlorococcum sp.	(García-Cubero et al., 2017)	Bubble PBR	Artificial – 12 hours illumination d^{-1}	12	Synthetic flue gas	
Chlorogleopsis sp.	(Ono and Cuello, 2007)	PBR	Artificial – 16 hour illumination d^{-1}	5	Pure CO_2 mixed in air	
Dunaliella sp.	(Kishimoto et al., 1994)	Water tank	Artificial	3	Flue gas	Diluted in air
Euglena gracilis	(Chae et al., 2006)	PBR	Artificial – 24 hour illumination	10	Flue gas	Kerosene flue gas
Fischerella	(Weissman et al., 1998)	Flask	N/A	– 0.5	Pure CO_2 mixed in air	
Haematococcus pulvaris	(Huntley and Redalje, 2007)	Tubular PBR	N/A	0.4	Air	
Hot spring algae	(Hsueh et al., 2007)	PBR	Artificial – 24 hour illumination	15	Synthetic flue gas	
Microcystis aeuginosa	(Jin et al., 2006)	Bubble PBR	Artificial	15	Pure CO_2 mixed in air	
Microcystis ichthyoblabe	(Jin et al., 2006)	Bubble PBR	Artificial	15	Pure CO_2 mixed in air	

Nannochloropsis gaditana	(Adamczyk et al., 2016)	PBR	Natural (day) & Artificial (night)	4 or 8	Pure CO_2 mixed in air	
nannochloropsis oculata	(Chiu et al., 2009a)	PBR	Artificial – 24 hour illumination	2 – 10	Pure CO_2 mixed in air	
	(Hsueh et al., 2009)	Bubble PBR	Artificial – 24 hour illumination	5 – 8	Synthetic flue gas	
Nostoc	(García-Cubero et al., 2017)	Bubble PBR	Artificial – 12 hours illumination d^{-1}	12	Synthetic flue gas	Cyanobacteria
Nostoc punctiform	(García-Cubero et al., 2017)	Bubble PBR	Artificial – 12 hours illumination d^{-1}	12	Synthetic flue gas	Cyanobacteria
Scenedesmus dimorphus	(Jiang et al., 2013)	PBR	Artificial	15	Flue gas	
Scenedesmus obliquus	(de Morais and Costa, 2007b)	PBR	Artificial – 12 hours illumination d^{-1}	0 – 12	Pure CO_2 mixed in air	
	(de Morais and Costa, 2007a)	PBR	Artificial – 12 hours illumination d^{-1}	6	Pure CO_2 mixed in air	
	(Ho et al., 2010)	PBR	Artificial – 24 hour illumination	10	Pure CO_2 mixed in air	
	(Toledo-Cervantes et al., 2013)	Bubble PBR	Artificial – 12 hours illumination d^{-1}	5 – 10	Pure CO_2 mixed in air	

Scenedesmus sp.	(de Godos et al., 2014)	Raceway pond	Natural	10.6	Flue gas	Diesel combustion flue gas
	(Jin et al., 2006)	Bubble PBR	Artificial	15	Pure CO_2 mixed in air	
	(Yoo et al., 2010)	N/A	Artificial – 24 hour illumination	10	Flue gas	Petroleum flue gas
Scenedesmus platensis	(Chen et al., 2012)	PBR	Mixed natural and artificial	12	Flue gas	De-sulphured flue gas
Scenedesmus vaculoatus	(García-Cubero et al., 2017)	Bubble PBR	Artificial – 12 hours illumination d^{-1}	12	Synthetic flue gas	
Spirulina sp.	(de Morais and Costa, 2007b)	PBR	Artificial – 12 hours illumination d^{-1}	0 – 12	Pure CO_2 mixed in air	
Thermosynechoccus sp.	(Hsueh et al., 2009)	Bubble PBR	Artificial – 24 hour illumination	10 or 20	Synthetic flue gas	

C. Depreciation and Cost of Assets

Depreciation of assets was calculated using a straight line model over the project lifetime (20 years) with a residual value of zero (Amer et al., 2011; Dunlop and Coaldrake, 2014; Huntley and Redalje, 2007). The item's lifespan (I_{LS}) and purchase price (I_C) (inflation adjusted if required) was used to calculate the annual depreciation value (DV):

$$DV = \frac{I_C}{I_{LS}} \quad \text{C.1}$$

The asset value in any given year (t_x) was calculated by removing the depreciation value from the initial purchase price for each year passed.

$$Asset\ value\ in\ t_X = I_C - (DV \times t_X) \quad \text{C.2}$$

As some items had longer lifespans than the project lifetime, their residual value at the end of the project was used to offset costs before the cost of treatment was calculated. Alongside this, land and buildings were not included in the depreciation schedule, as their values were likely to appreciate over the timespan, and so these were also included in the cost offsetting at the end of the project.

Several items required repurchasing throughout the 20-year period. For example, the harvesting tank had an expected lifespan of 10 years, meaning the equipment would require repurchasing at the beginning of the 11th year. To calculate which items required repurchasing and when, each item (I) was input to a table against year (0-20) and the =FLOOR formula was used to shown when a repurchase was necessary each year (t_x):

$$R = FLOOR(\frac{t_X}{LS}, 1) \quad \text{C.3}$$

The =FLOOR was used so that R (repurchase?) was rounded down to the nearest whole integer.

Another table was then produced, based of the results of the previous, to detail the cost incurred for each year, including an adjustment for inflation since the first year (t_0). This table used the =IF function. The statement checked if the corresponding cell in the previous table, and the year prior matched (no repurchase made) or not (repurchase made). If the cells matched, the value entered was £0, if they did not, the item was repurchased at a cost of the initial purchase price plus inflation over the number of years:

$$= IF((R_{X-1} = R_X, 0, (I_C * (1 + i)\text{^}t_X)) \quad \text{C.4}$$

Where X represents the financial year in question, R is the value calculated in Equation 1, I_c is the items initial purchase cost, *i* is the inflation rate, and *t* represents the time passed. These cost values were then pulled forwards to the net cash flow (NCF) table and included in the yearly direct capital costs.

D. Equipment and Reagent Costs

This appendix shows the base prices (in 2018 £) used within the techno-economic analysis (TEA) model. Major equipment such as the PBR, harvesting tank, pumps and probes are based on the true cost of the Phycoflow™ pilot scale PBR constructed at the Arthur Willis Environmental Centre:

Item	Cost per unit (£)	Life Span (Years)	Additional Information
Land	241,000.00	-	1 ha industrial land
Buildings & Facilities	43,625.00	-	Based on Tredici *et al.* (2016)
PhycoFlow™	31,107.60	25	300 L PBR, including installation and surrounding frame
Flow pump	802.80	20	ITT LOWARA 3 Phase SS pump
Aeration pump	50.00	20	BoyuAcq007
Tank (harvesting)	396.00	10	Made to order
Fluidic oscillator	100.00	20	Pandhal *et al.* 2018
Air diffusers	210.00	10	Pentair Aquatic Eco-system wedge lock diffuser
Lighting	70.00	4	
Thermoregulators	38.40	20	0.1W Thermoregulators from ThermoSenseDirect
Air compressor	99.98	20	SG2S, 24 Litre Air Compressor 9.6CFM, 2.5HP, 24L
Heater	15.00	5	Marko Electrical 2 kW Heater
Inoculum aerator	40.00	20	Aquarline Halilea Aco-9630 eight output air pump (20w), 1080l/h
Inoculum vessel	144.00	10	20 L Carboy with venting lid and accessories

The price of chemical reagents is based on bulk purchase from either Alibaba or Sigma Aldrich:

Item	Cost (£ tonne^{-1})
NaOCl (15%)	543.00
$Na_2S_2O_3$	2,452.00
Chitosan	4.84
$NaNO_3$	318.28
K_2HPO_4	909.36
KH_2PO_4	757.80
$MgSO_4 \cdot 7H_2O$	830.00
NaCl	190.00
$CaCl_2 \cdot 2H_2O$	580.00
$FeCl_3 \cdot 6H_2O$	1,894.50
$MnCl_2 \cdot 4H_2O$	1,136.70
$ZnCl_2$	3,789.00
$CoCl_2 \cdot 6H_2O$	12,124.80
$Na_2MoO_4 \cdot 2H_2O$	5,304.60
EDTA	2,273.40
Vitamin B12	18,945.00

The cost of producing the inoculum for each PBR is based on costs associated with production of 20 L dense culture within the lab:

Item	Amount Required (g)	Total Cost (£)
$NaNO_3$	15	0.00477
K_2HPO_4	1.5	0.00136
KH_2PO_4	3.5	0.00265
$MgSO_4 \cdot 7H_2O$	1.5	0.00125
NaCl	0.5	0.00010
$CaCl_2 \cdot 2H_2O$	0.5	0.00029
$FeCl_3 \cdot 6H_2O$	0.01164	0.00002
$MnCl_2 \cdot 4H_2O$	0.00492	0.00001
$ZnCl_2$	0.0006	0.00000
$CoCl_2 \cdot 6H_2O$	0.00024	0.00000
$Na_2MoO_4 \cdot 2H_2O$	0.00048	0.00000
EDTA	0.09	0.00020
Vitamin B12	0.0000002	0.00000
Make-up Water (L)	18.26	0.02404
Stock Water (L)	1.74	0.00229
Electricity (kWh)	3.36	0.48283
	Total cost for 1x 20 L inoculum	**0.52**

The cost of sterilising the PBR unit is based on the chemical sterilisation process utilising sodium hypochlorite and sodium thiosulfate. Firstly, the PBR is filled with tap water and 750 mL of 2 % sodium hypochlorite is added. The PBR is left for 24 hours. After the 24 hours, 900 mL of 5 % sodium thiosulfate is added to the PBR to neutralise the chlorine. The PBR is left for a further 24 hours. The PBR is then drained and filled with clean tap water until use.

Item	Amount Required Per Sterilisation	Total Cost (£)
NaOCl (15%)	0.1364 L	0.07
$Na_2S_2O_3$	0.045 kg	0.11
Water (L)	600 L	0.79
Electricity	9.6 kWh	1.38
	Total Cost Per Sterilisation	**2.35**

E. Net Cash Flow Table

A Net Cash Flow (NFC) table was created for each treatment and scenario. This documented all the costs accrued over the project lifetime as well as any revenue streams. The table shows changes in liquidity and costs year to year. It also gives the total cost value for each component at the end of the 20-year project lifetime which is then annualised for calculating the treatment cost and biomass selling prices. An example of an NFC table is presented here:

	A	B	C	D	E	U	V	W	X	Y	Z	AA
3				1	2	18	19	20	TOTAL	ANNUAL		
4	**TOTAL LIABILITIES**			£ -	£ -	£ -	£ -	£ -				
5	**TOTAL ASSETS**			£ 13,646,432.12	£ 13,082,301.47	£ 4,541,415.04	£ 3,977,284.38	£ 3,413,153.73	£ 2,849,023.08	Remaining		
6	***LIQUIDITY***			***£ 13,646,432.12***	***£ 13,082,301.47***	***£ 4,541,415.04***	***£ 3,977,284.38***	***£ 3,413,153.73***	***£ 2,849,023.08***	***£ 142,451.15***		
7	**Item**	**Total Cost (£)**	**Life Span**									
8	Land	241,000.00	100	£ 241,000.00	£ -	£ -	£ -	£ -	£ 241,000.00	£ 12,050.00		
9	Buildings&Facilities	43,625.00	100	£ 43,625.00	£ -	£ -	£ -	£ -	£ 43,625.00	£ 2,181.25		
22	**TDC (Total Direct Capital)**	**14,080,732.12**	*C*	**-£ 13,646,432.12**	**£ -**	**£ -**	**£ -**	**£ -**	**-£ 14,080,732.12**	**-£ 704,036.61**	*Correct*	*Correct*
23	Contingency	15%		£ 2,046,964.82	£ -	£ -	£ -	£ -	£ 2,112,109.82	£ 105,605.49		
24	Installation	5%		£ 682,321.61	£ -	£ -	£ -	£ -	£ 704,036.61	£ 35,201.83		
25	Loan Payments	£ -	£/yr	£ -	£ -	£ -	£ -	£ -	£ -	£ -	*Correct*	*Correct*
26	**TIC (Total Indirect Capital)**	**2,816,146.42**	*C*	**-£ 2,729,286.42**	**£ -**	**£ -**	**£ -**	**£ -**	**-£ 2,816,146.42**	**-£ 140,807.32**	*Correct*	*Correct*
27	**TOTAL CAPEX**	**16,896,878.54**	*C*	**-£ 16,375,718.54**	**£ -**	**£ -**	**£ -**	**£ -**	**-£ 16,896,878.54**	**-£ 844,843.93**	*Correct*	*Correct*
28	**TOTAL CAPEX *(inc. depreciation)***	**14,047,855.46**							**-£ 14,047,855.46**	**-£ 702,392.77**	*Correct*	*Correct*
29	*Operational load*	*100% Operation Figs*		*0*	*0.5*	*1*	*1*	*1*				
30	*Bwet produced (kg)*	*9,448.48*		*0.00*	*4724.24*	*9448.48*	*9448.48*	*9448.48*	*174796.86*	*8739.84*	*Correct*	
31	*CO2 in (tonnes)*	*5,223.63*		*0.00*	*2611.82*	*5223.63*	*5223.63*	*5223.63*	*96637.18*	*4831.86*	*4831.86*	*20%*
32	*CO2 out (tonnes)*	*4,203.39*		*0.00*	*2101.70*	*4203.39*	*4203.39*	*4203.39*	*77762.73*	*3888.14*	*3888.14*	
33	*CO2 captured (tonnes)*	*1,020.24*		*0*	*510*	*1020*	*1020*	*1020*	*18874.45*	*943.72*	*Correct*	
34	C(ster)	20,920.80		£ -	£ 10,460.40	£ 20,920.80	£ 20,920.80	£ 20,920.80	*£ 387,034.77*	*£ 19,351.74*		
35	C(inoc)	4,620.18		£ -	£ 2,310.09	£ 4,620.18	£ 4,620.18	£ 4,620.18	*£ 85,473.37*	*£ 4,273.67*		
41	Other consumables	36,912.59		£ -	£ 18,456.29	£ 36,912.59	£ 36,912.59	£ 36,912.59	*£ 682,882.86*	*£ 34,144.14*		
42	**DCO (Direct Cultivation OpEx)**	**406,038.45**		**£ -**	**-£ 203,019.23**	**-£ 406,038.45**	**-£ 406,038.45**	**-£ 406,038.45**	**-£ 7,511,711.41**	**-£ 375,585.57**	*Correct*	*£ 375,585.57*
44	Technicians	80,000.00		£ -	£ 80,000.00	£ 80,000.00	£ 80,000.00	£ 80,000.00	£ 1,520,000.00	£ 76,000.00		
45	Supervisors	30,000.00		£ -	£ 30,000.00	£ 30,000.00	£ 30,000.00	£ 30,000.00	£ 570,000.00	£ 28,500.00		
46	Overheads	66,000.00		£ -	£ 66,000.00	£ 66,000.00	£ 66,000.00	£ 66,000.00	£ 1,254,000.00	£ 62,700.00		
47	**LO (Labour OpEx)**	**176,000.00**		**£ -**	**-£ 176,000.00**	**-£ 176,000.00**	**-£ 176,000.00**	**-£ 176,000.00**	**-£ 3,344,000.00**	**-£ 167,200.00**	*Correct*	*Correct*
48	**TDO (Total Direct OpEx)**	**582,038.45**		**£ -**	**-£ 379,019.23**	**-£ 582,038.45**	**-£ 582,038.45**	**-£ 582,038.45**	**-£ 10,855,711.41**	**-£ 542,785.57**	*Correct*	*Correct*
49	Maintenance	5%		£ -	£ 18,950.96	£ 29,101.92	£ 29,101.92	£ 29,101.92	£ 542,785.57	£ 27,139.28		
50	Insurance	5%		£ -	£ 18,950.96	£ 29,101.92	£ 29,101.92	£ 29,101.92	£ 542,785.57	£ 27,139.28		
51	**TIO (Total Indirect OpEx)**	**58,203.85**		**£ -**	**-£ 37,901.92**	**-£ 58,203.85**	**-£ 58,203.85**	**-£ 58,203.85**	**-£ 1,085,571.14**	**-£ 54,278.56**	*Correct*	*Correct*
52	**TOTAL OPEX**	**640,242.30**		**£ -**	**-£ 416,921.15**	**-£ 640,242.30**	**-£ 640,242.30**	**-£ 640,242.30**	**-£ 11,941,282.55**	**-£ 597,064.13**	*Correct*	*-£ 597,064.13*
53	**TOTAL COST (CapEx+OpEx)**	**17,537,120.84**		**-£ 16,375,718.54**	**-£ 416,921.15**	**-£ 640,242.30**	**-£ 640,242.30**	**-£ 640,242.30**	**-£ 28,838,161.09**	**-£ 1,441,908.05**	*Correct*	*Correct*
54	**TOAL COST *(inc. depreciation)***	**14,688,097.76**							**-£ 25,989,138.01**	**-£ 1,299,456.90**	*Correct*	*Correct*
55	B(wet)			£ -	£ -	£ -	£ -	£ -	£ -	£ -		
56	CO2 Capture			£ -	£ -	£ -	£ -	£ -	£ -	£ -		
57	**PAR**			**£ -**	**£ -**	**£ -**	**£ -**	**£ -**	**£ -**	**£ -**	*Correct*	*Correct*
58	**NCF**	**NCF as Cost + Revenue**		**-£ 16,375,718.54**	**-£ 416,921.15**	**-£ 640,242.30**	**-£ 640,242.30**	**-£ 640,242.30**	**-£ 28,838,161.09**	**-£ 1,441,908.05**	*Correct*	*Correct*
59	**Cum.NCF**	**Cumulative Cash Flow**		**-£ 16,375,718.54**	**-£ 16,792,639.69**	**-£ 27,557,676.49**	**-£ 28,197,918.79**	**-£ 28,838,161.09**			*Correct*	
60	**TOTAL IN VS. OUT INC. DEP.**								**-£ 25,989,138.01**	**-£ 1,299,456.90**	*Correct*	
61	OVERALL Liquidity	s - Liabilities - Cost + Rev		-£ 2,729,286.42	£ 12,665,380.32	£ 3,901,172.74	£ 3,337,042.08	£ 2,772,911.43	**£ 141,757,697.43**	**£ 7,087,884.87**		
62	Cum.NCF	Cumulative Overall		-£ 2,729,286.42	£ 9,936,093.89	£ 135,647,743.91	£ 138,984,786.00	***£ 141,757,697.43***			*Correct*	
80												
81												
82												

... | 4. Capital Exp. | 5. Operational Exp. | 6. Benefits | **7. NCF** | 8. CostBreakdown | 9. Depreciation | 10. Financial Liabilities | 11.Density_Table | ⊕

F. Chapter 5 Scenarios

In Chapter 5, several different scenarios were input to the model to highlight sensitivity of cost of capture (CoC £ $tonne_{CO2}^{-1}$) to changes in capital and operational expense and system efficiencies. The changes made were described in Table 5.5 and a detailed summary of the changes is presented here.

Changes made	Scenario						
	1	2	3	4	5	6	7
Biomass productivity	0.11 g L^{-1} day^{-1}	0.15 g L^{-1} day^{-1}	0.15 g L^{-1} day^{-1}	0.15 g L^{-1} day^{-1}	0.15 g L^{-1} day^{-1}	0.15 g L^{-1} day^{-1}	0.15 g L^{-1} day^{-1}
Cultivation time	14 days	14 days	14 days	14 days	14 days	14 days	7 days
Capture efficiency	10 %	20 %	20 %	20 %	20 %	20 %	40 %
CapEx change	No change	No change	25 % reduction	No change	25 % reduction	No change	No change
OpEx change	No change	No change	No change	Reduced heat, chemical, energy	Reduced heat, chemical, energy	No change	No change
CO_2 credit	No	No	No	No	No	£50 $tonne_{CO2}^{-1}$	No

G. Chapter 6 Scenarios

Scenario based analysis was also utilised in Chapter 6 to show sensitivity of treatment cost (£ m^3 $leachate^{-1}$) to changes in capital and operational expense and system efficiencies. The changes made were described in Table 6.2 and a detailed summary of the changes is presented here.

Changes Made	Scenario					
	Baseline	**1**	**2**	**3**	**4**	**5**
MEC discount		Economies of Scale, 0.6		Economies of Scale, 0.6		Economies of Scale, 0.6
OpEx reductions			No additional heating, no additional NO_3	No additional heating, no additional NO_3		No additional heating, no additional NO_3
Batch cultivation time	42 Days	42 Days	42 Days	42 Days	21 Days	21 Days