

# Design of an optimal photobioreactor

*by*

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# ABSTRACT

Currently the three main algae strains that are manufactured commercially are *Chlorella*, *Spirulina* and *Dunaliella salina*, which are produced for biomass and bioproducts. Photobioreactors (PBR) allow the exploitation of over 50 000 known microalgae species with over 15 000 novel compounds having been chemically identified to date. Many of these algae could be sources of high-value products which are produced using a method that delivers them from renewable resources.

Designing an optimal photobioreactor is a complex process because a large array of variables is included in the design, with several of the variables interacting with each other directly. The interactions of most of these variables have not been established. The initial information that is available is inadequate because most photobioreactors have been tested on a laboratory scale and the information given does not include the manufacturing materials, the size of tubing used and other design variables.

Before designing a photobioreactor, it is important to understand the best conditions for the production of algae because these have a direct influence on the requirements. In order to produce algae biomass under the specific conditions, one has to investigate current photobioreactors that have been designed in order to establish whether they are capable of optimum production under the production conditions; determine possible factors that could influence the production negatively and how they could be prevented; and undertake a cost analysis to determine whether the production of algae is an economically viable process using the specific reactor. All of these criteria have to be met for a photobioreactor to be viable in the production of algae biomass.

Currently a Bubble column reactor is considered to be the best design for a photobioreactor and also the most scalable. Due to the limited information available, testing was conducted to determine the effect of: 1) different manufacturing materials, 2) the gas dispersion unit, 3) the diameters of the tubing and 4) the density. Bubble column reactors were used to test the effects of the four variables and were considered to be the most important aspects in the design. For testing these variables and their interaction, *Chlorella Vulgaris* was used because it is one of the most popular algae species used for production currently. As temperature and the availability of light play a large role in the production of algae, all testing was done in a laboratory environment to ensure small temperature changes and the constant availability of light.

The reactors that were tested were made of PVC couplings, with the clear tubing used being made of either PVC or acrylic tubing. Enriched air was supplied at a 5% volume per volume ratio of CO<sub>2</sub>, with a flow rate of 0.02 volume per volume per minute (vvm) for the 50 mm diameter reactors and 0.36 vvm for the 90 and 110 mm diameter reactors. Two gas dispersion units were used to determine whether they would have any effect on the production. The gas dispersion units create small bubbles to ensure a high surface area to volume ratio and thereby they allow for maximum CO<sub>2</sub> and O<sub>2</sub> mass transfer.

A growth rate of 0.14 gram per litre per day was found to yield the best production of all the reactors and configurations that were tested. The 50 mm diameter reactors showed the best growth followed by the 110 mm diameter reactors. The 90 mm diameter reactors all had a negative growth rate which appeared to be due to an insufficient gas flow rate. The 50 mm reactors had the best growth rate of 0.14 and 0.10 grams per litre per day for the acrylic tubing, while 0.08 grams per litre per day was achieved with PVC tubing. The 110 mm reactors had a highest growth rate of 0.05 grams per litre per day with PVC tubing.

It was found that the 50 mm and 90 mm reactors showed a better performance with acrylic tubing while the 110 mm reactors showed a better performance with PVC tubing. The gas dispersion unit is affected by the gas flow rate, the density, the diameter of the tubing and the material that is used. The gas dispersion units' effect is dependent on the diameter of the reactor seeing that the 50 mm reactor shows better performance with the small unit, while the 110 mm reactor shows better performance with the large unit, due to the gas flow rate that is required in the reactors. Because the gas flow rate and gas dispersion unit directly affect the agitation, the optimal density is affected directly due to the availability of light and therefore the tubing material. The gas dispersion units should fit properly into the reactor and be capable of handling the gas flow rate that is required. The diameter of the tubing does not show any effect but could have an effect under different testing conditions and could not be conclusively eliminated. The density of algae does have an effect, although most reactors showed a better production rate at a higher culture density.

The scale up of the bubble column reactor creates a dead zone when a module is constructed. The scale up of a bubble column reactor could range from increasing the vertical tubing length, increasing the diameter of the tubing to adding vertical tubing to a module. The dead zone is formed at the bottom of the reactor where the module interconnects the vertical growth tubes, because these fittings are not constructed from a

clear material, due to cost of such a construction. The dead zone that is created causes a large portion of algae to form a sediment, which directly affects the production of the system because it is in a dark zone of the reactor. Improved results would be obtained if the algae were kept at a homogeneous density that would ensure maximum exposure to light.

The ratio of gas flow rate to reactor volume and diameter of the tubing was found to be crucial. It is suspected that the 90 mm tubing reactor had a negative growth rate as this ratio was not correct. The 50 mm reactors had to be run at a much lower reactor volume per volume gas flow rate which could consist of air, carbon dioxide enriched air or other gases as required. The inclusion of the tubing diameter in the ratio is of vital importance and should be studied further.

A cost analysis shows that the bubble column reactors under the tested conditions are not financially viable. A large component of the cost is carbon dioxide and medium, which is a composition of nutrients. This could be removed if a free source were obtained, which would make the system financially viable. These sources could include waste water and flue gas from industrial processes.

It is recommended that a gas dispersion tube be positioned at the bottom of the reactor to ensure that no sedimentation occurs and that there is a homogeneous culture, and to maximise the production capabilities of a bubble column reactor. It is also recommended that the gas flow rate inside the reactor be studied to obtain a ratio where the volume of the reactor, the height of the reactor and the diameter of the tubing are included to obtain a sufficient rate of flow.

# OPSOMMING

Tans is daar drie belangrike alg stamme wat kommersieel geproduseer word, *Chlorella*, *Spirulina* en *Dunaliella salina*. Fotobioreaktors het meegebring dat meer as 50 000 bekende alg spesies met meer as 15 000 komponente tot op datum chemies geïdentifiseer is. Baie van hierdie alge kan hoë waarde produkte wees, wat met behulp van hernubare metodes geproduseer kan word.

Die ontwerp van 'n optimale fotobioreaktor is 'n komplekse proses aangesien 'n groot verskeidenheid veranderlikes ingesluit moet word wat 'n invloed op mekaar kan hê. Die interaksie van meeste van hierdie veranderlikes is nog nie vasgestel nie. Die inligting oor hierdie onderwerp is beperk aangesien die meeste fotobioreaktors in 'n laboratorium getoets is en dus nie die vervaardigingsmateriale, die grootte van buise en ander ontwerp veranderlikes insluit nie.

Voordat 'n fotobioreaktor ontwerp kan word, moet die ideale alg produksie toestande verstaan word, aangesien dit 'n direkte impak op die produksie vereistes kan hê. Om alg biomassa onder spesifieke omstandighede te produseer, moet die bestaande fotobioreaktor ontwerpe ondersoek word. Daar moet vasgestel word of die bepaalde ontwerp oor die kapasiteit beskik om optimale produksie te lewer; identifisering van faktore wat produksie negatief kan beïnvloed en hoe dit voorkom kan word; en 'n koste ontleding moet gedoen word om te bereken of die produksie van alge met die geïdentifiseerde ontwerp 'n ekonomies lewensvatbare proses is. Daar moet aan al die vereistes voldoen word om te bepaal of 'n fotobioreaktor lewensvatbaar is vir die produksie van alg biomassa.

'n Borrel-kolom reaktor ontwerp word tans as die beste ontwerp vir 'n fotobioreaktor geag, asook die mees aanpasbare ontwerp. As gevolg van die beperkte inligting wat beskikbaar is, is navorsing gedoen om die invloed van verskillende faktore te bepaal, naamlik: vervaardigingsmateriaal, gasverspreidingseenheid, buisdeursnee en digtheid. Borrel-kolom reaktors is gebruik om die vier belangrikste veranderlikes in die ontwerp te toets. Om die veranderlikes en hul interaksie te toets, is *Chlorella vulgaris* gebruik, aangesien dit een van die gewildste alg spesies is vir die produksie van biomassa. As gevolg van die belangrike rol wat temperatuur en lig beskikbaarheid in die produksie van alge speel, is al die toetse in 'n laboratorium-omgewing gedoen om temperatuur wisseling te beperk en konstante lig beskikbaarheid te verseker.

Die reaktors wat getoets is, is vervaardig uit PVC koppelstukke, met die deurskynende buise wat uit PVC of akriel vervaardig is. Verrykte lug is verskaf op 'n 5% volume per volume verhouding CO<sub>2</sub>, met 'n vloeitempo van 0,02 volume per volume per minuut (vvm) vir die 50 mm deursnee reaktors en 0,36 vvm vir die 90 mm en 110 mm reaktors. Twee gasverspreidingseenhede is gebruik om hul invloed op die produksie te bepaal. Die gasverspreidingseenhede skep kleiner borrels, om 'n hoër oppervlakte tot volume verhouding te skep en daardeur 'n maksimum CO<sub>2</sub> en O<sub>2</sub> massa-oordrag te verseker.

'n Groeikoers van 0,14 gram per liter per dag is gevind as die beste produksie van al die reaktors en konfigurasies wat getoets is. Die 50 mm deursnee reaktors het die beste groei getoon, gevolg deur die 110 mm deursnee reaktors. Die 90 mm deursnee reaktors het 'n negatiewe groeikoers getoon, wat moontlik toegeskryf kan word aan onvoldoende gas vloeitempo. Die 50 mm reaktors het die beste groeikoers van 0,14 en 0,10 gram per liter per dag vir die akriel buise getoon, terwyl 'n 0,08 gram per liter per dag behaal is met 'n PVC buis. Die 110 mm reaktors het die hoogste groeikoers aangedui van 0,05 gram per liter per dag met 'n PVC buis.

Daar is bevind dat die 50 mm en 90 mm reaktors 'n beter prestasie met akriel buise gehad het, terwyl die 110 mm reaktors 'n beter prestasie met 'n PVC buis gehad het. Die gasverspreidingseenheid word beïnvloed deur die gas vloeitempo, digtheid, buisdeursnee en die vervaardigingsmateriaal wat gebruik word. Die gasverspreidingseenhede word verder beïnvloed deur die reaktor se buisdeursnee aangesien die 50 mm reaktor 'n beter prestasie getoon het met die kleiner gas eenheid, terwyl die 110 mm reaktor 'n beter prestasie getoon het met die groter gas eenheid, as gevolg van die gas vloeitempo wat vereis is. Die gas vloeitempo en gasverspreidingseenheid het 'n direkte invloed op die groei van die kultuur, dus is die optimale digtheid afhanklik van die lig beskikbaarheid en dus die vervaardigingsmateriaal van die buise. Die gasverspreidingseenhede moet stewig in die reaktor pas en in staat wees om die gas vloeitempo wat vereis word te kan hanteer. Hoewel die deursnee van die buise nie 'n invloed getoon nie, kan dit 'n invloed onder verskillende toetsomstandighede toon en kon nie finaal uitgeskakel word. Die digtheid van die alge het wel 'n effek, hoewel die meeste reaktors 'n beter produksietempo op 'n hoër kultuur digtheid toon.

Die groter skaal borrelkolom reaktor ontwikkel 'n dooie sone indien 'n module saamgestel word. Die groter skaal borrelkolom reaktor kan insluit: die verhoging van die vertikale buislengte, 'n toename in deursnee van die buise en toevoeging van vertikale buise in die

module. Die dooie sone het gevorm aan die onderkant van die reaktor waar die module se vertikale groei buise met mekaar verbind is. Hierdie area is uit nie-deurskynende materiaal vervaardig as gevolg van die konstruksie koste. Die dooie sone het veroorsaak dat groot hoeveelhede van die alge 'n sediment gevorm het en 'n direkte invloed op die produksie van die stelsel gehad het aangesien dit 'n donker sone in die reaktor gevorm het. Beter resultate kan verwag word indien die alge op 'n homogeniese digtheid gehou kan word om maksimum lig blootstelling te verseker.

Daar is bevind dat die verhouding van gas vloeitempo tot reaktor volume en buisdeursnee deurslaggewend is. Die negatiewe groeikoers in die 90 mm reaktor word toegeskryf daaraan dat hierdie verhouding nie korrek was nie. Die 50 mm reaktors het op 'n laer reaktor volume per volume gas vloeitempo gefunksioneer wat kan bestaan uit die lug, verrykte lug of ander gasse soos benodig. Dit dui daarop dat die insluiting van die buisdeursnee in hierdie verhouding van kardinale belang is en verder bestudeer moet word.

'n Kosteontleding toon dat die borrelkolom reaktors onder hierdie getoets omstandighede nie finansiële lewensvatbaar is nie. 'n Groot deel van die koste is die medium, wat 'n samestelling van voedingstowwe is, en koolstofdioxide koste. Om finansiële lewensvatbaar te raak, moet hierdie kostes deur 'n gratis bron vervang word. Die bronne kan bestaan uit afvalwater en oortolige CO<sub>2</sub> uit industrie.

Daar word aanbeveel dat 'n gasverspreidingsbuisie aan die onderkant van die reaktor geplaas word. Dit sal verseker dat geen sediment vorm nie en 'n homogeniese kultuur gehandhaaf kan word om maksimum produksie in 'n borrelkolom reaktor te handhaaf. Verder word aanbeveel dat die gas vloeitempo binne die reaktor verder bestudeer word om 'n verhouding tussen die volume van die reaktor, die hoogte van die reaktor en die deursnee van die buise te bepaal deur sodoende 'n voldoende tempo van vloeitempo te verkry.



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# ABBREVIATION

3PG	3-phosphoglycerate
AA	Arachidonic acid
ABE	Acetone, butanol and ethanol
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
<i>Cyt<sub>b<sub>6</sub>f</sub></i>	Cytochrome <i>b<sub>6</sub>f</i>
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
Fd	Ferredoxin
FNR	Ferredoxin-NADP <sup>+</sup> oxidoreductase
G3P	Glyceradehyde-3-phosphate
GC	Gas chromatography
GLA	$\gamma$ -Linolenic acid
Hyd A	Hydrogenase
LHC	Light harvesting centre
NADPH	Nicotineamide adenine dinucleotide phosphate
OD	Outer diameter
PBR	Photobioreactor
PC	Plastocyanin
PQ	Plastoquinone
PS	Photosystem
PSI	Pounds per square inch
PUFA	Polyunsaturated fatty acids
PVC	Polyvinyl chloride
RBP	Rubilose 1,5-biphosphate
ROS	Reactive oxygen species
TAG	Triacylglycerides
TLC	Thin layer chromatography
UV	Ultraviolet
VAP	Vertical alveolar panel

# SYMBOLS

$\mu$	Specific growth rate	Days <sup>-1</sup>
$A_g$	Ground area utilised by PBR	m <sup>2</sup>
$dm_x$	Change in biomass weight	g
$dt$	Duration span	Days
$e^-$	Electron	
$hv$	Light energy	
kl	Kilolitre	
kWh	Kilowatt hours	
ms	Milliseconds	
pH	Potential of hydrogen	pH units
PSI	Pounds per square inch	
t	Time duration	Days
v/v	Volume per volume	%
vvm	Volume per volume per minute	Litres
wt%	Weight percentage	
$X_0$	Biomass initial concentration	g/l
$X_m$	Biomass end concentration	g/l
	Gram per litre	g/l
	Gram per litre per day	g/l/d
	Gram per square metre per day	g/m <sup>2</sup> /d
	Kilocalorie per gram	k.cal/g
	Kilogram per cubic metre	kg/m <sup>3</sup>
	Kilojoules	kJ
	Kilojoules per gram	kJ/g
	Kilowatt hour per ton	kWh/t
	Microgram per day	$\mu$ g/d
	Micrometre	$\mu$ m
	Milligram per litre per day	mg/l/d
	Milligram per millilitre	mg/ml
$\omega_3$	Omega-3	
$\omega_6$	Omega-6	

# CHEMICALS

$\text{Al}_2(\text{SO}_3)$	Aluminium sulfate
C	Carbon atom
<i>C. vulgaris</i>	<i>Chlorella Vulgaris</i>
$\text{C}_2\text{H}_4\text{O}_2$	Acetic acid
$\text{C}_6\text{H}_8\text{O}_7$	Citric acid
$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	Calcium nitrate tetrahydrate
$\text{CaCl}_2$	Calcium chloride
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	Calcium chloride dihydrate
$\text{CH}_2\text{O}$	Formaldehyde
$\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$	Sodium acetate trihydrate
$\text{CH}_4$	Methane
$\text{CH}_4\text{N}_2\text{O}$	Urea
CO	Carbon monoxide
$\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$	Cobalt nitrate hexahydrate
$\text{CO}_2$	Carbon dioxide
$\text{CO}_3^{2-}$	Carbonate
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	Copper sulphate pentahydrate
EDTA 2Na-Mg Salt	Ethylenediaminetetraacetic acid disodium dimagnesium
EDTA-FeNa	Ethylenediaminetetraacetic acid tetrasodium
EDTA-Na <sub>2</sub>	Ethylenediaminetetraacetic acid disodium
EDTA-Na <sub>4</sub>	Ethylenediaminetetraacetic acid tetrasodium
$\text{Fe}_2(\text{SO}_4)_3$	Ferric sulfate
$\text{FeCl}_3$	Ferric chloride
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	Ferrous sulphate heptahydrate
H	Hydrogen atom
$\text{H}^+$	Proton
$\text{H}_2\text{CO}_3$	Carbonic acid
$\text{H}_2\text{O}$	Hydrogen dioxide, water
$\text{H}_2\text{SO}_4$	Sulphuric acid
$\text{H}_3\text{BO}_4$	Boric acid
HCl	Hydrogen chloride
$\text{HCO}_3^-$	Bicarbonate
$\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$	Potassium phosphate dibasic trihydrate

# CHEMICALS (CONTINUE)

$\text{KH}_2\text{PO}_4$	Potassium phosphate monobasic	
KOH	Potassium hydroxide	
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	Magnesium sulphate heptahydrate	
$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	Manganese chloride tetrahydrate	
$\text{MoO}_3$	Molybdenum (VI) oxide	
N	Nitrogen atom	
$(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$	Ammonium molybdate tetrahydrate	
$\text{N}_2$	Nitrogen molecule (gas)	
$\text{Na}_2\text{CO}_3$	Sodium carbonate	
$\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$	Sodium phosphate dibasic dodecahydrate	
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	Sodium molybdate dihydrate	
$\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$	Sodium metasilicate nonahydrate	
NaCl	Sodium chloride	
$\text{NaHCO}_3$	Sodium bicarbonate	
$\text{NaNO}_3$	Sodium nitrate	
$\text{NH}_3$	Ammonia	
$\text{NO}_3^-$	Nitrates	
O	Oxygen atom	
$\text{O}_2$	Oxygen	
$\text{OH}^-$	Hydroxide ion	
P	Phosphorus	
<i>P. tricornutum</i>	<i>Phaeodactylum tricornutum</i>	
$P_i$	Phosphate	
S	Sulfur	
t	Time duration	Days
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	Zinc sulphate heptahydrate	

# 1. INTRODUCTION

## 1.1. Background

Sustainability is the most important principle in the management of natural resources. It involves operational efficiency, and environmental and economic impact, which are all interconnected (Singh and Sharma, 2012). The social, environmental and economic pressures of human activity require increasing energy resources, with an estimated increase in worldwide energy usage from 533 to 812 quadrillion ( $10^{15}$ ) kJ between 2008 and 2035 (Rogers et al., 2014). Biomass that is produced using photosynthesis can potentially become a renewable fuel source which will simultaneously use carbon dioxide and thereby reduce current greenhouse gas emissions and the carbon footprint. Microalgae are one of the most prolific sources of photosynthetic biomass on Earth and have been promoted as one of the most promising third-generation biofuel sources (Rogers et al., 2014), mainly due to its rate of production and because it can be produced on non-arable land.

The first unicellular algae culture was *Chlorella Vulgaris* which was achieved by Beijerinck in 1890. Research on algae production only started in Stanford in the USA, Essen in Germany and Tokyo after 1948. Hereafter, the commercial production of algae biomass began in Japan during the early 1960s with *Chlorella*, and during the 1970s research on *Spirulina* followed (Borowitzka, 1999).

The first attempts to grow algae occurred during World War II; algae were grown in open-pond systems by the Germans as a food supplement (Ugwu, Aoyagi and Uchiyama, 2008). By 1980 Asia had 46 commercial algae plants which produced in excess of 1000 kg of *Chlorella* monthly (Borowitzka, 1999). In 1996 the *Chlorella* traded in Japan alone was in the region of 2000 tons (Borowitzka, 1999).

Currently the three main algae strains produced commercially are *Chlorella*, *Spirulina* and *Dunaliella salina*, produced for biomass and bioproducts, although over 15 000 novel compounds from algae production have to date been chemically identified, with three main groups consisting of toxins, bioproducts and chemical ecology (Cardozo et al., 2007).

Algae are some of the most basic photosynthetic organisms on earth (Demirbas, 2010; Demirbas and Fatih Demirbas, 2011). They can live in either saline or fresh water and require mainly light energy, water and carbon dioxide for survival (Demirbas, 2010). Algae are part of a diverse group of organisms which can be found in simple unicellular to complex multicellular forms and can be found growing within most biotopes, due to their ecological diversity and physiological adaptability to the specific environments (Pulz, 2001).

Algae have mostly been seen as a problem: as a result of their rapid growth rate it costs millions annually to keep pools, dams, lakes and drinking water clean from algal infestation. Due to their rapid growth rate, algae show potential to produce biofuels, food and high-value molecules from a renewable source and supply the demand for specific natural and renewable products. Microalgae are cell factories that are sunlight-driven and could potentially produce an array of compounds which include polysaccharides, lipids, proteins, carotenoids, pigments, vitamins, steroids, pharmaceuticals and biofuels (Choi, Suh and Lee, 2003; Slegers et al., 2013).

Algae production on an industrial scale is currently expensive when compared to fossil fuels: the estimated production cost of algae oil is between \$1.40 and \$1.81 per litre and this cost needs to be reduced to around \$0.45 per litre in order to compete with petrol and diesel (Demirbas and Fatih Demirbas, 2011). In addition, high-value molecules are produced using algae, among them are the dietary supplements spirulina (*Spirulina*),  $\beta$ -carotene (*Dunaliella salina*),  $^{15}\text{N}$ -alanine, mixed fatty acids and allophycocyanin, to name a few currently produced (Spolaore et al., 2006). Worldwide, microalgae are produced at a commercial level with very little or no information available on the design, selection of locations, scaling, or constraints due to the competitive edge they provide in a commercial plant (Grobbelaar, 2009a). Several designs of photobioreactors (PBRs) have been developed. They would enable the production of new species' biomass and products at a commercial level (Borowitzka, 1999) with effective scaling creating the largest problem.

One of the main driving forces behind the research on algae is to find a replacement for the current fossil fuels. Because the use of algae-based biofuels would create a closed carbon system, it would thus have no net effect on the environmental levels of carbon dioxide or possibly even reduce the levels of carbon dioxide in the



atmosphere. An additional advantage of using algae is that the algal biomass can be produced using runoff, waste, saline or fresh water, depending on the algae. In addition the nutrients in the water may be collected and the water purified before it enters the natural ecosystem. Algae have the ability to deliver a renewable source of products with the ability to purify effluent from commercial processes, creating a lower carbon footprint and possibly reducing production costs.

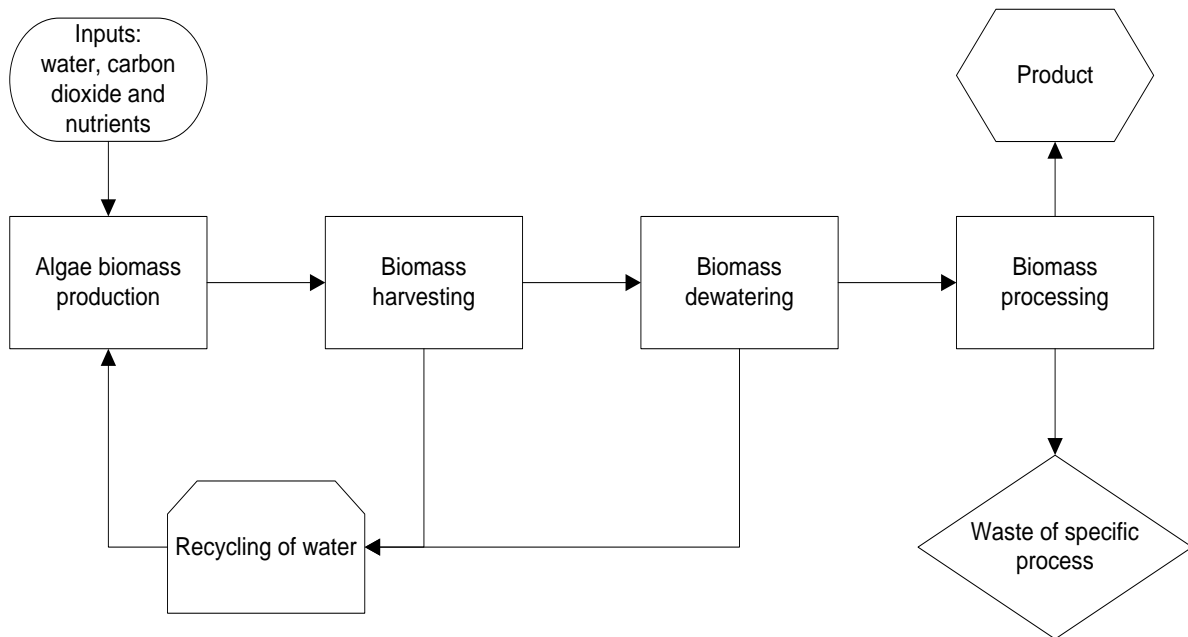


Figure 1.1: The basic process of algae production

Figure 1.1 shows the basic process for the production and processing of algae biomass. The recycling of water allows algae biomass to be grown with very high water efficiency when compared to other energy crops. This factor is becoming very important due to the limited freshwater sources that are available (Borowitzka and Moheimani, 2013). Control of the production system almost eliminates the possibility of having a failed harvest, unlike other energy crops which depend on natural weather cycles and could be negatively affected by unforeseen circumstances (Dominguez-Faus et al., 2009), e.g. drought could cause a complete failure of energy crops while algae production would not be affected or the effect could be limited by the proper management of the available resources.

The biggest benefit of algae biomass production is the ability to produce algae biomass on non-arable land. This does not directly threaten current food production,

while energy crops compete directly with food production for arable land. Waste created by a specific processing method could possibly be reprocessed to obtain another product, thus increasing the economical and processing viability of producing algae biomass, e.g. if the desired product from the biomass is the lipid content, the waste could be processed to obtain protein, carbohydrates or any other product that is produced by the specific strain of algae, making it more economical and environmentally friendly. Due to the possibilities for algae biomass, the design of a photobioreactor will play an important role.

Several factors in the design of photobioreactors influence their productivity as seen in Figure 1.2. The circular boxes represent the input into the reactor and the square boxes represent the calculations that have to be performed. The calculations are even more complicated by the fact that some of the inputs are interconnected and thus could cause problems when something is changed in the system. Due to the large quantity of algae species that are available and the different requirements of each species, the design of the reactor has to be modified to meet the specific alga's requirements for optimal production. Because there are so many variables as seen in Figure 1.2, the design of a bioreactor becomes fairly complicated and should be compatible with all species and conditions required, while providing high and efficient production levels.

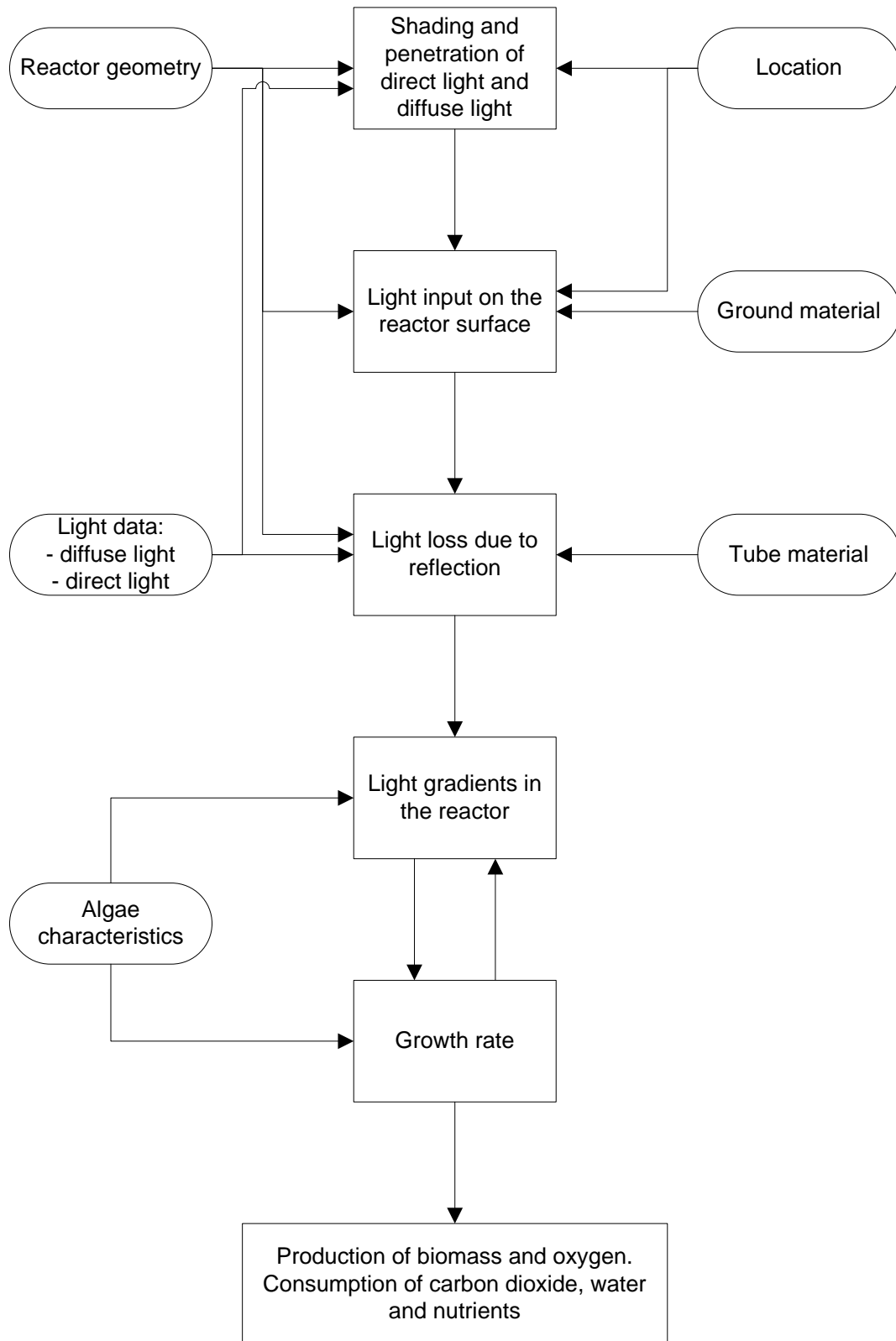


Figure 1.2: Tubular PBR calculation scheme to determine the productivity of algae biomass, redrawn from Slegers et al., 2013

## 1.2. Problem statement and objective

Currently most of the commercial plants producing algae use open pond systems with several closed photobioreactors also being used (Tredici, 2004). Many different designs for photobioreactors are available for the production of algae biomass. Many of the proposed designs have been tested on a laboratory scale, but have several very important factors that are affected by the scaling up of the design of the specific reactor, with light availability, carbon dioxide availability and agitation composing the top three factors for producing algae biomass (Molina Grima et al., 1999). The economic aspect of the design of the reactor is also important as it should be an economically viable process to produce algae biomass and thus the focus should not only be concentrated on the environmental aspect.

A greater difficulty is experienced in algae bioreactors because the effects of several external factors – consisting of the light characteristics inside the reactor, the temperature and the building materials – influence the design and viability of the reactor, because a living biomass is being produced. In some cases uncontaminated algae are required and this aspect complicates the problems because it is difficult to maintain certain production systems in a sterile state. With the aid of the knowledge obtained from the literature study (see chapter 2), several requirements were identified to be used as the backbone of the design of the bioreactor. The requirements of an optimal photobioreactor are listed below as suggested by (Tsoglin et al., 1996):

- The reactor should be able to produce algae biomass at an optimal growth rate constantly.
- The design must allow the maximum possible light penetration with little unilluminated areas and uniform illumination over the reactor.
- High mass transfer of algae biomass is required during the harvesting process, to ensure small disruption in biomass production.
- High mass transfer of CO<sub>2</sub> and O<sub>2</sub> is necessary to ensure that optimal growth is obtained.
- The reactor should have little or no mechanical parts.
- The reactor should be a system that is low cost and easy to maintain.

- The reactor should have limited bio-fouling and, in cases when bio-fouling does occur, it should be easily cleanable.

The objectives of the study are:

- to define the different algae production conditions;
- to determine the best photobioreactor design;
- to determine the input requirements for algae biomass production;
- to anticipate possible factors that influence the production negatively;
- to assess the influence of different factors and their interactions; and
- to determine the viability of algae biomass on the tested photobioreactor.

## 2. LITERATURE REVIEW

### 2.1. Introduction

This literature review starts by defining the main conditions of cultivation and the systems used in algae production, including the scale up of photobioreactors and their obstacles as described in the available literature. This is followed by researching algae's basic requirements, the growth potential and the cell compositions of different algae species. Specific nutrient requirement and the importance of the theory of these requirements will be reviewed. Potential problems that could be encountered and possible solutions to these problems will also be reviewed. The recovery of the algae biomass from its growth medium, including further processes to purify the biomass components and the uses of algae biomass will be compared. This will be followed by a summary at the end the chapter. This literature review attempts to highlight the engineering requirements of algae photobioreactors, including the overall process and to a limited degree the biochemistry and chemistry involved in algae production.

### 2.2. Algae cultivation conditions

The conditions under which algae are cultivated have a significant effect on the algae's composition, characteristics and the requirements of the reactor (Chojnacka, 2004; Chen et al., 2011). Photoautotrophic, (Chemo) heterotrophic, heteroautotrophic and mixotrophic are the four major cultivation conditions for algae (Chojnacka, 2004; Chen et al., 2011).

#### 2.2.1. Photoautotrophic

The photoautotrophic conditions are the most commonly used cultivation condition for the production of microalgae (Chen et al., 2011; Illman, Scragg and Shales, 2000; Yoo et al., 2010). Algae produced using photoautotrophic conditions use light as their sole energy source, while inorganic carbon is their sole carbon source, of which both are the limiting factors, to utilise during the photosynthesis process as seen in Figure 2.10 (Chojnacka, 2004). The advantage of using photoautotrophic cultivation for the production of algae biomass is that CO<sub>2</sub> from factories and power plants can

be used. This will reduce the carbon dioxide emissions of the factories making them more environmentally friendly (Chen et al., 2011).

Table 2.1: Stoichiometric equations and pH effect of major cultivation conditions

Condition	Stoichiometric equation	pH
Photoauto-trophic	$H_2O + HCO_3^- \xrightarrow{hv} C(biomass) + \frac{1}{2}O_2 + 3OH^-$	Increases
Heterotrophic	$(1 + a)CH_2O + O_2 \rightarrow C(biomass) + aCO_2 + (1 + a)H_2O$	Decrease
Mixotrophic	$bHCO_3^- + cH_2O \xrightarrow{hv} (b + (c - a))C(biomass) + 3OH^- + aCO_2$	Minor changes

Source: Chojnacka, 2004

### 2.2.2. Heterotrophic

(Chemo) Heterotrophic cultivations are the use of organic compounds as carbon and energy sources, eliminating the requirement of light as an energy source (Chojnacka, 2004). Microalgae can utilise an array of organic carbon sources including monosaccharides, disaccharides, acetates and glycerols (Chen et al., 2011; Liang, Sarkany and Cui, 2009). Liang, Sarkany and Cui (2009) find that a biomass production rate of 2 g/l/d and a lipid production of 932.0 mg/l/d are possible. The use of heterotrophic cultivation provides a lipid production of almost 20 times the production of photoautotrophic systems (Chen et al., 2011).

Table 2.2: Productivity of algae species under different culture conditions

Species	Collection number	Cultivation conditions	Biomass productivity (g/l/d)	Lipid productivity (mg/l/d)
<i>Botryococcus braunii</i>	UTEX 572	Phototrophic	0.03	5.5
<i>Chlorella protothecoides</i>		Heterotrophic	43.0-46.0	1881.3-1840.0
<i>Chlorella protothecoides</i>		Heterotrophic	50.3-57.8	1209.6-3701.1
<i>Chlorella protothecoides</i>		Heterotrophic	46.1	932.0
<i>Chlorella protothecoides</i>		Heterotrophic	43.0-48.7	732.7-932.0
<i>Chlorella</i> sp.		Phototrophic	0.37-0.53	121.3-178.8
<i>Chlorella vulgaris</i>	#259	Phototrophic	0.01	4.0
<i>Chlorella vulgaris</i>	#259	Heterotrophic	0.08-0.15	27.0-35.0
<i>Chlorella vulgaris</i>	#259	Mixotrophic	0.09-0.25	22.0-54.0
<i>Chlorella vulgaris</i>	CCAP 211/11B	Phototrophic	0.17	32.6
<i>Dunaliella tertioleca</i>	ATCC 30929	Phototrophic	0.1	60.6-69.8
<i>Nannochloris</i> sp.	UTEX LB1999	Phototrophic	0.04-0.35	15.6-109.3
<i>Nannochloropsis oculata</i>	NCTU-3	Phototrophic	0.37-0.48	84.0-142.0
<i>Neochloris oleoabundans</i>	UTEX 1185	Phototrophic	0.31-.63	38.0-133.0
<i>Scenedesmus obliquus</i>		Mixotrophic	0.1-0.51	11.6-58.6

Source: Chen et al., 2011



Photoheterotrophic cultivation uses light as its energy source and organic compounds as its carbon source instead of organic compounds as both energy and carbon source, while in mixotrophic cultivation the organic compounds act as the energy source (Chen et al., 2011).

### **2.2.3. Mixotrophic**

Mixotrophic cultivation exploits the ability of algae to use both organic and inorganic carbon sources, or their ability to live under both photoautotrophic or photoheterotrophic conditions (Chen et al., 2011). According to Chojnacka (2004) another definition of mixotrophy is the utilisation of organic compounds as a source of carbon, while the inorganic compounds merely act as electron donors. Table 2.2 shows that the use of mixotrophic systems is very limited, with the main research focus occurring on phototrophic and heterotrophic systems.

## **2.3. Algae cultivation: systems and designs**

There are two main types of photoautotrophic algae cultivation systems: open ponds and closed systems (photobioreactors). Open ponds will be mentioned to give some background, however, the main focus is on photobioreactors.

### **2.3.1. Open ponds**

Open ponds are currently the preferred commercial method because they are less expensive to construct and easier to operate and build, while at the same time they are more durable than commercial photobioreactors. Open pond systems have been designed and tested in a number of ways, utilising a number of different configurations and materials (Tredici, 2004). Natural ponds and raceway ponds will be discussed below, although other types of open ponds are also available.

#### **2.3.1.1. Lakes and natural ponds**

Microalgae will grow under suitable conditions for production – both climatic and nutritional. In certain temporary and permanent lakes, the conditions are suitable for the growth of a monospecific culture (Tredici, 2004). Lake Texcoco in Mexico was a near natural pond which produced significant amounts of *Spirulina*, but it was closed in 1994 – 1995 due to contamination. In Myanmar crater lakes were discovered to be suitable for the production of *Spirulina*. These are currently becoming

increasingly more important (Pulz, 2001). The biggest problem with the use of natural lakes and ponds for the production of algae is that the possible need for the addition of nutrients could cause an ecological imbalance in the specific body of water and could thus cause a large ecological problem in the area close to the lake.

#### **2.3.1.2. Raceway ponds**

Raceway ponds are usually closed oval channels that have a depth of between 0.25 and 0.4 metres, are open to the environment and in which the culture is agitated using a paddle-wheel system in order to circulate the algae culture, and prevent any sedimentation (Slade and Bauen, 2013). Raceway ponds are typically constructed of concrete or compacted earth and they could be lined with white plastic (Chisti, 2007) for its reflective properties, in order to increase the light absorption of the algae and prevent water loss. The open design of the raceway pond system allows nature to influence the growth of the algae because the growth environment can only be controlled in certain areas, while other factors, such as temperature, follow natural rhythms. From a financial point of view, the capital required to produce algal biomass in open ponds is low compared to photobioreactors, but open ponds have much higher operating costs due to their open design and external influences.

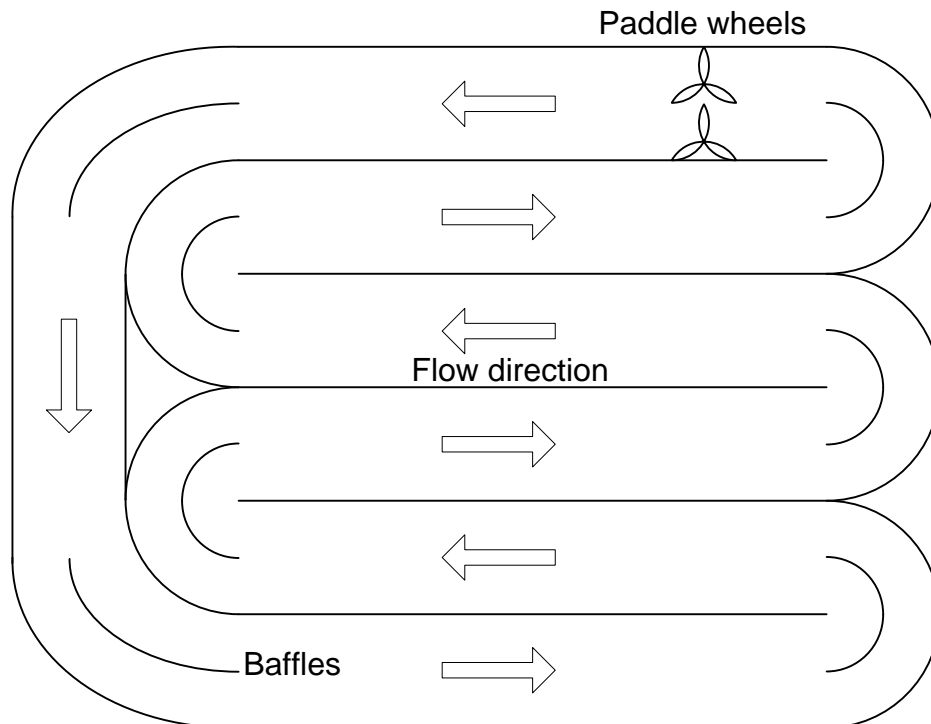


Figure 2.1: Schematic of a raceway pond system, redrawn from Chisti, 2007

Figure 2.1 is a schematic that shows the basic requirements and layout of a raceway pond system. Water depths of 0.15 – 0.20 metres are mainly used for operating conditions. These culture depths allow a 1000 mg/l biomass concentration to be maintained, allowing a production of 60 to 100 mg/l/day which is the equivalent of 10 to 25 g/m<sup>2</sup>/day of biomass. CO<sub>2</sub> diffusion to the atmosphere and evaporation losses are significant, with the possible contamination and pollution of the culture being the major drawback of the system (Pulz, 2001). The design of the paddle wheels in a raceway pond is very important as aeration would decrease the CO<sub>2</sub> levels in the system and thus decrease the growth rate of the algae (Grobbelaar, 2009a).

Table 2.3: Open culture systems currently used in commercial production of algae

System	Algae Specie	Location
Tanks	Aquaculture species	World wide
Open ponds	<i>Dunaliella salina</i> , <i>Chlorella</i> spp.	Australia Japan, Taiwan (circular ponds)
Raceway ponds	<i>Chlorella</i> spp., <i>Spirulina</i> spp  <i>Dunaliella salina</i>	Japan, Taiwan, Thailand, USA  Chile, China, India, Israel, USA, Vietnam
Cascade system	<i>Chlorella</i> spp.	Bulgaria, Czech Republic
Large bags	Aquaculture species	World wide
Fermenters	<i>Chlorella</i> spp.	Indonesia, Japan, Taiwan, USA
Two-stage system (PBR followed by open pond)	<i>Haematococcus pluvialis</i>	USA

Source: Borowitzka, 1999

### 2.3.2. Photobioreactors

Photobioreactors (PBR) allow the exploitation of over 50 000 known microalgae species, many of which could be interesting sources for high-value products which would then be produced using a renewable method. PBRs can be defined as systems where more than 90 % of the light has to enter the PBR through the transparent barrier in order to reach the culture medium. PBRs eliminate, or strongly limit, the direct interaction of gases and contaminants between the culture medium and the outer environment (Tredici, 2004).

PBRs enclose the algal broth in transparent tubes or plates with the culture being circulated from a reservoir through the reactor (Slade and Bauen, 2013). The available PBRs' configurations are numerous, but most PBRs can be divided into two main groups, namely reactors with either tubular or flat panels. It is possible to categorise PBRs further according to their design, including the orientation of the tubes, the mechanism for culture circulation, the lighting method and the construction materials, to name a few (Molina Grima et al., 1999).

The ability to control the environment of PBRs would ensure the constant optimal growth of the algal biomass. The use of artificial light could become beneficial when optimal growth is obtained as opposed to using normal sunlight growth durations, but would require additional energy and therefore additional costs would be incurred.

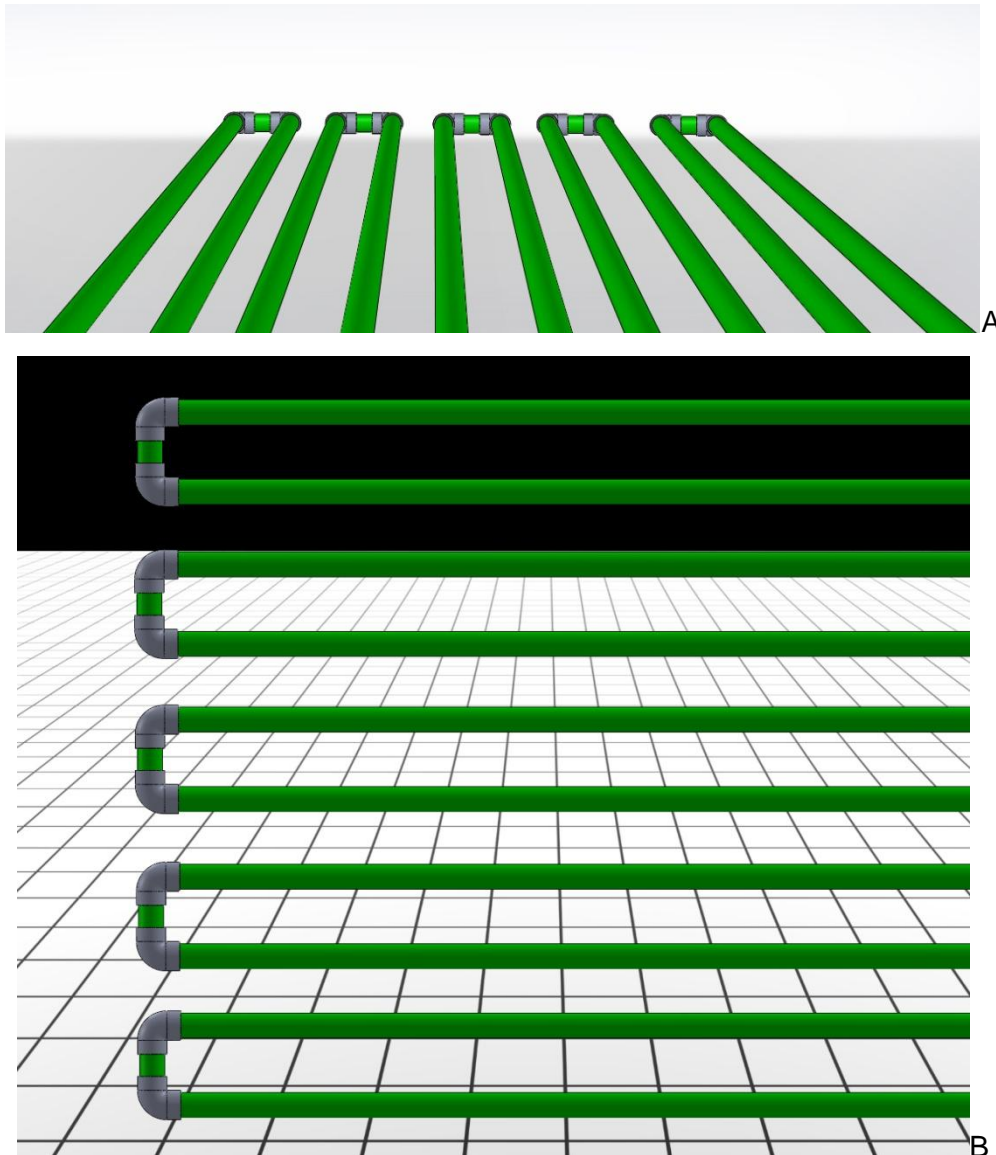


Figure 2.2: Photobioreactors currently used for experimental purposes:

A) horizontal, B) vertical PBR, redrawn from Slegers et al., 2013

Closed PBRs have the ability to regulate and control nearly all the biotechnological parameters. PBRs also have fundamental benefits which include a reduced risk of contamination, little loss of carbon dioxide, reproducible conditions, flexible designs, controllable hydrodynamics and temperatures (Pulz, 2001).

PBR efficiencies are determined by the utilisation of light and are defined by the ability to capture, transport, distribute and the usage of light energy. PBRs for biomass production should capture all the available light in a specific area to ensure both light- and area efficiency (Zijffers et al., 2008).

In Figure 2.2, the orientation of the PBRs can be seen in two different designs. By changing the designs of two very similar systems, several factors affecting the growth of algae biomass are influenced, including the availability of light and the hydrodynamics in the system. The changes in the design also entail additional requirements to the systems, for example the system in Figure 2.2B would require stronger pumps to circulate the culture around the entire reactor, which would increase the energy requirement and could cause damage to the algae cells due to pressure changes.

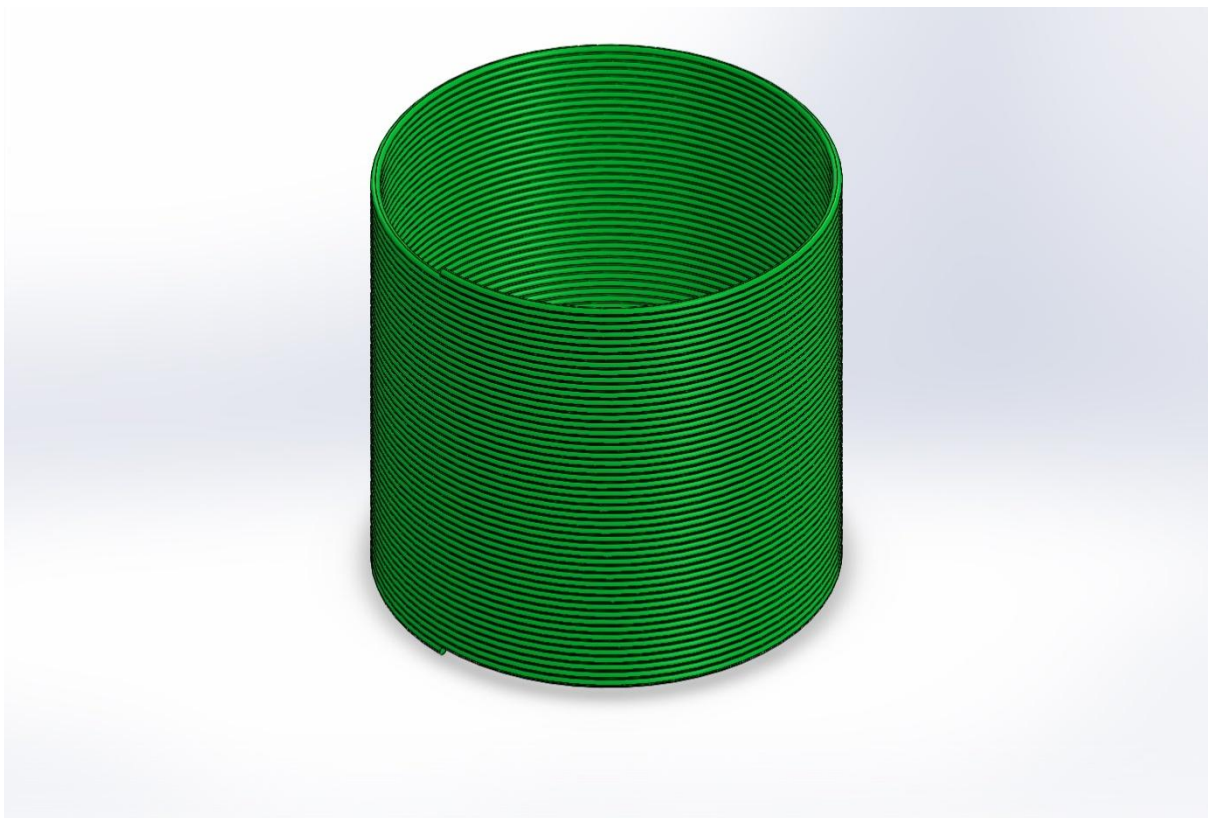


Figure 2.3: A helical tubular photobioreactor, redrawn from Chisti, 2007

The shape of PBRs does vary as seen in Figure 2.3 where the use of a very small surface area could ensure a high production of biomass when using a PBR instead of the raceway pond system.

Tubular reactors are most likely to be used as the next generation of enclosed algae culture systems. They allow effective illumination, high biomass concentration, effective bioreactor sterilisation, high CO<sub>2</sub> conversion efficiencies, low contamination levels, easy monitoring and easy control of operational parameters (Torzillo, 1997).

Three known commercial scale PBRs are utilised at full scale although very little information can currently be found on these plants. Two of the commercial scale plants were built in Hawaii (USA) by Micro Gaia Inc. and Aquasearch Inc. while the third was built in Germany by Ökologische Produkte Altmark GmbH. A commercial scale PBR was built, but operations were stopped after only a few months. The main reasons for the stop of operations were (Tredici, 2004):

- production process instability;
- contamination;
- improper circulation;
- inadequate degassing;
- improper management;
- biofouling;
- unequal culture distribution; and
- inadequate materials.

#### **2.3.2.1. Tubular PBR**

Vertical tubular PBRs are made from clear vertical tubing and use gas transfer to agitate the algae growth mixture gently, thus minimising the shear stress. The height is usually limited to four metres because a high concentration of CO<sub>2</sub> and pH gradients form in the reactor. The addition of gas (i.e. bubbling from the bottom end of the reactor) allows for efficient CO<sub>2</sub> utilisation and optimal O<sub>2</sub> removal, while simultaneously agitating the culture and reducing the energy requirements of the reactor (Wang, Lan and Horsman, 2012).

Vertical tubular PBRs consist of two types of sub-reactor, namely Bubble column and airlift. The main difference is that a Bubble column PBR has no internal division inside the reactor tube, while an Airlift PBR makes use of dividers to create and mix the flow inside the reactor tube, as seen in Figure 2.4. It is also possible to use an external loop in an airlift reactor by using the external loop for the returning mixture although this system would increase the cost of the PBR due to the extra work required during the building process, but the surface area to volume ratio is increased substantially (Wang, Lan and Horsman, 2012).

Tubular PBRs are one of the most preferred designs and can be found as horizontal, helical or bent reactors, as seen in Figure 2.2 and Figure 2.3. The transparent tubes are generally made from glass or clear plastic which have an outer diameter of 0.1 m or less – generally for optimal light penetration. The typical arrangement of tubular reactors differs in order to collect maximum light (Chisti, 2007).

Horizontal PBRs' incident light angle is much better when compared to that of vertical PBRs – this angle causes excess heat requiring expensive cooling systems. Difficulty arises when scaling up horizontal PBRs due to the excess heat, especially when large areas are occupied by the reactor. The scaling up of tubular PBRs also causes other major problems which are explained next (Wang, Lan and Horsman, 2012).



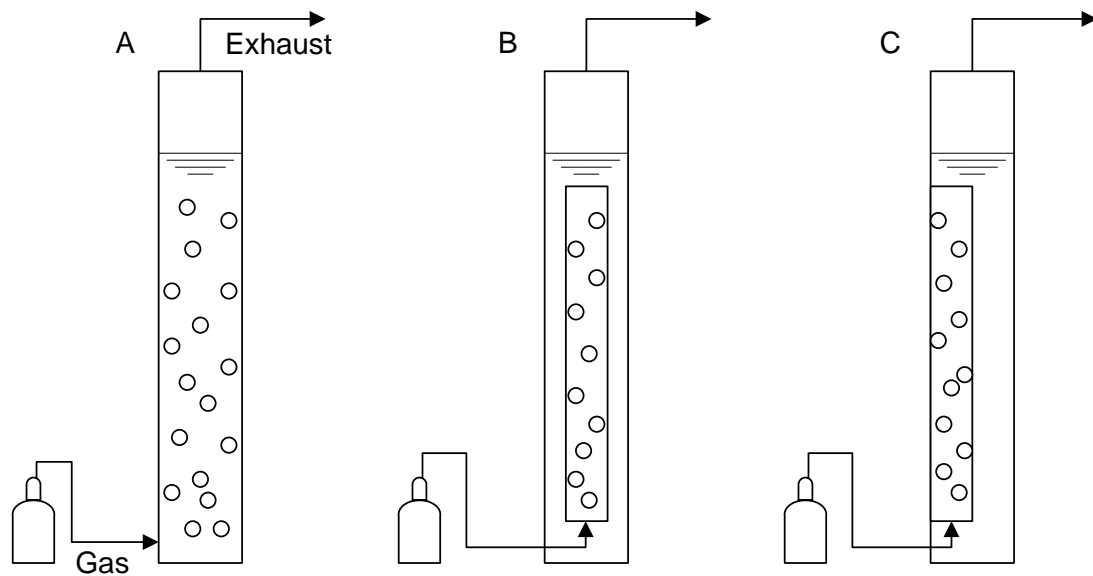


Figure 2.4: Diagram of A) bubble column reactor, B) internal loop airlift reactor, and C) split airlift reactor, redrawn from Wang, Lan and Horsman, 2012

### 2.3.2.2. Flat panel PBRs

Flat panel PBRs normally have a cuboidal shape that is designed to obtain a minimal light path to ensure complete penetration. Transparent materials are used, including glass, Plexiglas and polycarbonate. Similar to vertical tubular PBRs, flat panel PBRs have a high surface area to volume ratio and agitation is provided either through gas bubbling from the bottom of the reactor or through a mechanical motor (Singh and Sharma, 2012).

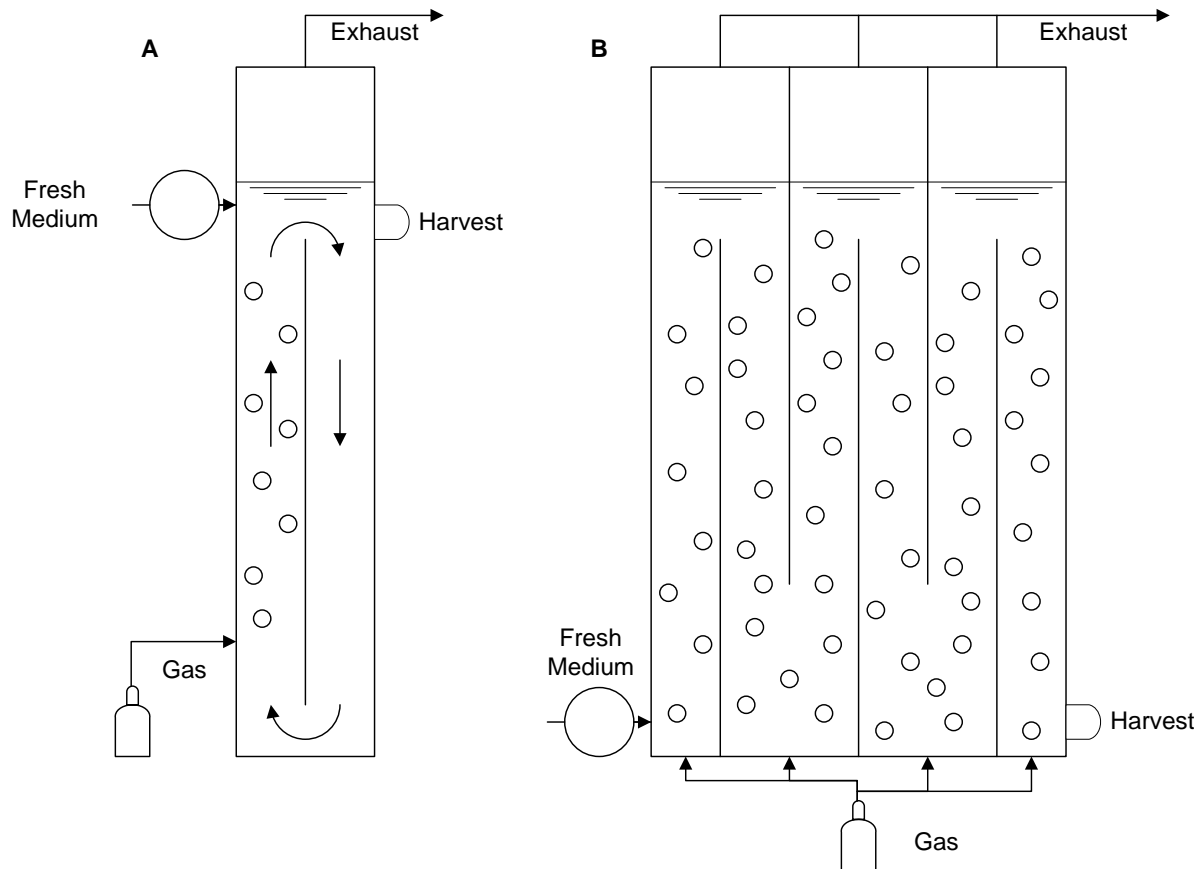


Figure 2.5: Diagram of: A) an airlift flat panel PBR; and B) a pump driven flat panel PBR, redrawn from Wang, Lan and Horsman, 2012

(Tredici et al., 1991) constructed a vertical alveolar panel (VAP) reactor that had a surface area of  $80 \text{ m}^2/\text{m}^3$  with surface areas ranging from  $0.5$  to  $2.2 \text{ m}^2$  using bubbling gas for agitation and  $\text{O}_2$  removal. The Plexiglas sheets used to build the reactor had a transparency of 95 %. However, two problems arose: firstly, the temperature of the algae mixture had to be regulated as heat generation occurred rapidly, and secondly the gas bubbles filled approximately 2 to 4 % of the reactor. Both problems arose due to the high surface area to volume ratio (Tredici et al., 1991).

### 2.3.2.3. Scaling up

There is no reliable scale up method for photobioreactors. Models could not predict the condition and performance of larger reactors particularly if the diameter of the tube was changed substantially. Other than using multiples of identical tubular

modules, the volume could only be enlarged by increasing the length of the tube and/or the internal diameter of the tube. By changing the length or the diameter of the tube, the mass transfer and the light regime are changed and the product is thus not a scale up of the original model. Increasing the length of the tube and using a constant tube diameter would cause a change in pH at the exit of the tube due to a change in the concentrations of O<sub>2</sub> and CO<sub>2</sub>. A scaled-up reactor should produce the same exit composition (oxygen, carbon dioxide and biomass contents) as a smaller reactor (Molina Grima et al., 1999) and therefore it is difficult to achieve a scale up of a reactor.

The residence time of a longer tube should be the same as that of a shorter tube when using a constant diameter. In order to obtain the same residence time, the flow velocity has to be increased by a factor of the length between the two (Molina Grima et al., 1999). The fact that higher flow velocities could have an increase in pressure to obtain the desired velocity should be included in the calculation. The increase in velocity and pressure would also cause the algal culture to experience an increase in drag which is not taken into account when increasing the flow rate by the ratios of the longer to shorter tubes. These factors could easily cause damage to the algae cells, providing a lower product grade or the loss of a significant amount of the product.

No detailed study has been conducted to determine the effect that design variables have on a large scale PBR. Tubular PBR performance is mainly dependant on the light profile internally, which is in turn also dependent on the location, the reactor configuration (including tube arrangement, distances and diameter), the biomass concentration and the characteristics of the algae growth (Slegers et al., 2013).

Table 2.4: Summary of culture systems' advantages and disadvantages

<b>Culture Systems</b>	<b>Advantage</b>	<b>Disadvantage</b>
Open ponds	<ul style="list-style-type: none"> <li>• Economical</li> <li>• Easy to clean</li> <li>• Low energy</li> <li>• Low maintenance</li> <li>• Good for large-scale production</li> </ul>	<ul style="list-style-type: none"> <li>• Little culture control</li> <li>• Low productivity</li> <li>• Easy contaminated</li> <li>• Limited strain potential</li> <li>• Occupy large area</li> <li>• Poor mixing</li> </ul>
Column photobioreactor	<ul style="list-style-type: none"> <li>• High mass transfer</li> <li>• Good mixing</li> <li>• Easy to sterilise</li> <li>• Compact</li> <li>• Potential for scalability</li> <li>• Reduce photo inhibition and photo-oxidation</li> <li>• Low energy consumption</li> </ul>	<ul style="list-style-type: none"> <li>• Small illuminated area</li> <li>• Expensive compared to open ponds</li> <li>• Sophisticated materials</li> </ul>
Tubular photobioreactor	<ul style="list-style-type: none"> <li>• Large illuminated areas</li> <li>• Suitable for outdoors</li> <li>• Good productivity</li> <li>• Relatively cheap</li> <li>• Reasonable scalable</li> <li>• Easy species control</li> <li>• Uniform mixing</li> <li>• Good temperature control</li> </ul>	<ul style="list-style-type: none"> <li>• pH gradient</li> <li>• Fouling</li> <li>• Wall growth</li> <li>• Requires large area</li> <li>• Hydrodynamic stress</li> <li>• Possible low gas transfer</li> </ul>
Flat plate photobioreactor	<ul style="list-style-type: none"> <li>• Large illuminated areas</li> <li>• Suitable for outdoor</li> <li>• Good light path</li> <li>• Good productivity</li> <li>• Relatively cheap</li> <li>• Easy to clean</li> <li>• Low oxygen build up</li> </ul>	<ul style="list-style-type: none"> <li>• Scale up difficult</li> <li>• Temperature control difficult</li> <li>• Wall growth</li> <li>• Possible hydrodynamic stress</li> </ul>

Source: Borowitzka, 1999; Ugwu, Aoyagi and Uchiyama, 2008; Brennan and Owende, 2010

#### 2.3.2.4. Consideration for designing PBRs

It was suggested by Tsoglin et al. (1996) that the following points should be considered when designing a PBR:

- The cultivation of various algae species should be permitted by the reactor.
- Uniform illuminations and fast mass transfer of CO<sub>2</sub> and O<sub>2</sub> must be provided.
- The design should prevent or minimise the fouling of the PBR, especially the light-transmitting surface because microalgae are highly adhesive and could cause frequent shutdowns of the reactor.
- Mass should be transferable at high rates without damaging the cell or suppressing the growth in the reactor.
- High rates of mass transfer often cause intensive foaming – a condition in which the reactor must be able to work.
- The PBR should have no or minimal non-illuminated areas.

Developing a bioreactor requires a detailed knowledge of its key aspects, including light distribution, mass transfer, stresses, the ability to scale up and the biological requirements of algae. No single bioreactor has all the properties required for a complete bioreactor, but combining reactor designs to form a hybrid reactor could combine the strengths of several reactor types to produce a suitable bioreactor for the production of algae biomass (Singh and Sharma, 2012).

### 2.4. Algae

Algae are some of the most basic photosynthetic organisms that exist on Earth, and microalgae are unicellular organisms. Algae live in saline or fresh water and require energy (mainly in the form of sunlight), water and carbon dioxide as their main conditions for survival (Demirbas, 2010).

Each species of alga produces a different ratio of oils, carbohydrates and proteins (Haag, 2007). The composition of the oil content that is produced is directly affected by the temperature at which the algal biomass is grown (Naoki et al., 1979). Table 2.5 shows the different amounts of oil that are found in different microalgae species. This shows how important the environment and species type are when considering the desired final product. In higher order plants, structural carbohydrates

predominate in the biomass, while in microalgae the major components are proteins (Ginzburg, 1993).

Microalgae are aquatic organisms that form part of a large and diversified group. Due to their aquatic nature, they lack the complex structures normally seen in higher order plants. Depending on the species of microalgae, useful quantities of polysaccharides, triacylglycerides and proteins can be produced (Slade and Bauen, 2013).

Table 2.5: Oil content of microalgae species

<b>Species</b>	<b>% dry weight</b>
<i>Botryococcus braunii</i>	25-75
<i>Chlorella sp.</i>	28-32
<i>Cryptocodinium cohnii</i>	20
<i>Cylindrotheca sp.</i>	16-37
<i>Dunaliella primolecta</i>	23
<i>Isochrysis sp.</i>	25-33
<i>Monallanthus salina N</i>	20
<i>Nannochloris sp.</i>	20-35
<i>Nannochloropsis sp.</i>	31-68
<i>Neochloris oleoabundans</i>	35-54
<i>Nitzschia sp.</i>	45-47
<i>Phaeodactylum tricornutum</i>	20-30
<i>Schizochytrium sp.</i>	50-77
<i>Tetraselmis sueica</i>	15-23

Source: Chisti, 2007

While the productivity of these organisms makes them look attractive, the complexity of the systems required for their cultivation are an obstacle, reflecting the research cost, the capital cost for their implementation and the recurring cost for their operation (Terry and Raymond, 1985). It is expected that algae biomass production could reach 40 to 80 tons of dry matter production per hectare per year using closed systems (Slegers et al., 2013).

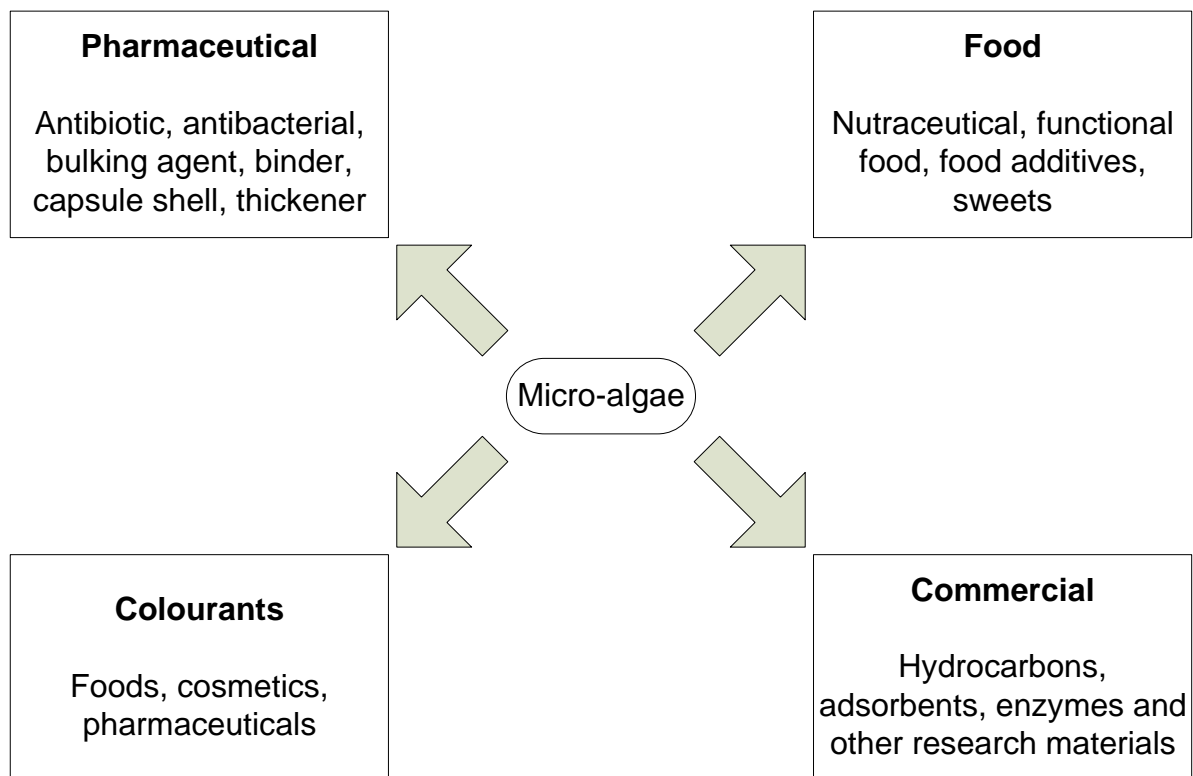


Figure 2.6: Possible uses for microalgae biomass, redrawn from Dufossé et al., 2005

In Figure 2.6 some of the potential uses of algae biomass can be seen. Algae biomass can be used in such a large array of products that it increases the desire to use algae as a replacement for fossil fuels and synthetic products. As will be seen later in the review, several factors influence the final yield as well as the cost of production.

#### 2.4.1. Growth rate and composition

Algae are very sensitive and can be killed by too much sunlight. The optimum growth environment is where temperatures are kept constant and oxygen is removed continuously. In addition, algae cells can be ruptured by carbon dioxide bubbles that are present when additional carbon dioxide is added to the culture (Haag, 2007). Macroalgae contain a lower concentration of lipids and produce less biomass than microalgae, making microalgae a more desirable option for large-scale systems (Hossain et al., 2008).

By controlling the environment in which the algae biomass is grown, the biochemical composition can be manipulated beneficially to produce higher quantities of the desired products (Mirón et al., 2003). By reducing the growth temperature, the production of higher-order unsaturated fatty acids increases, while lower-order unsaturated fatty acids decrease. A thin-layer chromatography (TLC) analysis of the lipids indicated that monogalactosyldiglyceride, degalactosyldiglyceride, sulfoquinovosyldiglyceride and phosphatidylglycerol make up the major lipid components of blue-green algae. Monogalactosyldiglyceride accounts for approximately half of the composition of the lipids. It was noted that unsaturation as well as chain length were affected by the growth temperatures of the culture. At higher growth temperatures, the C18 acids increased while the C14 acids decreased (Naoki et al., 1979).

The lipid gas chromatography (GC) profile of *P. tricornutum* showed that the algae contains over 20 different fatty acids. Four of these fatty acids were at a level of above 8 %. Eicosapentaenoic acid (EPA, 20:5n3) contributed 27 – 30 %; palmitic acid (16:0) contributed 16.9 %; palmoleic acid (16:1n7) contributed 14.0 %; and myristic acid (14:0) contributed 9.4 % to the total fatty acid content (Mirón et al., 2003).



Table 2.6: Change of lipid concentrations at different temperatures

Fatty acid	Molar percentage											
	Monogalactosyl-diglyceride			Digalactosyl-diglyceride			Sulfoquinovosyl-diglyceride			Phosphatidyl-glycerol		
	38°C	28°C	22°C	38°C	28°C	22°C	38°C	28°C	22°C	38°C	28°C	22°C
16:0	23.1	24.4	51.5	19.8	21.1	18.7	52.2	54.8	51.1	52.3	51.7	50.0
16:1	27.4	27.1	28.5	32.4	27.4	29.3	3.8	2.7	7.8	2.3	1.9	3.9
16:2	0.6	1.6	3.8	1.0	2.6	5.86	0.0	0.0	0.5	0.0	0.0	0.0
18:0	0.1	0.0	0.0	0.2	0.0	0.3	4.4	2.2	0.9	0.4	0.0	0.2
18:1	23.5	10.2	6.7	18.6	6.1	3.1	27.1	19.2	13.2	30.7	16.2	9.2
18:2	24.9	20.1	15.3	26.8	19.1	12.7	12.6	13.4	12.5	14.2	19.1	16.9
18:3	0.6	16.5	24.3	1.1	23.7	30.3	0.0	7.9	14.0	0.0	11.1	19.8
Average double bonds per lipid	2.07	2.71	2.92	2.20	2.96	3.20	1.12	1.44	1.78	1.23	1.79	2.12
C <sub>16</sub>	51.0	53.1	53.8	53.3	51.1	53.7	56.0	57.5	59.4	54.7	53.6	53.9
C <sub>18</sub>	49.0	46.9	46.2	46.7	48.9	46.3	44.0	42.5	40.6	45.3	46.4	46.1

Source: Naoki et al., 1979

Using a chemostat with artificial light under laboratory conditions, a growth rate was obtained in which the doubling time of the biomass was less than two hours. Assuming a 14-hour growth day, a 100-fold increase in biomass content may be theoretically possible (Ginzburg, 1993).

An important consideration is the thermodynamics, where hydrocarbons have a heat of formation of 11 k.cal/g, while carbohydrates have a heat of formation of 4 k.cal/g. The conversion of lipids into hydrocarbons is relatively difficult and high quantities of lipids are only found in very old algae, making it undesirable from an economical point of view (Ginzburg, 1993).

Microalgae grow extremely rapidly compared to other energy crops and many algae have higher energy potential due to a high oil content as seen in Table 2.5. Microalgae commonly double their biomass content within a 24-hour period. During an exponential growth phase, it has been found that a doubling time of around 3.5 hours is a common occurrence (Chisti, 2007). Blue-green algae have a proportional

relationship between their exhibited growth rate and the effective light duration available to the growing culture (Foy, Gibson and Smith, 1976). The fast doubling times in combination with the high oil content of some of the algae species seen in Table 2.5 make algae biomass a more viable renewable fuel source than other energy crops that only produce once a year and have little to no impact on food production.

Continuous production methods showed a considerable increase in production over semi-continuous and batch production methods, including a more constant nutrient yield (Terigar and Theegala, 2014; James and Abu-Rezeq, 1989). The specific growth rates and productivity of outdoor cultures are directly dependent on the nutrient addition rate, incident light and dilution in the reactor. Little information is available on the effect that dilution rates have on the productivity of microalgae in a continuous outdoor growth reactor. A study indicates that cultures close to their physiological cell density limit diverted the incident light more toward the production of lipids (Terigar and Theegala, 2014).

The composition of the algae is mainly affected by the direct environment of the culture which includes temperature, pH, the irradiance history and the nitrogen present in the culture. Nitrogen and sulfur are mainly found in the proteins of the algae cells. During the night, the portion of protein in the cells increases because some of the carbohydrates are consumed and new proteins are synthesised (Mirón et al., 2003). The increase in protein could be due to the lack of energy during the night as the synthesis of carbohydrates and the splitting of cells cease, but the production of proteins continues during the night.

*Neochloris oleoabundans* is an alga that combines a high specific growth rate at optimal growth conditions with an accumulation of lipids and a large content of saturated fatty acids during nitrogen starvation conditions (Sousa et al., 2013).

Nitrogen fixation does occur in certain blue-green algae species. Observations have shown a  $N_2$  growth rate of approximately 75 % of that seen on nitrates at both light-limiting and light-saturating intensities (Kratz and Myers, 1955). The nitrogen-fixing species could have the ability to produce bio-hydrogen. This will be discussed later.

## **2.4.2. Growth phases**

The algae undergo different growth phases because there are changes in the biomass composition or in the environment to which the algae are exposed either internally or externally.

### **2.4.2.1. Lag phase**

The lag phase is observed when non-viable cells are present in the inocula and so little growth is seen in the initial stages. The lag phase is most commonly observed when the cells are introduced to a new environment and have to first undergo physiological changes to adapt to the specific environment. The lag phase could be eliminated by using cells that are already in the exponential phase for the specific environment (Lee and Shen, 2004).

### **2.4.2.2. Exponential phase**

The exponential phase is usually observed after the lag phase. The cells are fully adapted to their new environment and start to grow at such a high rate that they create an exponential growth curve as a function of time. For the algae to remain in the exponential phase, it is vital to ensure that the medium and light are at saturated levels (Lee and Shen, 2004).

### **2.4.2.3. Linear growth phase**

The linear growth phase usually happens when the cell density reaches a point where all the light photons are absorbed by the culture. This would cause the culture to be in a linear growth rate up to the point where another factor becomes the limiting factor. A linear growth rate would usually indicate a maximum concentration of biomass for the setup of a specific reactor. By reducing the density of the culture to a point where an excess of light photons is available to the culture, the exponential phase would resume (Lee and Shen, 2004).

### **2.4.2.4. Death phase**

The death phase can be seen as a much more severe form of the linear growth phase. The reason for this is the loss of algae cells and this is usually caused by a complete deficiency of any of the factors. The most common deficiencies are very

limited light or a deficiency of the minerals that are required for growth. The death phase is mainly seen in batch cultures as a result of a mineral deficiency because no harvesting occurs and therefore no fresh medium is added to the culture.

## **2.5. Nutrient requirements:**

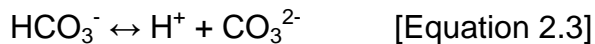
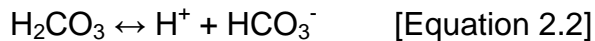
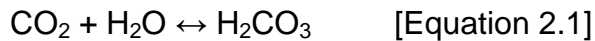
The average elemental composition of freshwater algae is  $\text{CH}_{1.7}\text{O}_{0.4}\text{N}_{0.15}\text{P}_{0.0094}$ , with the N content being particularly sensitive to its environment. To achieve the best growth rate, these nutrients have to be supplied in an optimum quantity in order not to limit the growth rate in any way. If saline water is used, the addition of other salts may be required for maximum algal growth (Borowitzka and Moheimani, 2013). Grobbelaar (2004) suggests that a minimum requirement for balanced nutrition of  $\text{CH}_{1.83}\text{O}_{0.48}\text{N}_{0.11}\text{P}_{0.01}$  is necessary for the production of microalgae.

### **2.5.1. Carbon dioxide**

Algae are photosynthetic organisms that fix carbon from a main source of carbon dioxide to produce algal biomass (Demirbas, 2010). Because carbon dioxide is a requirement for the growth of algae biomass, it could potentially be used to reduce excess carbon dioxide in the environment (Gavrilescu and Chisti, 2005). The ability of microalgae to remove excess carbon dioxide from the environment could potentially allow for the habitation of other planets that are currently too hostile for most life forms (Gòdia et al., 2002) or could reduce and manage the increase of carbon dioxide levels on Earth in an attempt to decrease global warming and its effects.

Algal biomass contains approximately 50 % carbon which is mainly contained in the carbohydrates and lipids. Carbon is derived from the fixation of carbon dioxide through the process of photosynthesis (Chisti, 2007; Mirón et al., 2003). Mirón et al. (2003) calculate that the fixation rate was 2.5 mg carbon per litre per hour in their system. Although this seems low, at a commercial level the fixation could allow power plants to become carbon neutral. It can be calculated that it would require approximately 183 tons of carbon dioxide to produce 100 tons of algae biomass (Borowitzka and Moheimani, 2013; Chisti, 2007). Carbon dioxide has to be fed continuously during growth periods. The addition of carbon dioxide requires the monitoring of the pH levels of the broth (Chisti, 2007). The addition of carbon

dioxide to the broth would form carbonic acid ( $\text{H}_2\text{CO}_3$ ) because it is in equilibrium. The  $\text{CO}_2$  levels in the broth can be monitored and controlled relatively easily as the carbonic acid equilibrium changes the pH of the algae broth, depending on which side of the equilibrium has the higher concentration.



Light transfer is the most important factor for syntheses to occur and for the removal of the  $\text{O}_2$  produced during photosynthesis, followed by the feeding of  $\text{CO}_2$  to the algae culture. The requirement of  $\text{CO}_2$  can be calculated from the total carbon content in the biomass, using a stoichiometric basis. The carbon fraction varies between species and normally ranges between 0.45 for high carbohydrate algae and 0.8 for algae with high oil contents. This gives a minimum requirement of 1.85 g of  $\text{CO}_2$  per gram of biomass that should be fed continuously. This is similar to the previously stated 1.83g  $\text{CO}_2$  per gram biomass, with the addition of pure  $\text{CO}_2$  when required (Posten, 2009).

The addition of  $\text{NaHCO}_3$  as an inorganic carbon source showed an increased productivity of *Chlorella vulgaris*, with an optimal level of 1g/litre. The addition of  $\text{NaHCO}_3$  could have a double effect because it could serve as both a carbon source and act as a buffer in the system, limiting the pH fluctuations and allowing for more continuous growth (Yeh, Chang and chen, 2010).

#### **2.5.1.1. Optimal concentration of carbon dioxide**

The growth rate of *C. vulgaris* was shown to be directly affected by the concentration of  $\text{CO}_2$ . Achieving the best growth through the use of a 5 % (v/v)  $\text{CO}_2$  to air mixture, with an enhancement in growth found in 15 % (v/v)  $\text{CO}_2$  adaption over air. The addition of  $\text{CO}_2$ -enriched air did cause a sharp initial drop in the pH of the mixture compared to when normal air is used (Yun et al., 1997).

Table 2.7: Growth rates at different CO<sub>2</sub> concentrations using different culture densities.

	Biomass (dry weight, g/l)	Growth rate (µg/d)
Low-density (8x10 <sup>5</sup> cells/ml)		
Air	0.537 ± 0.016	0.230
2%	1.211 ± 0.031	0.492
5%	0.062 ± 0.027	0.127
10%	0.010 ± 0.003	-
15%	0.009 ± 0.001	-
High-density (8x10 <sup>6</sup> cells/ml)		
Air	0.682 ± 0.007	0.248
2%	1.445 ± 0.015	0.605
5%	0.899 ± 0.003	0.343
10%	0.106 ± 0.001	-
15%	0.099 ± 0.001	-

Source: Chiu et al., 2008

In Table 2.7, Chiu et al. (2008) find that using a 2 % (v/v) for the growth of *Chlorella* sp. obtained the best results. The use of 10 and 15 % (v/v) inhibited the growth after four days. The process was repeated by first incubating *Chlorella* sp., previously grown at 2 % (v/v), with the respective CO<sub>2</sub> concentrations. This showed that the environmental stress of higher CO<sub>2</sub> could be overcome. The initial results also show that the cell density is in relationship with the CO<sub>2</sub> tolerance (Chiu et al., 2008).

Table 2.8: Maximum temperature and CO<sub>2</sub> concentration of several algae species

Algae specie	Maximum temperature (°C)	Maximum CO <sub>2</sub> % (v/v)
<i>Cyanidium caldarium</i>	60	100
<i>Scenedesmus</i> sp.	30	80
<i>Chlorococcum littorale</i>	-	70
<i>Synechococcus elongates</i>	60	60
<i>Euglena gracilis</i>	-	45
<i>Chlorella</i> sp.	45	40
<i>Chlorella</i> sp. HA-1	-	15
<i>Chlorella</i> sp. T-1	35	-
<i>Endorina</i> sp.	30	20
<i>Sunaliella tertiolecta</i>	-	15
<i>Chlamydomonas</i> sp. MGA161	35	15
<i>Nannochloris</i> sp.	25	15
<i>Tetraselmis</i> sp.	-	14
<i>Monoraphidium minutum</i>	25	13.6
<i>Spirulina</i> sp.	-	12

Source: Kumar et al., 2011

In Table 2.8, the maximum CO<sub>2</sub> concentration of several algae species can be seen. This information should therefore be taken into account when deciding which algae should be used because, as can be seen in the table, different strains of the *Chlorella* sp. have significantly different CO<sub>2</sub> tolerances.

#### 2.5.1.2. Volume of air supplied

Chiu et al. (2008) and Chiu et al. (2009) used a flow rate of the previously mentioned CO<sub>2</sub>/air mixture at a rate of 0.25 vvm and used a vertical tubular photobioreactor for growing *Chlorella* sp. and *Nannochloropsis oculata*. Figure 2.7 shows the setup used to control the flow rate of the gas into the PBR as well as to control the mixture of gas that enters the reactor.

Using a similar system as seen in Figure 2.7, Morris et al. (2010) state that pure CO<sub>2</sub> gas was added for 2 to 5 minutes at a rate of 20 litres per minute, but they do not state whether it was for a single tubular reactor or for the entire module of 45 tubular

reactors. The duration of the CO<sub>2</sub> addition is controlled by the pH of the growth medium to ensure optimal levels for the particular algae species. The rate at which air is bubbled through is not stipulated. If the flow rate of CO<sub>2</sub> is used to calculate the flow rate in a vvm, the flow rate will be 0.008 vvm or 0.36 vvm for the module or for the single tube reactor respectively (Morris et al., 2010).

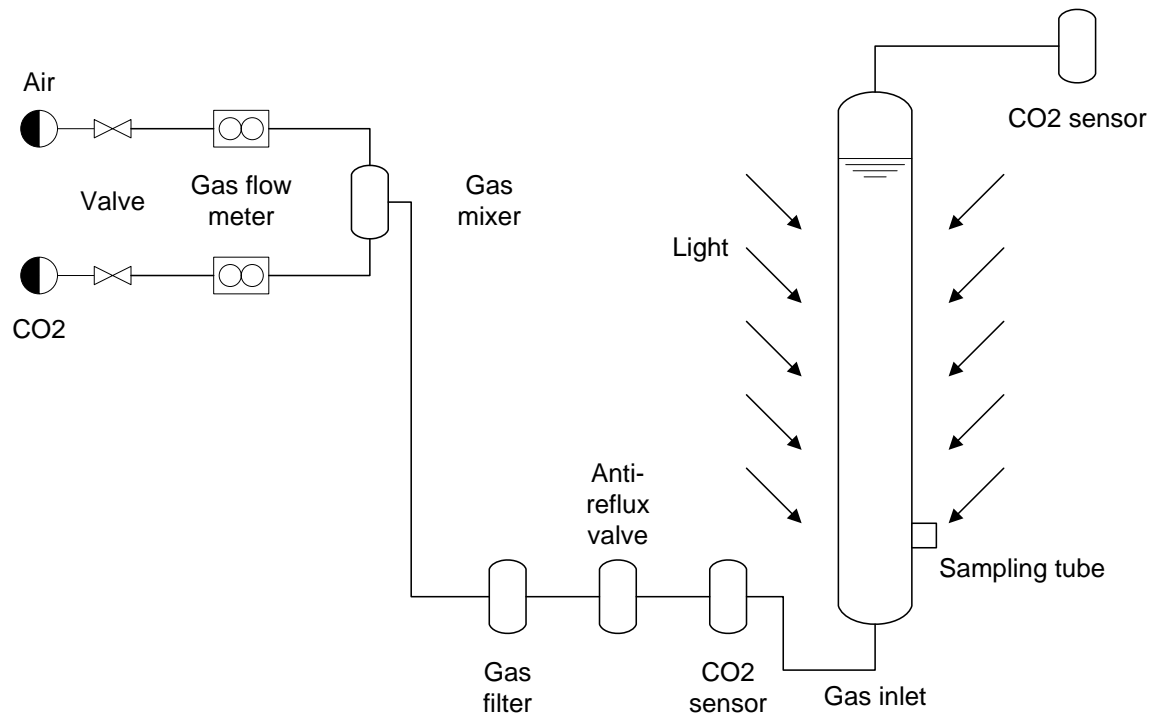


Figure 2.7: Schematic of the setup to control CO<sub>2</sub>/air mixture and flow speed of the gas, redrawn from Chiu et al., 2008

Zhang, Miyachi and Kurano (2001) used a flow rate of 0.05 vvm on a flat panel PBR at a CO<sub>2</sub> concentration of 10 % (v/v) while working with *Synechocystis aquatilis*. Similarly a flow rate of 0.5 vvm was used for the production of 14 species, using air and 5 % (v/v) CO<sub>2</sub> although the type of reactor is not mentioned.

*Anabaena* sp. were grown using a bubble column PBR with air bubbling through it continuously at a rate of 0.2 vvm (González López et al., 2009). Little work has been done on the optimal flow rate of gas in a reactor. This is due to the different needs of different reactors. The flow rate is complicated because the gas that enters the PBR could have different concentrations of CO<sub>2</sub> and this might lead to a lower flow rate.



As is reported in the literature, the flow rates that were used were often applied to several species to eliminate the specific variable, although the requirements of the species were not taken into account and therefore some of the cells could have been damaged – and this would reduce the specific production. Consequently a PBR would be required to have a variable flow rate to ensure the optimal conditions for production if different species were grown in a reactor at different times.

### **2.5.2. Water**

Currently the water requirements of other biofuel sources are between 500 and 4000 litres, which is enough to produce feedstock for one litre of bioethanol. Typically, sugar cane and corn refineries require 2 – 10 litres of water to process the feedstock into one litre of bioethanol (Dominguez-Faus et al., 2009). Water requirements for biomass feedstock crops will depend on the type of crop, the location where the crop is grown at and how the crops are managed (Gopalakrishnan et al., 2009). These limitations of other biomass crops, give algae a solid foundation as they can be produced using waste or saline water and non-agricultural lands, allowing for food security (Singh and Olsen, 2011).

The production of energy sources requires water while the production of bioenergy sources requires large amounts of water because the biomass has to be produced first before it is converted into the desired biofuel. Since limited fresh water is available in the areas where the production of algae using sunlight is at its highest, the use of saline water is required. Due to the limitation of fresh water, the use of fresh water algae for biofuel production is highly unlikely. This shifts the focus to algae that can be produced using saline water (Borowitzka and Moheimani, 2013).

Open pond systems that are situated in regions with high solar irradiation can experience a rate of evaporation of up to 1.5 m. Replacing evaporated water with water from a saline water source would be more beneficial than from a fresh water source due to the high volume of evaporation in a large-scale plant. If saline water were used to replace the evaporated water in the pond, the saline levels of the pond water would increase over time which could cause production problems and ultimately the complete loss of production due to the death of the algae. The range of salinity tolerance in many marine algae species is very narrow and would require regular, partial or complete discharge of the pond water. The discharge creates a

new problem because the pond water still contains nutrients and thus creates additional costs and problems that have to be overcome for the system to operate sustainably (Borowitzka and Moheimani, 2013) especially when regular discharges are required. The use of saline water could also affect the nutrient requirements for the production of the algae biomass as some of the components in the saline water could inhibit the uptake of nutrients due to the complexation of the specific nutrient which would require an excess of the nutrient in order to obtain maximum growth.

The use of fresh water in closed photobioreactors is possible because evaporated water can potentially be recovered. Recycling in closed systems would still require the disposal of water because the build-up of undesirable compounds including salts may affect the productivity and safety of the biomass, especially if it is used as a source in the production of human and animal foods. Because discarded water may still contain nutrients, the disposal could potentially have a large effect on the environment (Borowitzka and Moheimani, 2013).

If the target of EISA2022 is to be met for the production of 136 million m<sup>3</sup> of biofuel, the water required for the entire production process will be between 91 and 420 million m<sup>3</sup> of water. If fresh water are used, these values will be in the range of 0.7 to 3 times the current usage for grain farming in the US. The highest water requirement is found for the production of bioelectricity, while the lowest is for aquaculture production. The study by Batan, Quinn and Bradley (2013) shows that the water requirement for the production of algae-based biofuel is less than for oil-seed biofuels, similar to most bioethanol production from biomass and higher than for petroleum-based fuels. The water requirements for algae biofuels are directly dependant on the usage of the biofuel (Batan, Quinn and Bradley, 2013).

### **2.5.3. Phosphates**

Similar to oil, phosphorus is a non-renewable resource, which is currently extracted at such rates that the global phosphate reserves will be depleted in as little as 50 – 100 years. Algae production would therefore be in direct competition with the phosphorous fertilisers that are required for food production. This increases the importance of using phosphorous efficiently in algae production by recycling the nutrients in algae production systems wherever possible (Borowitzka and

Moheimani, 2013) and possibly using algae to recover phosphorous from run-off water.

Like all living organisms, algae require phosphorous for growth and development. Inorganic phosphate is the preferred method for the addition of phosphorus to algae culture, although many algae can utilise polyphosphates and organic phosphorous sources. It is possible to use phosphorous from waste water, but reliable growth is harder to achieve due to variations in waste water composition and the generally high ammonia concentrations (Borowitzka and Moheimani, 2013).

Alternative sources of phosphorous could include waste water, manure or even ashes containing phosphorous from the combustion of biomass. Bone meal, which is rich in both phosphorous and calcium, has been used successfully in cultures (Borowitzka and Moheimani, 2013).

Phosphorus-starved cells take longer to start their growth cycle, as the reparation of damaged functional macromolecules has to be completed first. Phosphorus stored intracellularly can mainly be found in the form of a polyphosphate as it forms part of the main energy transporter in living cells (Qi, Wang and Wang, 2013).

Phosphorus is one of the most important nutrients as it regulates both growth and metabolism in cells. It plays a significant role in energy transport, biosynthesis, photosynthesis and respiration. Phosphorus is also a key factor for microalgae to accumulate lipids. Only a few studies have been done, although no common conclusion could be drawn since the process appears to be species dependant (Liang et al., 2013). Benning, Huang and Gage (1995) find that algae that are grown in a phosphorus-deficient environment showed a rapid decrease in membrane phospholipids and replaced the missing phospholipids with non-phosphorous acidic glycolipids and ornithine lipids. This shows how well algae adapt to their environments.

#### **2.5.4. Nitrogen**

Nitrogen in several forms can be added, such as ammonia ( $\text{NH}_3$ ), nitrates ( $\text{NO}_3$ ), urea ( $\text{CH}_4\text{N}_2\text{O}$ ) and  $\text{N}_2$  for nitrogen-fixing species. The form of nitrogen that is added has the possibility of directly affecting the cell's composition, its growth rate and the stability of the culture. The use of  $\text{NH}_3$  should be carefully managed as it can react

with other components in the culture mixture due to its alkalinity and also because of the algae's sensitivity towards  $\text{NH}_3$  (Borowitzka and Moheimani, 2013).

According to Bilanovic et al. (2009), microalgae should be grown in a concentration of between 285 and 427 g N/l, irrespective of the  $\text{CO}_2$  concentrations that are used. The high nitrogen requirement for the production of algae biomass makes the recycling and conservation of nutrients essential for the sustainable production of biomass. The cost of the production of nitrogen fertiliser is not only financial but also environmental because the production of nitrogen generates carbon dioxide and other greenhouse gases, with the production of 1 kg of nitrogen producing 2 kg of carbon dioxide. A possible alternative source of renewable nitrogen is the use of *Anabaena* which is a nitrogen-fixing algae. Cultivation of nitrogen-fixing algae could have the potential of producing fertiliser in a sustainable matter for the agricultural sector and/or algae culture for biofuel production (Borowitzka and Moheimani, 2013).

Algae cells that are nitrogen starved can completely or greatly deplete compounds such as nitrates, ammonium, amino acids and proteins (Qi, Wang and Wang, 2013). Protein forms the major intracellular nitrogen pool and thus the consumption of nitrogen directly affects the formation of proteins and could indirectly affect the formation of lipids and carbohydrates. The biochemical composition of proteins varies considerably during growth periods. In nitrogen-limited environments the protein content of algae was lower than algae growing in a non-limiting environment (Liang et al., 2013).

It was found that *C. vulgaris* has a calorific value of 18 kJ/g but it increases to 23 kJ/g in an environment that was nitrogen limiting. The limitations of nitrogen have been shown in several algae species to cause an increase in lipids. This suggests that the lipid content of the biomass could be increased, by prolonging the cultivation period after nitrogen depletion has occurred (Wang et al., 2008).

The deprivation of nitrogen diverts the flow of fixed carbon from the synthesis of protein to the synthesis of either lipids or carbohydrates. There are indications that cellular lipids accumulated during nitrogen deprivation could be derived from newly fixed carbon. It is possible for the level of carbohydrates to be up to 70 % of the dry mass without reducing the productivity of lipids, although the productivity of biomass

is often affected at the expense of other biological components, mainly proteins (Rodolfi et al., 2009).

Some algae have been found to increase their carbohydrate content while others have been found to increase their lipid content under nitrogen deprivation. In the *Chlorella* genus, it has been found that some strains accumulate large amounts of carbohydrates while others accumulated large amounts of lipids. The accumulation of secondary carotenoids is also a main characteristic in many species, combined with a reduction in chlorophyll content during nitrogen starvation (Hu, 2004).

### **2.5.5. Nutrient sources**

With the expansion of the commercial microalgae-growing industry, competition with the agricultural sector for inorganic fertilisers is expected to increase. Coupled with the fact that fossil fuel prices are likely to increase, this could mean that inorganic fertilisers may be an economically unviable source of nutrients for microalgal production systems. Approximately 50% of the required fossil energy input for the growth of algae for biofuel is linked to inorganic fertilisers (Fenton and Ó hUallacháin, 2012).

Flue gas from thermal power stations and steel-making plants has a carbon dioxide concentration that is approximately 500 times higher than that of atmospheric levels. The use of waste water from the steel-making plants requires the addition of phosphates, while allowing a calculated rate of 23 100 kg of CO<sub>2</sub> to be fixed daily and the production of 12 430 kg of algal biomass daily. The use of steel-making waste water is likely to cause the algal biomass to be contaminated by heavy metals (Yun et al., 1997). The contamination could have no or little effect on the commercial value of the algae but this would depend on the intended use of the specific algae biomass that was produced.

The nutrient content of agriculturally derived organic fertilisers, runoff and drainage water have the potential ability to facilitate the growth of algal biomass (Fenton and Ó hUallacháin, 2012). Agricultural waste water characteristically contains a large amount of both organic matter and nutrients. Wetlands have a finite ability for nutrients to be recovered and stored by aquatic plants, sedimentation, detritus, microbes and fauna. Removal of both N and P was more efficient here than in

unplanted wetlands, when using waste water from a dairy farm. Studies which included unplanted control wetlands found that planted wetlands removed more nitrogen from waste water which was rich in ammonia (Tanner, Clayton and Upsdell, 1995). The biggest potential problem when using agricultural runoff water is the inclusion of pest-control poisons that could kill the algae.

Using algae to capture the nutrients in waste water is possible and could become a viable source not only of cultivated algae but also of agricultural fertiliser. Benthic algae are produced on vertical screens in an ecological water treatment system using waste water which is harvested from fish grazing on the benthic algae. Fish faeces are then collected in the conical base of the tank. Nitrogen and phosphorous recovery is achieved by the collection of both the fish and their faeces (Wilkie and Mulbry, 2002). A 23 % reduction in N was found in municipal waste water with a reduction of 82 % in P content (Wilkie and Mulbry, 2002; Rectenwald and Drenner, 2000). In future the technology could be utilised as an alternative to current tertiary treatment for the removal of phosphorus in waste-water facilities (Rectenwald and Drenner, 2000).

### **2.5.6. Light**

The two major factors that control the productivity of photosynthesis in algae cultures are the availability and intensity of the light (Molina Grima et al., 1999; Yeh, Chang and chen, 2010). Cultures that use natural light are subjected to natural cyclic changes in irradiation with two or more distinct cycles: a daily cycle and a seasonal cycle. A third cycle should also be included. It is evident in the movement of the fluid between different illumination zones within the biophotoreactor (Molina Grima et al., 1999).

Plants and algae have several mechanisms to manage the fluctuation in both quality and intensity of the light that is available. The number of light-harvesting pigments increases in light-limiting conditions, which increase the amount of photosynthetic units in the algae cell (Grobbelaar, 2006). In general, limitations cause a reduction of the pigment concentration in the cell and increase the number of photosynthetic units and enzymes. Algae grown at high levels of light generally have lower photosynthetic units per cell, but have a higher maximal photosynthetic rate when compared to algae grown at low irradiation (Sukenic, Bennett and Falkowski, 1987).

Cells at the front surface of the reactor have a very low efficiency because they are exposed to the high intensity of sunlight while the dense culture may cause the cells at the back surface of the reactor to be in the absence of light. Theoretical studies have shown that intermittent light has an effect on the rate of photosynthesis. This means that high intensities of flashing light can be used with high efficiency by the algae cells (Phillips and Myers, 1954). By using a “two dark, one light” cycle of 10 ms each, the photosynthetic rate increases 6.7 times on average (Grobbelaar, 2009b). Productivity could be increased substantially by utilising light-dark cycles and low turbulent biomass mixtures to allow equal light availability and overcome the light penetration differences, as seen in Figure 2.8.

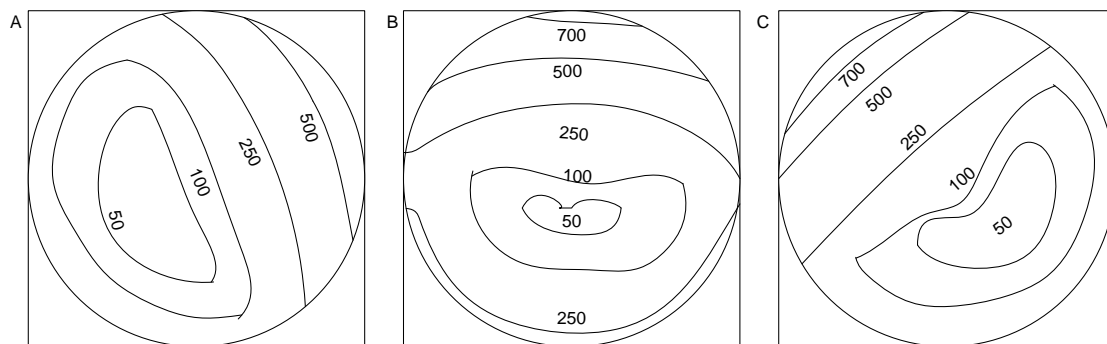


Figure 2.8: Cross section of the light profile ( $\text{W}/\text{m}^2$ ) inside a horizontal tubular reactor at A) 08:00, B) 12:30 and C) 16:00 using a 0.06m tube diameter, redrawn from Slegers et al., 2013

The use of artificial light would allow both the availability and the intensity of the light that is required for optimal growth to be controlled. A major obstacle to the use of artificial light is the cost of the electricity required. A possible compromise would be to use sunlight during the day and artificial light at night. The compromise could allow for more cost-effective growth although it could affect the composition of the algae biomass in an undesirable way due to the drastic changes in light intensities.

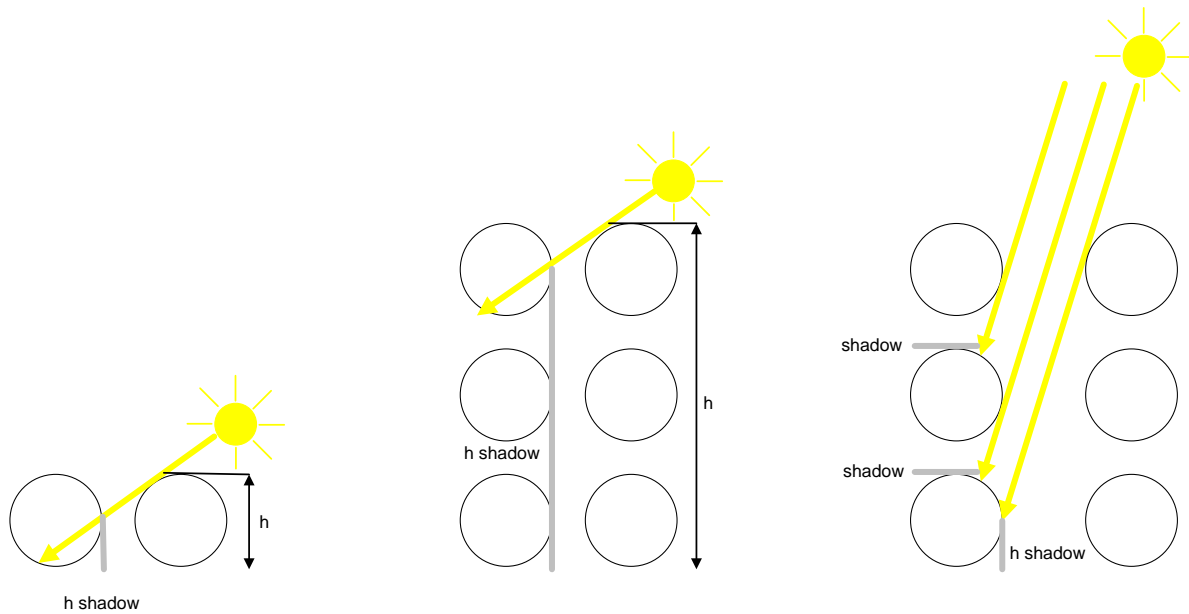


Figure 2.9: Shading effects on different PBRs designs, redrawn from Slegers et al., 2013

In Figure 2.8, the light profile inside a horizontal PBR shows that the deeper the penetration, the lower the intensity of the light. It can be seen that the middle part of the PBR only gets light of a very low intensity. The arrangement of the PBR tubes has to allow for maximum penetration. As seen in Figure 2.9, the design of the PBR could limit the light exposure of certain tubes at particular times and this would reduce the growth ability of the algae. In Figure 2.9 C, the bottom tubes would receive very little to almost no light. This would affect the light profiles, seen in Figure 2.8, negatively (Slegers et al., 2013).

There are three distinct regions on the photosynthetic vs. irradiance response curve. The first is the light-limiting region where the photosynthetic rate increases with an increase in the irradiance. The second is a region where saturation has occurred and where the photosynthetic rate is independent of the irradiation levels. The third is a photo-inhibition region where the rate of photosynthesis decreases with an increase in irradiation (Grobbelaar, 2013). Simionato et al. (2013) find that high light intensities that are well beyond the total saturation limit could be harvested, provided that a sufficiently light dark cycle is maintained.



The main factor affecting the biomass yield is the light regime that is found in the reactor. It consists of three characteristics:

1. the light intensity on the reactor's surface;
2. the duration of light exposure of cell in the light regions of the reactor; and
3. the frequency that the cell travel between the light and dark regions of the reactor (Barbosa et al., 2005).

Figure 2.10 highlights the path that light energy has to travel during photosynthesis. The figure shows that light energy is captured by the light harvesting complex (LHC) and used to split water to obtain protons. The protons allow the formation of adonine triphosphate and NADPH, which are both energy carries. The energy carriers are utilised by the Calvin cycle or Hydrogenase A enzymes to form hydrogen gas, oils, charbohydrates and other carbon containing products. The long path that the electrons have to follow can be seen; also evident is the fact that the rate of utilisation by the Calvin cycle and Hydrogenase A (HydA) is the limiting factor on the amount of light that can be harvested by chlorophyll in the cell.

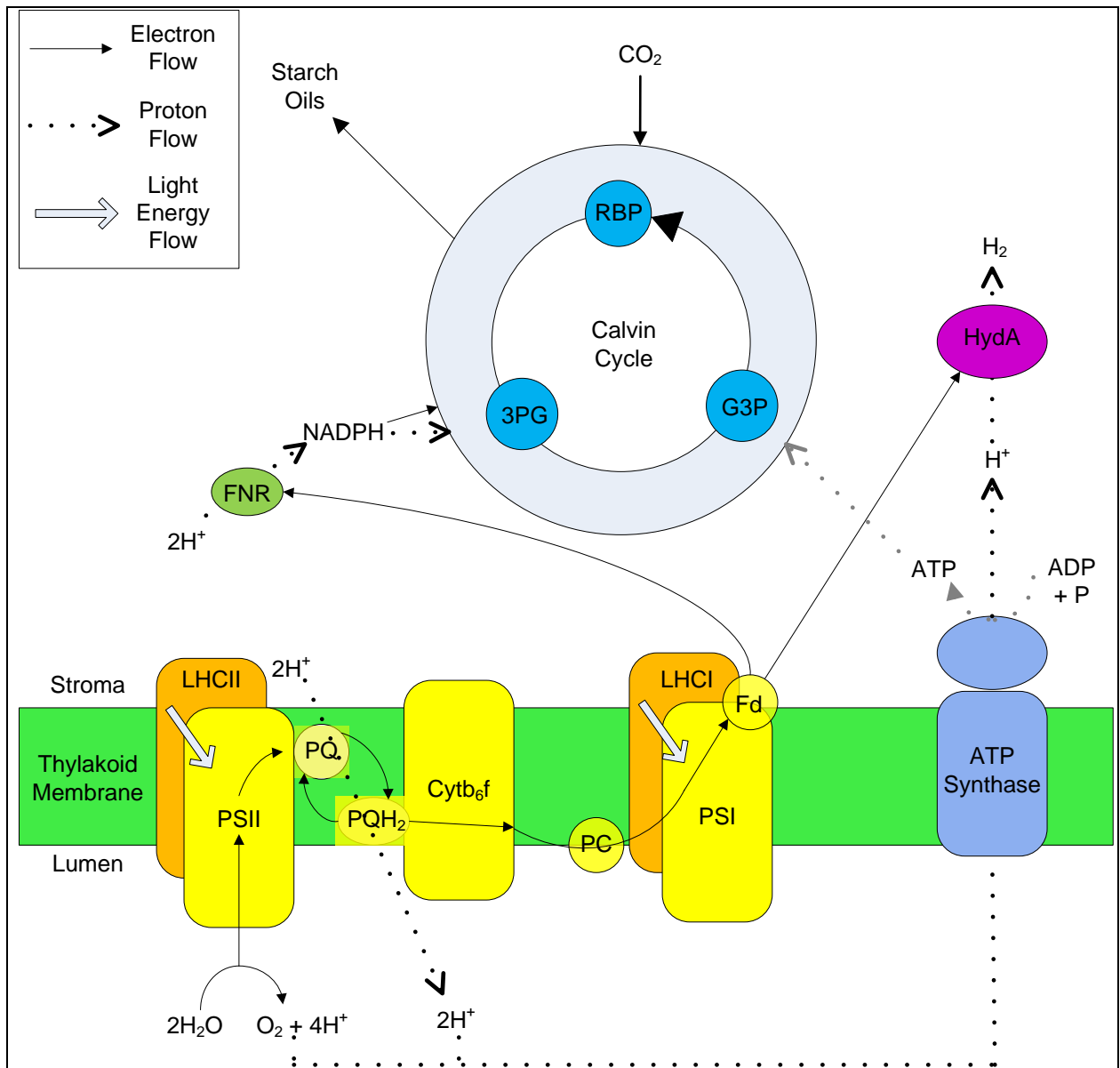


Figure 2.10: Schematic of the photosynthesis process, redrawn from Schenk et al., 2008

### 2.5.7. Growth medium

Many recipes for algae growth nutrients are available, with special formulations often being used but not reported due to their value in maintaining a commercial competitive advantage. N, P and C are commonly the limiting nutrients because an excess of these nutrients will cause stress to the cells and a decrease in the growth rate. Each type of alga has different requirements and these can be seen in Table

2.9 which shows the growth medium recipes that were designed for specific algae types or genus (Grobelaar, 2004).

Table 2.9: Media recipes commonly used for algae production. All concentration in g/l unless otherwise stated

Substrate	BG11	Modified Allen's	Bold's Basal
NaNO <sub>3</sub>	1.5	1.5	0.25
K <sub>2</sub> HPO <sub>4</sub> ·3H <sub>2</sub> O	0.04	0.039	0.075
KH <sub>2</sub> PO <sub>4</sub>			0.175
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.075	0.075	0.075
CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.036	0.025	0.084
Ca(NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O		0.02	
Na <sub>2</sub> SiO <sub>3</sub> ·9H <sub>2</sub> O		0.058	
Citric acid	0.006	0.006	
Fe-Ammonium citrate	0.006		
FeCl <sub>3</sub>		0.002	
FeSO <sub>4</sub> ·7H <sub>2</sub> O			0.005
EDTA. 2Na-Mg salt	0.001	0.001	0.05
Na <sub>2</sub> CO <sub>3</sub>	0.02	0.02	
NaCl			0.025
KOH			0.031
H <sub>3</sub> BO <sub>4</sub> (µg/l)	2.86	2.86	11.41
MnCl <sub>2</sub> ·4H <sub>2</sub> O (µg/l)	1.81	1.81	1.44
ZnSO <sub>4</sub> ·7H <sub>2</sub> O (µg/l)	0.222	0.222	8.82
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O (µg/l)	0.391	0.391	
CuSO <sub>4</sub> ·5H <sub>2</sub> O (µg/l)	0.079	0.079	1.57
Co(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O (µg/l)	0.0494	0.0494	0.49
MoO <sub>3</sub> (µg/l)			0.71
Adjust final pH	7.4	7.8	

Source: Grobelaar, 2004

The BG11 is a mixture that is used mainly for the production of green freshwater algae. Cyanobacteria are grown by using both BG11 and modified Allen's mixture, while Bold's Basal mixture targets most algae species and can be supplemented with soil extracts (Grobbelaar, 2004). As seen in Table 2.9, the makeup of the growth medium can differ considerably. These differences should be taken into account when growing the algae biomass.

## **2.6. Shortcomings**

### **2.6.1. Oxygen**

In photo-bioreactors, the oxygen that is produced during photosynthesis accumulates and induces processes like photorespiration and photo-inhibition which decreases the growth rate and yield (Sousa et al., 2013). The oxygenase activity of the Rubisco enzyme is mainly associated with photorespiration. An accumulation of oxygen in the cells would cause a change in the local  $O_2/CO_2$  ratios and inhibit the carboxylase activity of the Rubisco enzymes and increase their oxygenase activity (Sousa et al., 2012).

Photoinhibition can be defined as the suppression of the process of photosynthesis which is caused by light. It results in a reduction of the maximum carbon dioxide uptake, causing a reduction in the maximum total product (Torzillo, Bernardini and Masojídek, 1998). Photoinhibition occurs mainly at highly over-saturated intensities. These conditions generate an excess of electrons in the Photosystem II as seen in Figure 2.10, which uses energy from the light harvesting complex to split water. The excess of electrons react with the oxygen that is produced during the photosynthesis process, forming radicals and other reactive oxygen species (ROS) (Sousa et al., 2013; Sousa et al., 2012; Murata et al., 2007). Light stimulates the formation of the highly reactive singlet oxygen. Singlet oxygen causes damage to the water-oxidising centres and deactivates the electron transport chain, resulting in a loss of photosynthetic activity – this could ultimately cause cell death (Sousa et al., 2013). The formation of radicals and ROS could also cause substantial damage to the reactor over a period of time, destroying it completely or increasing the maintenance requirements drastically.

Microalgae have developed a mechanism, which is generally referred to as photoacclimation, with which to overcome the photo-oxidative damage caused by photoinhibition at high light intensities. Photoacclimation can be recognised by changes in the pigmentation. This will result in a lower chlorophyll content and a higher carotenoid content. Carotenoid content normally increases to dissipate the energy of excited chlorophyll and eliminated ROS. At very high light irradiation, the protective mechanism cannot sufficiently deal with the surplus of electrons, singlet oxygen and ROS, leading to cell damage (Sousa et al., 2013). Photoinhibition could potentially be induced for the formation of carotenoid production, in the cases where carotenoids are the desired product by creating a two part system.

## **2.7. Seperation of algal biomass**

The process of harvesting biomass usually requires several solid-liquid separation steps. The small size of an algae cell (3 - 30  $\mu\text{m}$ ) causes significant problems with the recovery process. Algae cultures are generally fairly dilute (less than  $0.5 \text{ kg/m}^3$ ), and so they require a large volume of culture to be processed for the recovery of the biomass. Recovering the biomass from the culture can contribute to between 20 and 30 % of the costs (Molina Grima et al., 2003).

Commonly deployed techniques for the recovery of microalgae biomass have proved uneconomical, although certain biomass processes do not require the separation of the growth medium and the biomass-like hydrothermal liquefaction. The principle harvesting techniques include centrifugation, filtration, flocculation, sedimentation and electrophoresis (Coward, Lee and Caldwell, 2013).

### **2.7.1. Centrifugal recovery**

It is possible to harvest most microalgae species from suspension using centrifugation (Molina Grima et al., 2003). It is the fastest and most reliable method, although an energy input of up to 3000 kWh/t is required (Coward, Lee and Caldwell, 2013, Molina Grima et al., 2003). Centrifugation is the method of choice to recover algae biomass, especially for the production of extended shelf-life concentrations (Molina Grima et al., 2003).

Biomass recovery, using a sedimenting centrifuge, is directly affected by the settling characteristic of the algae, their settling depth and the residence time of the slurry.

Proper design of the centrifuge will keep the depth to a minimum, while the residence times can be manipulated by the flow rate of the slurry. Cell viability is dependent on the species in question and the method of centrifugation that is used (Molina Grima et al., 2003).

### **2.7.2. Filtration**

Filtration is common, but is dependent on the size of the microalgae that are harvested (Coward, Lee and Caldwell, 2013). The use of membrane filtration, microfiltration and ultrafiltration are alternatives to conventional filtration methods. Microfiltration is a suitable option for the recovery of fragile cells, but large scale processes generally avoid using a membrane filtration process (Molina Grima et al., 2003).

Filtration is very abrasive; it causes the cells to rupture and reduces the quality of the cellular content. Operation costs are also high due to pumping and frequent replacement of the filters, coupled with long processing times and low recovery rates (Coward, Lee and Caldwell, 2013).

To increase the filtration ability, the filter cloth was first used to filter diatomaceous earth or cellulose to form a precoat to aid the filtration process. Settled biomass is recovered together with the precoat layer. The recovery of biomass using the precoat filtration method is an unsuitable option if the biomass product may not be contaminated with filter aid, which is the case if further processing is required or for the use of the biomass in animal feed (Molina Grima et al., 2003).

### **2.7.3. Flocculation**

Flocculation can be effectively used for the agitation of the microalgae cells, increasing their effective “particle” size. By applying various methods of flocculation, the recovery of biomass (using sedimentation, centrifugal or filtration methods) is eased significantly (Molina Grima et al., 2003).

Microalgae have a negative charge which prevents the agitation of cells because they repel each other. The negative charge can be reduced significantly or overcome by the addition of a flocculent that includes multivalent cations or cationic polymers to the culture. The factors to consider when using flocculants are that they

should ideally be inexpensive, non-toxic (especially for food or feed sources), effective in low concentrations and be selective so that downstream processes are not adversely affected (Molina Grima et al., 2003).

Multivalent metal salts are currently considered to be most effective with ferric chloride ( $\text{FeCl}_3$ ), aluminium sulfate ( $\text{Al}_2(\text{SO}_4)_3$ ) and ferric sulfate ( $\text{Fe}_2(\text{SO}_4)_3$ ) being the most commonly used. The efficiency of the electrolyte coagulation induction is measured by the concentration at which rapid coagulation occurs. This is also known as the critical coagulation concentration. Because prepolymerised metal salts have a wider pH range, they are more effective than non-polymerised metal salts. The use of prepolymerised metal salts produces flocs of the product which can be easily dewatered (Molina Grima et al., 2003). This assists in the processing of the biomass later.

Cationic polymers (polyelectrolytes) can be considered as an alternative option to the conventional metal salts. In addition to manipulating the charge of the cells, polyelectrolytes can form large chains of particles by creating bridges which connect the chains to each other. Cationic polymers can induce flocculation in fresh water algae at concentrations of between 1 – 10 mg/ml. The marine environment's high salinity can inhibit the flocculation ability of polyelectrolytes. Effective flocculation is possible at a salinity level of 5 kg/m<sup>3</sup> or less while seawater has a salinity level of approximately 37 kg/m<sup>3</sup> (Molina Grima et al., 2003).

#### **2.7.4. Purification of algal biomass**

Harvesting of algal biomass generally results in an increased concentration of between 50- to 200- fold. Freshly harvested biomass (5 – 15% solids) requires rapid processing as degradation can occur in hours, especially in hot climates. The particular post-harvesting process that is used depends on the product that is to be obtained (Molina Grima et al., 2003). The post-harvesting process can be seen in Figure 2.11 for the production of eicosapentaenoic acid (EPA) from the microalgae *P. tricornutum* as described by Molina Grima et al., (2003).

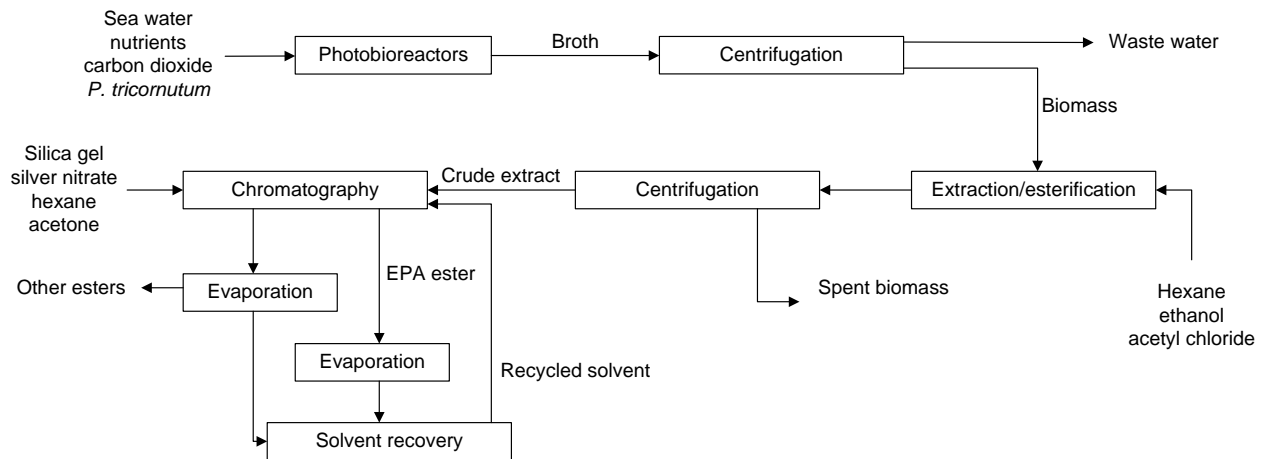


Figure 2.11: Production of EPA from the microalgae *P. tricornutum*, redrawn from Molina Grima et al., 2003

## 2.8. Bioenergy

Algae biomass can be processed in several different ways. The different processing methods have different harvesting requirements and therefore different design requirements, all of which should be taken into consideration.



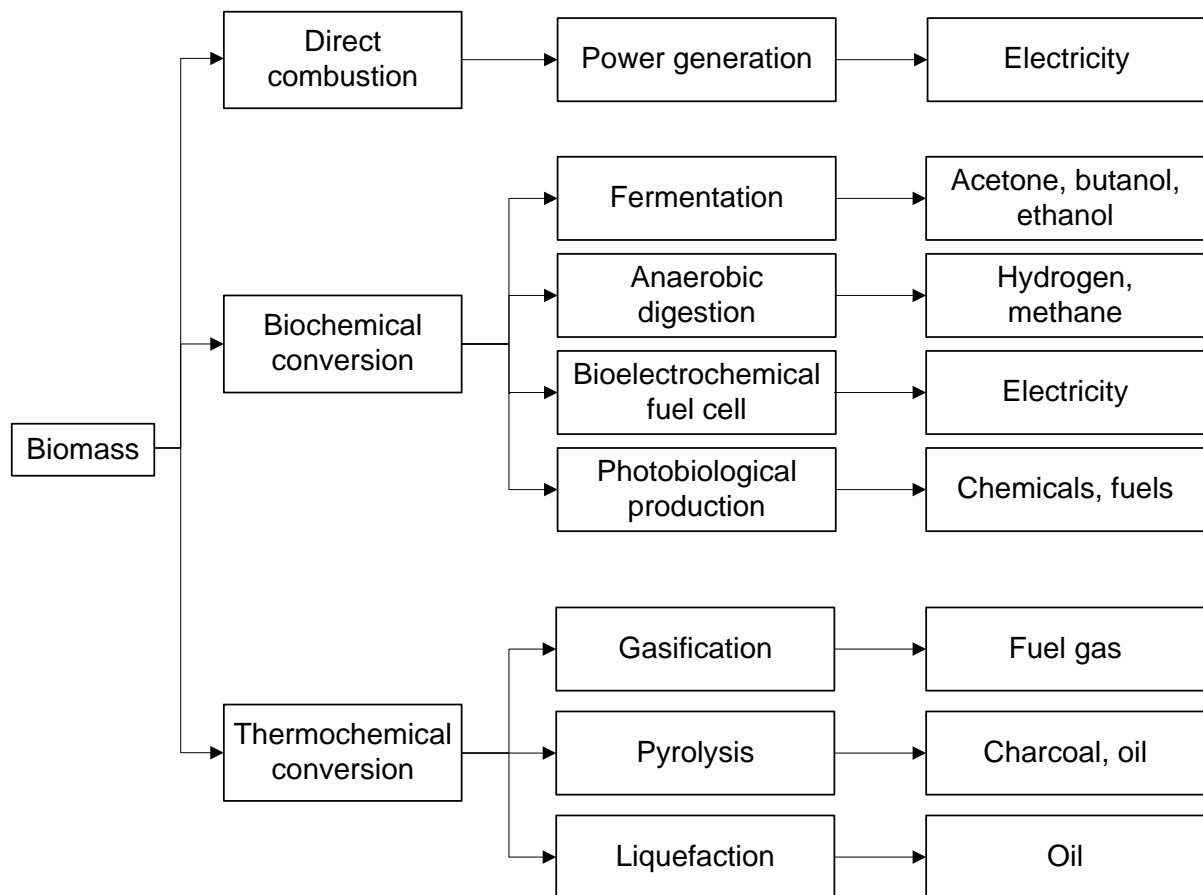


Figure 2.12: Overview of algae biomass conversion into energy sources, redrawn from Brennan and Owende, 2010; Tsukahara and Sawayama, 2005

It has been found that microalgae produce between 15 and 300 times more oil for the production of biofuel sources than energy crops. The short life cycle of algae compared to energy crops makes it much more favourable because energy crops can only be harvested a few times a year (Schenk et al., 2008).

Table 2.10: Comparison of the biodiesel production of energy crops and algae

Plant source	Biodiesel (L/ha/year)	Area required (ha x 10 <sup>6</sup> )	Area required of arable land (%)
Cotton	325	15 002	756.9
Soybean	446	10 932	551.6
Sunflower	952	5 121	258.4
Canola	1 190	4 097	206.7
Oil palm	5 950	819	41.6
Algae (10g/m <sup>2</sup> /day at 30% TAG)	12 000	406	20.5 <sup>a</sup>
Algae (50g/m <sup>2</sup> /day at 50% TAG)	98 500	49	2.5 <sup>a</sup>

<sup>a</sup> If algae are produced on non-arable land, the required arable land becomes 0%  
Source: Schenk et al., 2008

### 2.8.1. Hydrothermal liquefaction

Hydrothermal liquefaction (HTL) is the process of converting a biomass source into a liquid bio-crude. Temperatures ranging from 200 to 350 °C with pressure ranging from 15 to 20 MPa (150 – 200 bar) are standard practice for this process. These conditions cause the breakdown of complex molecules and their repolymerisation to oily compounds. HTL eliminates the drying process as it allows the conversion of biomass with a high moisture content into bio-crude. HTL provides four products: bio-crude oil, solid residue, processed water and gas. The gas consists mainly of CO<sub>2</sub>, which can be cleaned and recycled for the cultivation of algae. Solid residues are high in nitrogen and minerals and could be used as a source or supplement to the fertiliser. The processed water is rich in nitrogen and carbon, which can be used to provide growth nutrients for microalgae in the growth stage. Bio-crude oil is ready to be separated and refined to obtain the desired products. The properties and yield are largely dependent on the composition of the feedstock as well as the temperature and pressure used during the HTL process (Biller et al., 2012).

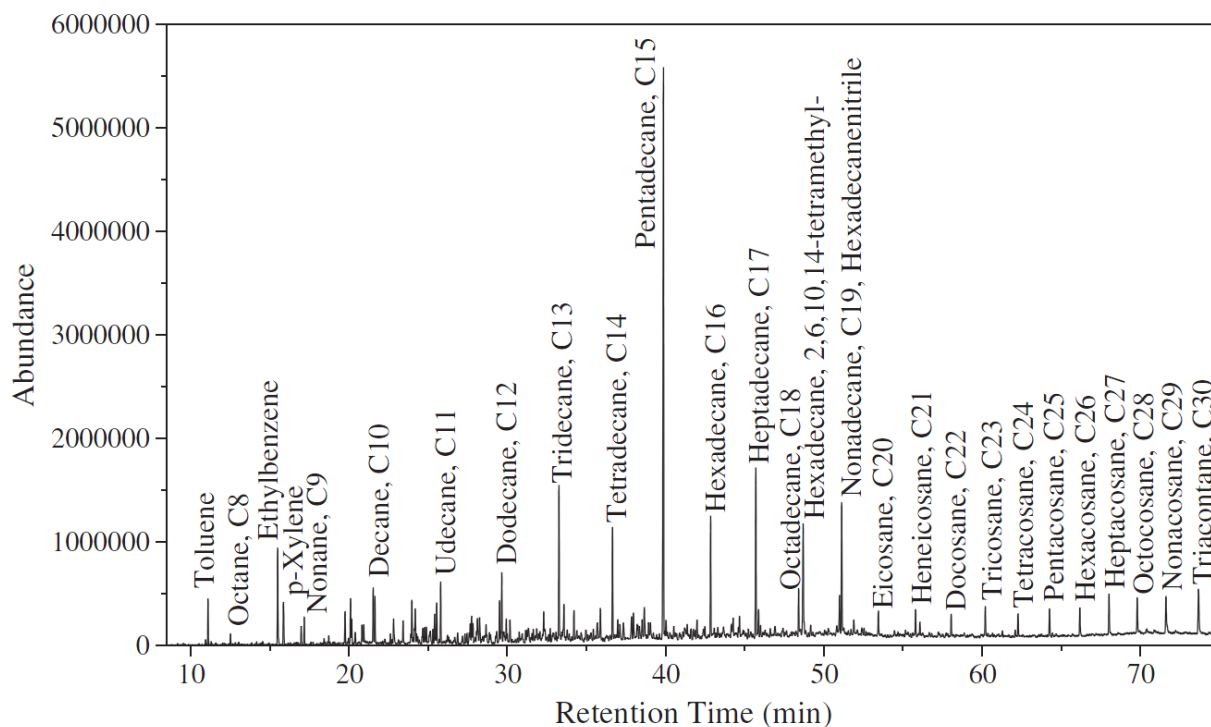


Figure 2.13: Total ion chromatogram of light oil produced at 400 °C using 50 % HZSM-5 (aluminosilicate zeolite) catalyst. Copyright Li and Savage, 2013. Reproduced with permission

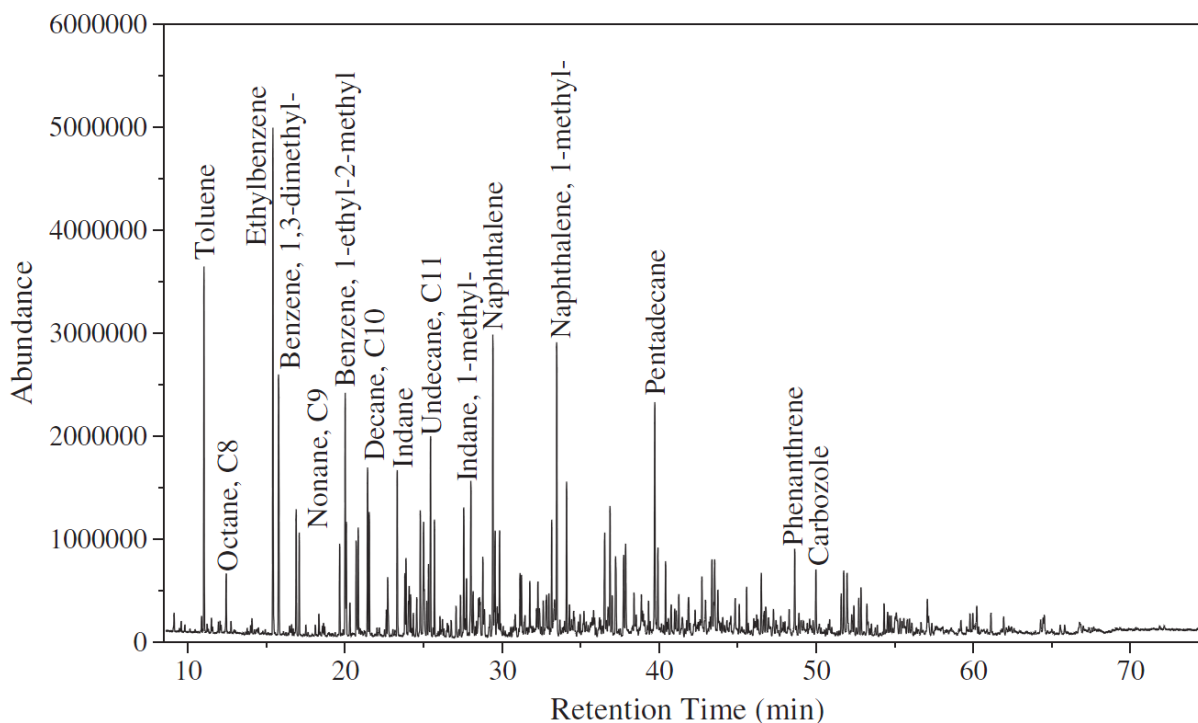


Figure 2.14: Total ion chromatogram of light oil produced at 450 °C using 50 % HZSM-5 (aluminosilicate zeolite) catalyst. Copyright Li and Savage, 2013. Reproduced with permission

The formation of bio-oil follows the trend of lipids > proteins > carbohydrate seen in the results. Protein and lipid conversions occur most efficiently in the absence of a catalyst, while carbohydrate processing showed the best results in the presence of a catalyst,  $\text{Na}_2\text{CO}_3$ . Bio-oil is generated from all the available components; this gives HTL a distinct advantage (Biller and Ross, 2011). The difference in the composition of the oil and processing time can easily be seen in Figure 2.13 and Figure 2.14 where the only difference is the reaction temperature that was used during processing.

At temperatures of around 300 °C, the hydrothermal liquefaction process produces a crude bio-oil that is viscous and contains approximately 10 – 15 wt.% heteroatoms (primarily O and N). The heteroatoms prevent the use of bio-oil as a fuel for transportation applications and have to be processed further (Li and Savage, 2013).

### **2.8.2. Bioethanol**

The feasibility of using lignocelluloses biomass as feedstock for bioethanol is often limited by its low yields. Algae have the ability use inexpensive raw materials to produce large quantities of carbohydrate and this could increase its feasibility for bioethanol production. Certain species use photosynthates in dark anaerobic fermentation to produce ethanol naturally (Singh and Olsen, 2011).

Although macroalgae appear to be similar to land plants, they lack the same lignin cross-linking molecules in their cellulose structure, primarily because their aquatic environments allow them to use their buoyancy to grow upright. While macroalgae have a low lignin content, they also have a significant sugar content (at least 50 %) that could be fermented to produce bioethanol. The carbohydrate content of certain marine algae, including red algae, is influenced by agar, a polymer made from galactose and galactopyranose. These could increase the bioethanol production if the polymer could be reduced to its building blocks (Jones and Mayfield, 2012).

The fermentation changes the carbohydrates in algae biomass to acetone, butanol and ethanol (ABE). This is achieved by using spore-forming anaerobic gram-positive bacteria. The production of valuable industrial solvents like ABE from a waste-water source could have beneficial effects in domestic economies, as could the utilisation of lagoons or ponds for the treatment of waste water in rural areas (Ellis et al., 2012).

### 2.8.3. Biofuels

#### 2.8.3.1. Biodiesel

Biodiesel can be seen as one of the main algae-based biofuels with future commercial feasibility because the world is turning to a renewable fuel source. Many algae have the capability of both producing and storing large amounts of lipids. These amounts could be as high as 50 – 60%. From a chemical point of view, upon transesterification the biomass lipids are similar to those in the other oil-seed crops (Jones and Mayfield, 2012).

Biodiesel is a sought-after product due to its renewable nature and the increasing price of fossil fuels. Its ability to be grown without interfering with food production and because it can be cultivated on non-arable land give it a major advantage over oil-seed crops.

#### 2.8.3.2. Gasification

Gasification is the partial oxidation of biomass feedstock using high temperatures for the production of syngas (Biller and Ross, 2011). The partial oxidation of the biomass occurs in the presence of oxygen and steam from water. The syngas obtained from the process consists mainly of “carbon monoxide (CO), hydrogen (H<sub>2</sub>), carbon dioxide (CO<sub>2</sub>), nitrogen (N<sub>2</sub>) and methane (CH<sub>4</sub>)” and the product becomes highly combustible at temperatures between 800 – 1000 °C (Singh and Olsen, 2011).

The efficiency can be increased significantly by using higher temperatures, low algae concentration and longer residence time. Using low, supercritical temperatures typically results in gas that is rich in CH<sub>4</sub> and CO<sub>2</sub>. Theoretical calculations estimate that algae biomass that has been gasified at a temperature of 1000 °C produces the highest methanol yield of 64 % (Singh and Olsen, 2011).

#### 2.8.3.3. Pyrolysis

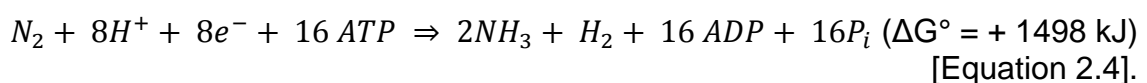
Pyrolysis is a process in which the anaerobic formation of bio-oil, syngas and charcoal occurs in a medium to high temperature environment (Biller and Ross, 2011). It follows the same principles as gasification, with the only difference being the absence of air and halogens.

#### 2.8.4. Biohydrogen and biogas

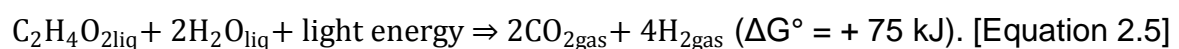
Hydrogen gas is considered to be a clean-burning fuel source seeing that it emits no CO<sub>2</sub>, only water. Electricity can be generated by using hydrogen in a fuel cell; however, combustion is possible, but highly dangerous. Hydrogen's very high energy capacity of 122 kJ/g, is approximately 2.75 times the amount present in hydrocarbon fuels. The main obstacles to the use of hydrogen are its high production cost and low availability in nature. The production of hydrogen gas using biomass and water would produce a renewable source of hydrogen production (Kapdan and Kargi, 2006). Typical industrial-sized facilities producing solar hydrogen require an area of between 500 and 1000 acres (approximately 202 – 405 ha) for a cost-effective operation according to models (Melis, 2002).

Under normal photoautotrophic conditions, the microalgae use carbon dioxide, water and light to produce an energy-rich organic compound and oxygen. Under anaerobic conditions, microalgae can produce hydrogen gas (H<sub>2</sub>) through the pyrolysis of water, by using light as an energy source to provide H<sub>2</sub> and O<sub>2</sub>. Hydrogenase acts as a catalyst, but is extremely sensitive to oxygen which is one of by-products of photosynthesis (Singh and Olsen, 2011; Akkerman et al., 2002).

The ability of nitrogen (N<sub>2</sub>) fixation is catalysed by the nitrogenase enzyme of the photoheterotrophic bacteria, which can also catalyse the production of H<sub>2</sub>, especially in the N<sub>2</sub> free environment. The reaction equation is (Jones and Mayfield, 2012; Akkerman et al., 2002):



Similar to the hydrogenase enzyme, the nitrogenase enzyme is oxygen sensitive and ammonium ions act as an inhibitor, but this can be overcome by using an anaerobic environment which is free of both oxygen and nitrogen. These conditions allow the following example of a reaction to take place (Jones and Mayfield, 2012; Akkerman et al., 2002):



Macroalgae have the potential to act as a production source for biohydrogen and biogas due to their high growth rates, their ability to grow in saline environments and

their low lignin content. A high carbohydrate content is one of the main characteristics of macroalgae, which in many cases consists of nonglucose monosaccharides. Biogas and biohydrogen are obtained by means of the anaerobic fermentation of algal biomass (Jones and Mayfield, 2012). The anaerobic fermentation (digestion) of algae biomass would produce methane gas as its major component, with hydrogen gas being produced as a by-product, at less than 5 % of the total gas produced. The majority of studies have shown that the methane portion of the biogas ranges from between 69 – 75 % of the total gas produced (Sialve, Bernet and Bernard, 2009). As anaerobic fermentation only uses the volatile solids present in the biomass, the post-fermented materials could be used as a source of fertiliser. Post-fermented materials have been concentrated and sterilised so that they may reduce the risk of introducing unwanted bacteria into the reactor.

Table 2.11: The theoretical volume of methane produced from different algae species.

<b>Algae Species</b>	<b>Protein (%)</b>	<b>Lipids (%)</b>	<b>Carbo- hydrates (%)</b>	<b>CH<sub>4</sub> (l(CH<sub>4</sub>)/g (VS))<sup>a</sup></b>
<i>Euglena gracilis</i>	39-61	14-20	14-18	0.53-0.8
<i>Chlamydomonas Reinhardtii</i>	48	21	17	0.69
<i>Chlorella Pyrenoidosa</i>	57	2	26	0.8
<i>Chlorella vulgaris</i>	51-58	14-22	12-17	0.63-0.79
<i>Dunaliella salina</i>	57	6	32	0.68
<i>Spirulina maxima</i>	60-71	6-7	13-16	0.63-0.74
<i>Spirulina platensis</i>	46-63	4-9	8-14	0.47-0.69
<i>Scenedesmus obliquus</i>	50-56	12-14	10-17	0.59-0.69

<sup>a</sup> l(CH<sub>4</sub>)/g(VS) = litre methane per gram volatile solids.

Source: Sialve, Bernet and Bernard, 2009

The production of methane in Table 2.11 was calculated by using the specific yield of each of the major components as seen in Table 2.12. The use of anaerobic processing after the extraction of lipids for other uses could still produce a valuable product, especially when high protein levels are available.

Table 2.12: Specific methane yield of major organic components

Component	Composition	I(CH <sub>4</sub> )/g(VS) <sup>a</sup>
Protein	C <sub>6</sub> H <sub>13.1</sub> ON <sub>0.6</sub>	0.851
Lipids	C <sub>57</sub> H <sub>104</sub> O <sub>6</sub>	1.014
Carbohydrates	(C <sub>6</sub> H <sub>10</sub> O <sub>5</sub> ) <sub>n</sub>	0.415

<sup>a</sup>I.(CH<sub>4</sub>)/g(VS) = litre methane per gram volatile solids.

Source: Sialve, Bernet and Bernard, 2009

The cost of drying harvested algae is a major drawback. When considering an anaerobic digestion system which allows for the recovery of energy and nutrients from biomass, the drying cost becomes minimal. The anaerobic digestion would also increase the bio-availability of nutrients, the algae production and anaerobic digestion combination could have a synergetic effect (Wilkie and Mulbry, 2002).

If the production of biogas could follow the extraction of lipids, it would allow the harvesting of maximum energy as well as the recycling of nutrients (Singh and Olsen, 2011). The production of biogas, biofuel and fewer nutrient requirements could make the process more sustainable and cost effective.

## 2.9. Uses of algae biomass

Table 2.13: Uses of algae biomass for non-bioenergy sources

	Product	Uses	Relevance
Human and animal nutrition	<ul style="list-style-type: none"> <li>• Tables</li> <li>• Capsules</li> <li>• Liquids</li> </ul>	<ul style="list-style-type: none"> <li>• Nutritional supplements</li> <li>• Natural food colourant</li> </ul>	Table 2.14 High quality protein and energy source
Dehydration	<ul style="list-style-type: none"> <li>• Powder</li> </ul>	<ul style="list-style-type: none"> <li>• Extraction of solvents</li> </ul>	High value product extraction from algae biomass
High value molecules	<ul style="list-style-type: none"> <li>• Fatty acids</li> <li>• Pigments</li> <li>• Fertiliser</li> </ul>	<ul style="list-style-type: none"> <li>• PUFAs</li> <li>• Colourant</li> <li>• Slow releasing biofertiliser</li> </ul>	Table 2.15 B-carotene production Nitrogen fixing algae



Table 2.14: Comparison between the compositions of traditional foods and algae

	<b>Protein</b>	<b>Carbohydrate</b>	<b>Lipid</b>
Bakers' yeast	39	38	1
Meat	43	1	34
Milk	26	38	28
Rice	8	77	2
Soybean	37	30	20
<i>Anabaena cylindrica</i>	43-56	25-30	4-7
<i>Chlorella vulgaris</i>	51-58	12-17	14-22
<i>Porphyridium cruentum</i>	28-39	40-57	9-14
<i>Scenedesmus obliquus</i>	50-56	10-17	12-14
<i>Spirulina maxima</i>	60-71	13-16	6-7

Source: Spolaore et al., 2006

Table 2.15: Microalgae PUFA of interest

<b>PUFA</b>	<b>Structure</b>	<b>Microorganism</b>
$\gamma$ -Linolenic acid (GLA)	18:3 $\omega$ 6, 9, 12	<i>Arthrospira</i>
Arachidonic acid (AA)	20:4 $\omega$ 6, 9, 12, 15	<i>Porphyridium</i>
Eicosapentaenoic acid (EPA)	20: 5 $\omega$ 3, 6, 9, 12, 15	<i>Nannochloropsis</i> , <i>Phaeodactylum</i> , <i>Nitzschia</i>
Docosahexaenoic acid (DHA)	22: 6 $\omega$ 3, 6, 9, 12, 15, 18	<i>Cryptocodinium</i> , <i>Schizochytrium</i>

Source: Spolaore et al., 2006

## 2.10. Conclusions

The design of a photobioreactor does not only entail the optimal growth of algae biomass but also includes the overall production costs and the overall production process. This means that the design of each type of photobioreactor is unique to a certain type of algae and its end product and allows for a photobioreactor that is easily adaptable.

The design of the photobioreactor is based on all the requirements of the algae biomass and the post-harvesting processing to obtain the desired product. The photobioreactor has to be designed for optimal efficiency in the entire production process as well as for the optimal growth of algae.

The choice of algae is based on the decision of what the desired product is. This is followed by the post-harvest processing (it could be several processes) requirements in order to obtain the best product, because the process and its conditions could result in a different (undesirable) final product. The harvesting of algae biomass should also be considered because the harvesting method could affect the post-harvesting processes to be used. The environment and all its variables have to be taken into account because different compositions would affect post-harvesting processes differently. By changing the availability of light, nutrients or carbon dioxide, the composition of the algae biomass is directly affected and could possibly increase the production of the final product by changing the environment inside the reactor – and this could lead to the more efficient production of the required biomass.

The use of waste water or the capturing of nutrients in waste water through processes like growing benthic algae for N and P collection could reduce the cost drastically. The recycling of nutrients and the use of solar technology to reduce the energy requirement of the reactor is a condition for sustainable production – both financially and environmentally – of algae biomass.

The cost of an optimal photobioreactor cannot be calculated because there are too many variables involved. To calculate the cost of operating a photobioreactor at optimal levels, all the variables have to be considered as small changes in the overall process could have a large effect on the cost and profitability of the system. It is therefore only possible to calculate the production cost of the photobioreactor on the tested algae species and conditions.

## 3. REACTOR DESIGN

### 3.1. Introduction

The design of the reactor commenced after a through literature study had been done. All the advantages, disadvantages and the objectives of this study were taken into account when planning the reactor. The design of a reactor is a very complex process because there are a large array of possible combinations. What is more, the literature gives very little information on the construction materials and whether there is any difference between them.

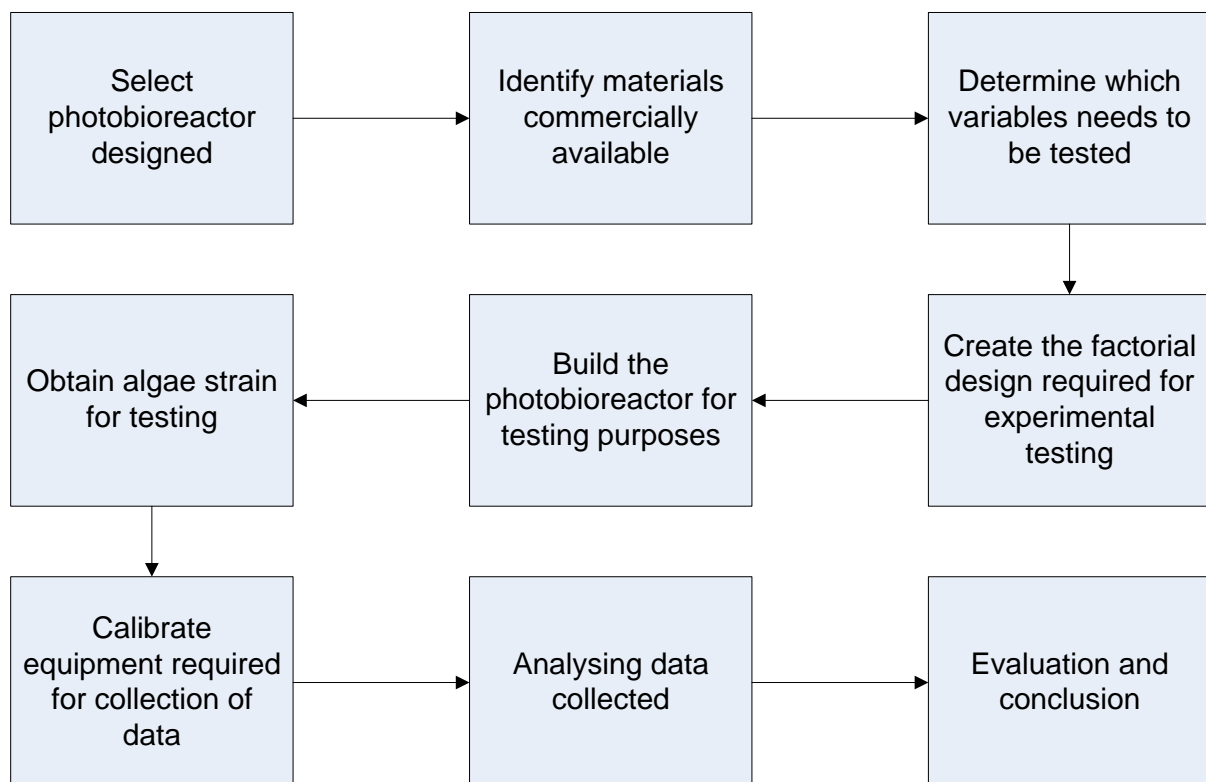


Figure 3.1: Flow diagram of experimental setup

### 3.2. Design of the reactor

The design that was chosen took into consideration the requirements for a good bioreactor, the type of algae to be cultivated, the processing requirements and the energy usage. Based on these constraints, it was decided that a Bubble column

reactor would be the best option because its system has a fairly simple construction, with high surface-area-to-volume ratio. This type of reactor uses very few mechanical components, a factor which is considered beneficial because it does not place extra stress on the algae cells and it reduces both the cost and the energy required for running the reactor or plant.

The use of gas to agitate the algae culture is seen as a positive aspect of the reactor's design because it simultaneously removes the O<sub>2</sub> from the system and adds CO<sub>2</sub>, thus ensuring that a small or no pH gradient forms in the reactor and because it eliminates the need for a mechanical stirrer. Similarly, the use of gravity allows the fast and effective harvesting of biomass with limited damage being caused to the harvested and the remaining culture. The quality of the biomass that is available for processing is important from the point of view of the system's profitability, especially when high-value products are produced.

For the system to work well, only two mechanical components are required: an air compressor or air blower to ensure that sufficient air flow can be achieved; and a pump to replace the medium that is lost during the harvesting process. Two major problems with the use of a compressor are that the required volume of air could surpass the maximum ability of a standard compressor, or that the cost of running a compressor could become too high, in which case an air blower would be the alternative. Air blowers provide the volume of air at a low pressure; however, this could cause a problem in overcoming the water pressure when too many units are supplied by the same air blower. Because the tubing length is a maximum of 5 metres (as stated on the pricelist in APPENDIX B), the pressure that has to be overcome is 0.5 bar which is easily achievable using an air blower. If required, additional lighting could be added to increase the productivity of the system although this would increase the cost and energy requirements of the system.

One of the major benefits of the reactor is that it is possible to automate the entire algae production system. The inputs and harvesting automation could be achieved by using solenoids which would allow the addition of the growth medium as required and the harvesting of the algae in chronological order. This could be beneficial for the downstream processing as it would allow the continuous supply of algae biomass and reduce the requirement of large holding tanks for the biomass while it is waiting

to be processed. The automation of the system would be particularly beneficial to large-scale systems as it would need little human interaction, thus reducing the possibility of contaminants' entering the reactor and lowering the operating cost because fewer salaries have to be paid.

One requirement of the PBR is that it should be able to create several environmental conditions for the growth of algae. As seen in the literature study, there are thousands of species, each with its own requirements for optimal growth. The PBR should be able to handle all these requirements in order to be considered efficient for the production of algae biomass. As a result of all these possible requirements, the design should allow for a system that can adapt to the specific requirements of a species with few or no changes to the design, especially if different species are grown at different times during the seasonal calendar.

Figure 3.2 shows the proposed design of a 36-tube reactor module. The design is compact and easy to maintain because a supporting structure would be built around the module. This would provide easy access to most areas of the module. If a reactor taller in height was produced, the need for the support structure would become very important because the weight of the culture could cause problems and possibly break if stress was placed on the tubing. The reactor also has a high surface-area-to-volume ratio, which could be changed by using different diameters and lengths of tubing and would theoretically create a better environment for the growth of biomass caused by optimal light conditions.

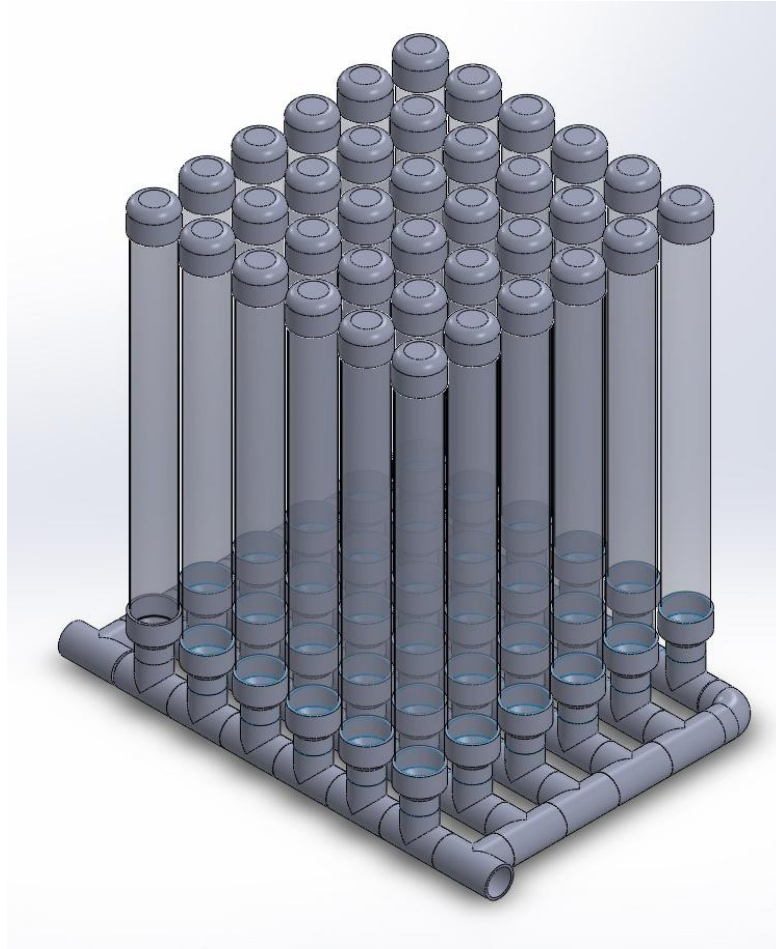


Figure 3.2: Design of the proposed PBR

The PBR can be made from any transparent material, especially glass, acrylic tubing and PVC. Because they cost less than glass tubing and would not break as easily as glass, PVC and acrylic tubing are considered a better option for a PBR. Both materials (PVC and acrylic) are very resilient to many chemicals and the chemicals which could cause problems are not commonly used for biomass production as they are mainly very strong acids, bases, oxidising agents and solvents. The physical characteristics (tensile strength, elasticity, thermal conductivity and friction coefficient to name a few) of the tubing (PVC and acrylic) are very similar to each other, with one being stronger in a specific area and the other being stronger in another area (APPENDIX B). One of the major obstacles when using glass or acrylic tubing is that special fittings or adaptations need to be made to install the available fittings made from other materials if different diameters are used. The second problem that was found is the bonding of different materials to each other. After testing, it was found

that acrylic tubing can be glued to PVC fittings using PVC adhesive, but that acrylic adhesive did not form a proper bond between the two materials.

In the event where a reactor has to be disassembled, it would be recommended that O-rings be used to create a seal as it would allow for the disassembly of the reactor with the ability to reuse all the tubing and fittings for reconstruction. It has the additional advantage that all the tubing and fittings could be used again for reconstruction. It would also be advisable to use O-rings when different materials are used in order to ensure that a proper seal is formed. In the case where a single material is used for the entire reactor, it is suggested that the best sealing method is to determine the requirement of the reactor because a complete PVC reactor, which can be PVC welded, glued or O-rings could be used to create a seal between components in the system.

The design seen in Figure 3.2 has the benefit that it can easily be changed to allow the use of the reactor in specific areas. The length of the clear PVC tubing can be increased or decreased as required, allowing the system to be used in abandoned hot houses or other limited spaces. The number of tubes that are placed in a module can also be altered to allow the PBR to be used in smaller spaces or to increase the productivity of a confined space by customising the reactor for the specific space. Figure 3.2 shows a module consisting of 36 vertical tubes that are interconnected. Theoretically the number of vertical tubes could be infinite – although this might create several problems in a commercial plant. Ideally a commercial plant should have as large a number of these modules as possible in order to allow better control over the plant, especially when it is necessary to clean the reactor and a problem in one module would not affect overall production. By using modules and having the correct setup, a specific module could be replaced with another module and thus will not influence the productivity of the plant if cleaning or repairs are required.

Harvesting will occur through a port on the system which could be fitted with either a ball valve or a solenoid valve. Depending on the size of the plant, it would allow the harvesting of individual modules or all the modules through a network of pipes leading to the processing plant. Similarly, a port could be created for replacing the medium that was lost through the harvesting process. This is very important in order to obtain optimal growth in the reactor. The medium replacement port could be

removed if the system so required to create a more manual system. Creating these networks that interconnect the PBR modules reduces the labour requirements and allows for the central processing of both the medium production and algae processing or storage. This would increase the potential productivity of the entire system, either by being more efficient or allowing a larger area for modules in the plant.

In the literature study it was noted that some of the commercial plants use a two-stage process. This is done primarily to produce the algae biomass; this is followed by a stage in which the biomass is placed in a different environment in order to produce more of the desired product. If the algae species that is used requires a two-stage system, the module can be altered easily to accommodate the two stages. However, this option would only be viable in a small-scale system, because in a large scale system separate modules will be utilised for the different stages. The ability of the specific PBR to be manipulated easily gives it an advantage over other PBR designs because the other types of PBRs require more complex alterations to obtain a two-stage setup and this would increase the cost substantially.



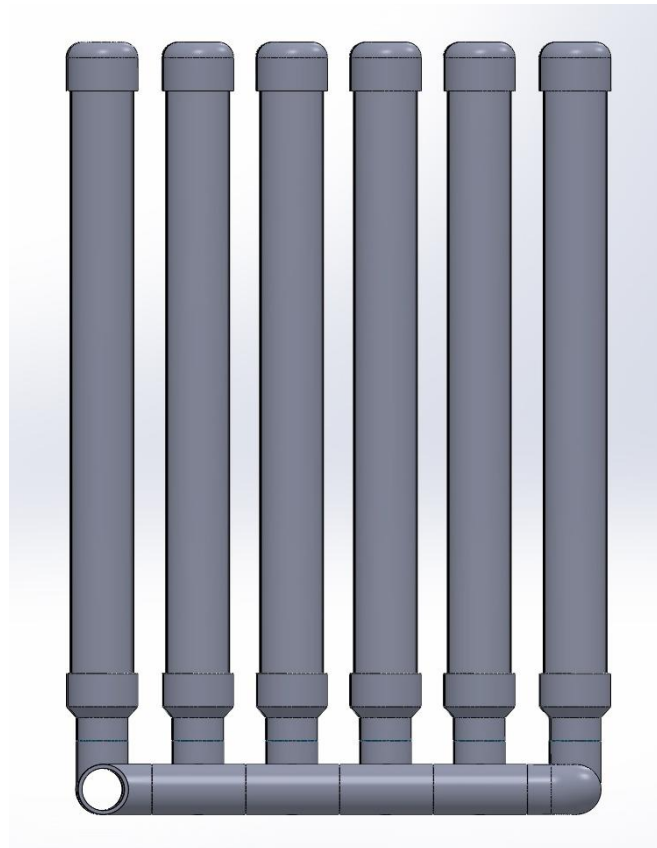


Figure 3.3: Side view of PBR

The diameter of clear tubing available can be seen in APPENDIX B. From this one can observe that the PVC tubing corresponds to standard PVC fittings, but that the acrylic tubing is only available in a few diameters that are the same diameter as the standard PVC fittings. This limits the use of acrylic tubing as it would require expensive fittings. In this connection, a cost analysis will be done in chapter 6 to compare the materials with each other and determine the profitability of the specific algae species using a bubble column system, as suggested in Figure 3.2. The costing will, from now on, only compare PVC and acrylic tubing of similar diameters, thereby excluding the cost of special fittings which could be required by acrylic tubing with another diameter.

Larger tubing was chosen because, despite the fact that the light would not completely penetrate the algae culture, it would allow the algae cells to go through light/dark cycles and thereby possibly increase the productivity as stated by (Grobbelaar, 2009b). The light/dark cycles would also have a beneficial effect during

high irradiation times, and prevent damage to the algae cells and decrease in the productivity. The 110 mm tubing allows sufficient area between the vertical clear tubes for light to reach all of the tubes, as seen in Figure 3.3. If a larger space between the reactor tubes is required, the length of piping between the tees of the base can be increased and thereby increase the space between the vertical tubes. By using larger diameter tubing to create the base of the module, the productivity on the footprint of the PBR is dramatically decreased, but the cost of building the reactor increases substantially due to the more expensive materials required. This is especially the case when using tubing with diameters that are larger than 110 mm. The tubing that is used can theoretically have any diameter, but the specific diameters were chosen as they would show how the diameter of the tubing affects the growth in the reactor and they would thereby indicate which would be the better option to use in the manufacture of the reactors.

The PBR use PVC end caps on top of the clear PVC tubing. The reason for this is to keep contaminants out of the system and thus ensure that a monoculture is maintained. The end cap could house a filter which would allow the exchange of clean gases between the atmosphere and the PBR. The filter is primarily for a system that is switched off at night, allowing the system to breathe as required. In the case where the system is run continuously, the filter can easily be substituted for a relief valve because the PBR would always have a higher pressure on the inside. This would cause air to flow outward and prevent a large portion of contaminants to enter the PBR. The benefit of using a relief valve is the fact that it does not have to be replaced, while the filters have to be replaced regularly. This would increase the running cost of the plant. Replacing the filters could cause additional problems as they could be fairly difficult to put back because of their position on top of the system. A solution to the problem would be to have tubing that is connected to every vertical tube that would then go through one filter, which could be placed in an area that is easily accessible for replacement purposes. Using one central filter ensures that the system is not placed under any additional stress when replacing the filters, as the final setup of the reactor could cause problems.

Due to the limited knowledge concerning the choice of the material and the tubing diameter, both PVC and acrylic tubing were employed, using 50 mm, 90 mm and 110 mm diameter tubing with a length of 500 mm. These diameters were chosen

because they were among those commercially available. The thicknesses of the wall of the tubing are different for some of the diameters but they were chosen in such a way as to keep the difference to a minimum. The tubing with a minimal wall thickness was chosen. This allowed the maximum amount of light to penetrate the materials. All the additional fittings were made out of PVC that is capable of withstanding pressure of up to 16 bar, and that had the same dimensions as those of the specified clear tubing. All the tubing and all fittings, excluding the end caps, were glued using PVC adhesive. The end caps were not glued to the tubing, to allow for the replacement of the medium after the algae concentrations had been readjusted.

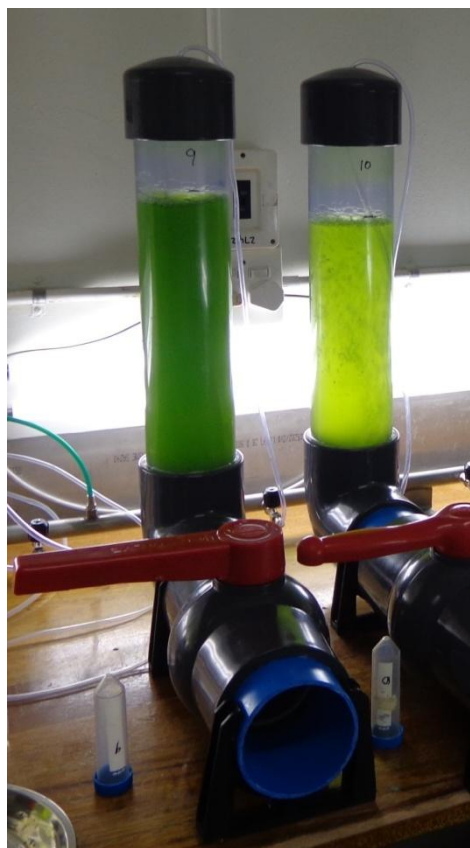


Figure 3.4: 110 mm tubing reactors used for testing purposes

PVC ball valves were installed for use when sampling and adjusting the concentration of the algae, but they could not be used because the valves were too stiff to use in the small laboratory-scale systems that were being tested. The ball valve would be usable when used in a fullscale reactor module as it would have a higher weight and would therefore be easier to use. The ball valves were also

required to simulate the area between the vertical tubes in a module setup. Due to the problems with the ball valves, the reactors were removed from their stands and emptied using the top of the tube after the end cap had been removed.

The end caps fitted tightly on most of the tubes and required some effort to be removed to empty and refill the reactor. A hole was drilled into the centre of the dome of the end caps through which the gas supply could enter. The drilled hole was slightly larger than the gas supply tubing, allowing the excess gas to escape from the reactor. The extra space from the hole was not closed because the gas supply was only stopped for sampling and adjustments. It was decided not to add a filter because unnecessary holes would be created and, with the continuous gas supply, this would create unnecessary problems. In the case of the end cap in the 50 mm tubing, the space for a filter was very limited and would probably not have fitted if it had been slightly oversized.

### **3.3. Gas mixing and supply**

To achieve optimal growth, the system should be fed CO<sub>2</sub> continuously. The CO<sub>2</sub> feed should be adjusted according to the specific algae's needs for CO<sub>2</sub>. As seen in Table 2.8, each species has the highest level of CO<sub>2</sub> (v/v) that it can survive. The continuous feed of CO<sub>2</sub> will provide optimal growth conditions because of the higher availability of carbon for fixation and a better mass transfer. It would also limit the pH fluctuations that are obtained through the batch addition of CO<sub>2</sub>.

It has been found in other living organisms, especially dairy cows, that a higher feeding frequency with lower quantities gave better control of the internal pH which results in higher and more economical productivity, while a low pH also causes a decrease in the energy intake and health problems (Krause, Combs and Beauchemin, 2002). A similar effect should be seen in algae due to the sensitivity of the algae cell to its environment and the need to add CO<sub>2</sub> to ensure growth throughout the growth period. By continuously supplying CO<sub>2</sub>, the running cost of the plant can be decreased substantially as the requirement for additional labour would be reduced (because the need to test the pH to determine CO<sub>2</sub> levels in the algae medium is eliminated). The cost would be reduced especially when several modules are in operation or in a commercial plant.

If it is necessary to add batches of CO<sub>2</sub>, this can be done without any modification as the setup will remain the same. The addition of the CO<sub>2</sub> and air will be controlled by valves which allow only air or CO<sub>2</sub> to be bubbled through the system at a given time. In Figure 3.5 it is seen that the gas is mixed by using a flow meter with the gas at the same pressure. To obtain a batch addition of one of the gases, the valves can simply be altered and thus enable not only a specific gas to be added continuously, but also allow the increase of a specific gas if required during continuous addition.

The setup in Figure 3.5 was considered to be the best as it should have the smallest change in pressure after mixing has occurred. If the pressure did change after mixing, it could change the composition of the gas dramatically and would over- or undersupply the reactor leading to incorrect gas compositions and directly affect the growth of the biomass. The position of the pressure regulators in front of the flow meters should be set to have identical pressures. This will allow the calculation of the volume required by each flow meter. The main reason for having the gas at the same pressure when it reaches the flow meters is that, in the case of a change in pressure after mixing has occurred, it would have the smallest effect on the composition. If one gas was added at a higher pressure, it would increase the pressure of the downstream system in the case of a problem and could completely stop the flow of the other gas. This could kill all the algae if only pure CO<sub>2</sub> had been added for a long time. Downstream of the gas filter the addition of a CO<sub>2</sub> analyser would ensure that any error in either the pressure regulators or gas flow meters can be monitored to ensure the most accurate gas composition. This would also allow for more economical production conditions in the reactors.

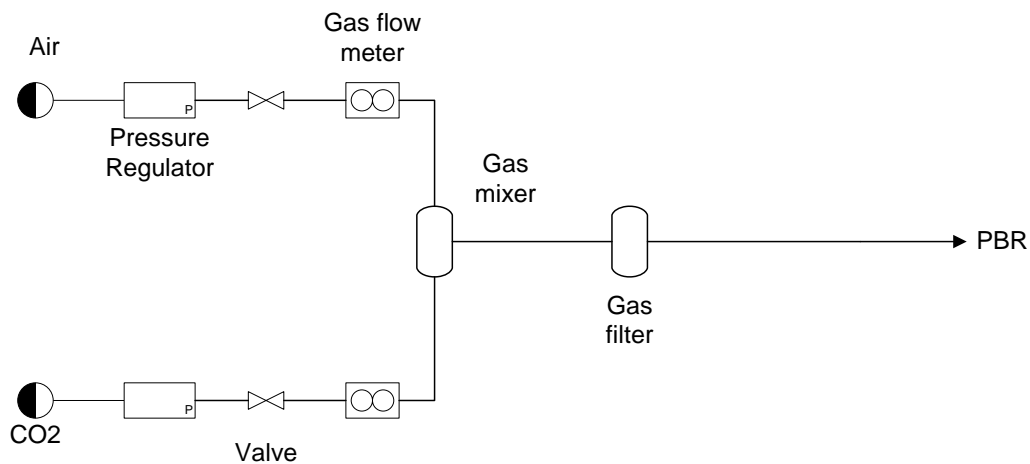


Figure 3.5: A schematic of the gas-mixing unit

The gas delivery system has to allow for the efficient agitation of the medium as well as for a high mass transfer of CO<sub>2</sub> and O<sub>2</sub>. To ensure the optimal growth of algae, this is one of the most important aspects of the reactor's design. The gas delivery lines would enter the vertical tubing from the top end. By entering from the top end, they will allow the entry point to be above the algae medium and therefore eliminate the formation of any possible leaks and allow for easier maintenance.

Due to the high gas flow rate that is required, the air supply has to be taken into consideration because it is the major component of the design. Taking the mass balance (APPENDIX C) and the values obtained in the literature study into consideration, it was calculated that a maximum gas flow rate of 600 litres per minute could be required for a 1200 litre reactor. To obtain an adequate air-flow rate, a compressor was considered. Although it is possible to obtain a compressor capable of supplying sufficient air flow, the size of the compressor motor would be a very large three-phase motor requiring a large amount of electricity because it would run continuously. An alternative is the use of an air blower which has the air capacity and is able to produce enough pressure to overcome the water pressure while using a smaller, single-phase motor. The supply of air should be considered carefully as the requirements of the reactors could cause either the compressor or air blower to have an insufficient supply for the specific reactor's setup.

The air component used in the mixture was obtained from a compressed air supply. The air pressure was reduced by means of a regulator because the initial 8 bar was

too high for the valves of the manifold that was being used. CO<sub>2</sub> was mixed directly into the air supply by using a tee piece. This was done at a specific volumetric flow rate which was controlled using a flow meter. The CO<sub>2</sub> used was obtained from Afrox (CO<sub>2</sub> Technical RC-40 dry). The CO<sub>2</sub> pressure was reduced from the bottle pressure to 1 bar and passed through a flow meter at the required flow rate before entering the air supply through the tee piece to ensure that the enriched air had the correct concentration (% v/v) and that the concentration could be reproduced.

The air pressure was reduced from the 8 bar of the compressed air supply, as the metering valves (seen in Figure 3.7) could not handle the high pressure effectively. It was found that the high pressure closed the valve a fair amount and in the case when pressure was reduced, the valves increased the flow rate through them, causing a sudden increase of flow inside the reactors. The sudden large increase in the gas flow rate caused a large volume of the algae culture to blow out of the top of each reactor, thereby influencing the results of the test. The blowout could also potentially damage the cells in the reactors as it increased the pressure drastically. The excess flow was corrected after the pressure of the air supply had been reduced while still supplying sufficient volume of gas to each reactor.

The gas was bubbled through air dispersion units to create smaller bubbles and to allow for a higher surface-area-to-volume ratio and therefore a better mass transfer between the culture and the gas. To keep uniformity, it has been decided to use air stones that are commonly used in fishponds because their design allows a large number of small bubbles to be created. These air stones are easily available and are fairly cheap. Two types of air stone were compared and, although a large variety are available, these covered the spectrum the best. Figure 3.6 shows that the large air stone (A) has a much larger surface area for the formation of bubbles compared to the small air stone (B). These differences could have benefits for the tubing sizes of the specific reactor. The reason for using different gas dispersion units was to determine whether the shape size of the units had any effect on the growth rate of the culture.





Figure 3.6: Gas dispersion units that were used during testing, A) large air stone, B) small air stone.

The gas supply entered a manifold after having been mixed. Each reactor was connected to the manifold individually using flexible 6 mm tubing. The flow rate was calibrated for every reactor and checked daily to ensure that a constant flow rate was being maintained. When the air stones were changed, the flow rates were checked and adjusted to ensure that the correct flow rate was supplied to the reactors. The 6 mm tubing provided sufficient gas flow for the test reactors, but if larger reactors were used they might require tubing with a larger diameter for the gas supply.



Figure 3.7: Two valves extruding from the manifold that was used to ensure a constant gas flow rate



# 4. MATERIALS, METHODS AND EXPERIMENTAL

## 4.1. Materials

As discussed in Chapter 3, PVC and acrylic tubing will be used for the construction of the PBR. It was decided to use these materials because of their durability and comparability to each other. It also allowed for easy assembly.

Compressed air was enriched with carbon dioxide and distributed using a manifold with needle valves to control the gas flow rate to each reactor. The gas was bubbled through air stones as per Figure 3.6, consisting of a large and a small air stone, because air stones create smaller gas bubbles.

The specie of algae that was used will be discussed in section 4.3. All experimental procedures were repeated four times to ensure reproducibility of the results. The experiments were done in a batch setup as the use of natural light does not allow the continuous production of algae due to change in light availability and intensity as discussed in Chapter 2. Due to the effect of light over harvesting will occur during the continuous harvesting of algae biomass and would therefore affect the production dramatically.

Continuous light were supplied to the reactors using fluorescent lights, placed at equidistance from the reactors to obtain comparable results. Enriched air was supplied at a volume per volume percentage of 5%.

## 4.2. Factorial design

A factorial design was chosen because it would allow the researcher to see which interactions were most important. From the literature it is clear that a large range of conditions can cause problems in the design of an efficient PBR. The reason for this is that there are several standard designs that can be used.

It was initially decided to use a factorial design of four on two levels and one on three levels. The three-on-two-level variables are density, gas dispersion units and materials of the clear tubing. The three-level variables are the tubing diameters that

were used. The testing conditions that were used, was to determine if the parameters had an effect on the growth as limited information was available in literature, these are not limits for the reactor design.

Table 4.1: Variables and their possibilities that were used in testing the factorial design

Variable	Low	Medium	High
Gas dispersion unit	Small		Large
Culture density	0.5 g/l		1.0 g/l
Material	PVC		Acrylic
Tubing diameter	50 mm	90 mm	110 mm

The 50 mm tubing was tested using a factorial design which included the following factors from Table 4.1: gas dispersion units, density and material. The 50 mm tubing was not compared to the other diameters because the change in flow rate would cause a change in the available carbon dioxide which could affect the growth rate and would thus not be a fair comparison. The 90 mm and 110 mm tubes were tested on the following factors: gas dispersion units, density, material and tubing diameter. It was decided to use a gas flow rate of 0.36 vvm for the 90 mm and 110 mm diameter reactors and 0.02 vvm for the 50 mm diameter reactors.

The experimental work for the factorial designs were done in a laboratory. The main reason was to ensure a constant illumination of the reactor's surface area, which would vary if natural light were used. The laboratory also allows the temperature to remain fairly constant, while outdoor cultures would experience large temperature fluctuations. As these two factors are known to have a large influence on the growth of algae, it is necessary to keep them as stable as possible. Some experimental work was done using natural light to see how it would affect the growth of the algae.

### 4.3. Algae cultivation

For testing the photobioreactor, the algae specie *C. Vulgaris* was used. The *C. Vulgaris* CCAP 211/11B strain was obtained from the Culture Collection of Algae and Protozoa, Dunbeg, Scotland.

The strain was obtained in EG:JM medium and was further grown using Bold's Basal Medium (see APPENDIX D). The medium was replaced because the algae concentration was readjusted for testing purposes. After each run, the algae were allowed to form a complete sediment and additional medium with its small amount of algae in suspension were removed. After this new dilutions were created to ensure that sufficient medium was available for the algae and to allow for accurate results, in case a specific reactor had too little medium or if there was another problem (e.g. algae cells which were dying or in a dormant state) which could affect the results.

The algae were grown using continuous artificial light with the addition of pure CO<sub>2</sub> added for five minutes, twice a day at the same flow rate as tested in the specific reactor. Due to large changes in pH and the death of large amount of algae, the addition of CO<sub>2</sub> was changed to two minutes four times a day, although this still led to the death of a large amount of algae. These experiments were done to determine if a continuous supply of CO<sub>2</sub> have an advantage over batch addition.

It was then decided to buffer the culture using sodium bicarbonate as suggested by Grobelaar, JU (APPENDIX E), with a continuous supply of CO<sub>2</sub>. at a 10 % (v/v) CO<sub>2</sub> concentration. It was observed that production was negatively affected and it was decided to add a 5 % (v/v) CO<sub>2</sub> concentration for testing the factorial design. The algae were allowed an adaption period of several days before results were taken. This was done to ensure that the cells had climatized to their new culture conditions.

#### **4.4. Operation the UV-Vis spectrometer**

A Varian Cary E1 UV-Vis spectrometer (Palo Alto, California, USA) was used. The spectrometer was switched on approximately one hour before use in order to ensure that all the components had sufficient time to warm up. Once the spectrometer had had sufficient time to warm up, the 690 nm wavelength was selected, as *Chlorella vulgaris* shows the best response at that specific wavelength. Before any measurements were taken the spectrometer had been zeroed, using two cuvettes filled with reverse osmosis water.

The 690 nm wavelength was chosen because it is the only individual peak as is seen in Figure 4.1. It is clear that the lower wavelength region has several peaks that interconnect and could easily cause a large error. Moreover, the 690 nm wavelength

was used in the available literature and so it was chosen in this case (Yeh, Chang and Chen, 2010).

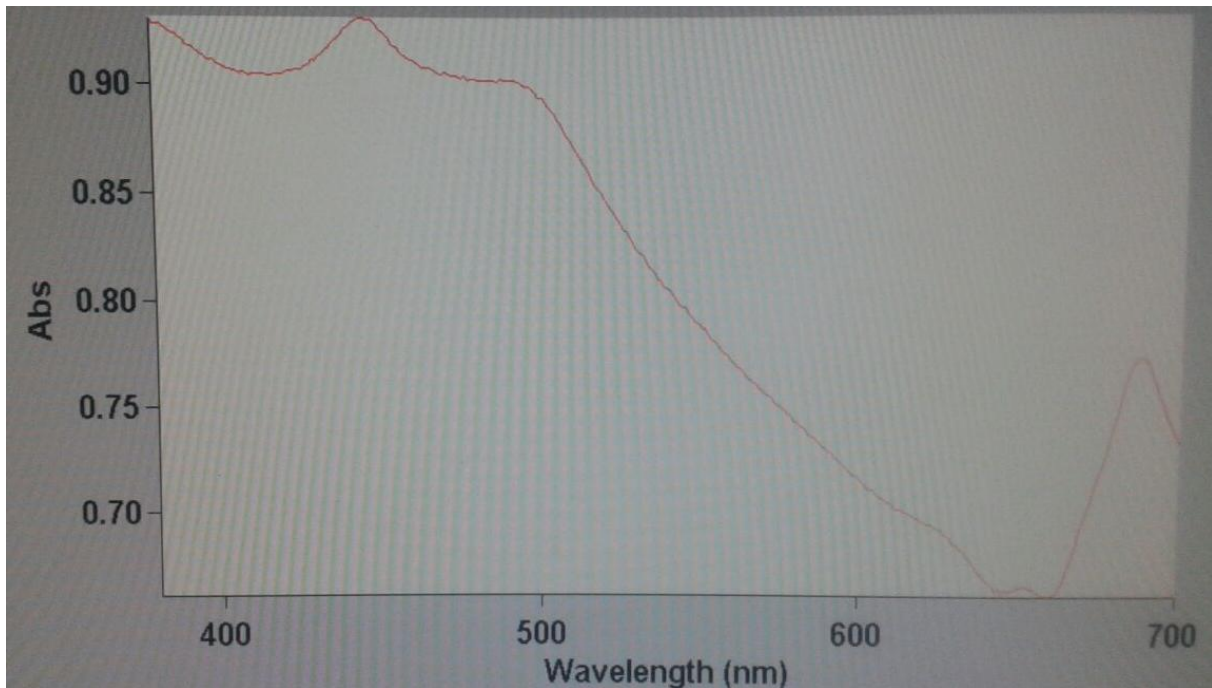


Figure 4.1: Scanning absorption curve between 370 and 700 nm to determine algae growth

To prepare samples for analysis, the content of each algae culture used was mixed to ensure uniform cell distribution in the culture before initial samples were taken. The sample taken of a given culture was mixed using a vortex mixer before taking the sample to be used in the spectrometer. The samples used in the spectrometer were diluted to obtain more accurate readings from the correlation curve. The ideal absorbance for the case in point is between 0.0000 and 1.0000. A dilution factor of 1:4 of algae to reverse osmosis water was used because this placed both cell densities in the ideal range of the correlation curve and allowed for easy calculation during experimental work. All samples volumes were accurately measured using a micropipette to ensure that little error is introduced when preparing the dilution and in creating the correlation curve.

#### 4.5. Correlation curve

The correlation curve was created so that Beer-Lambert's law could be applied, i.e. the absorption of a sample is equal to its concentration, multiplied by a specific factor and its path length, as is shown in Equation 3.1.

$$A = \epsilon cl \quad [\text{Equation 4.1}]$$

As the path length of the cuvette is one centimetre, it can be eliminated. This allows the creation of the correlation curve to obtain  $\epsilon$  because the absorbance and concentration of algae were known.

To create the correlation table, 30 ml of algae culture were used. This is important because it is required in order to calculate the initial concentration of algae that were in the culture. Dilutions were made using reverse osmosis water and the product was then added to an oven-dried and pre-weighed piece of glassware. After all the dilutions had been done and all the dilution information recorded, the specific cultures were oven-dried overnight at 100 °C and reweighed to obtain the mass of the algae in the culture.

The initial 30 ml of algae culture was used to make several dilutions because it was found that this reduces any error in the weighing process. The major problem was that, when working at very low concentrations, small errors in the absorbance values and in the weighing are multiplied significantly and this renders the results unusable. Because reverse osmosis water was used to create the dilutions, it would simply evaporate and so not affect the results obtained, while simultaneously creating a very accurate set of results for the plotting of the correlation curve.

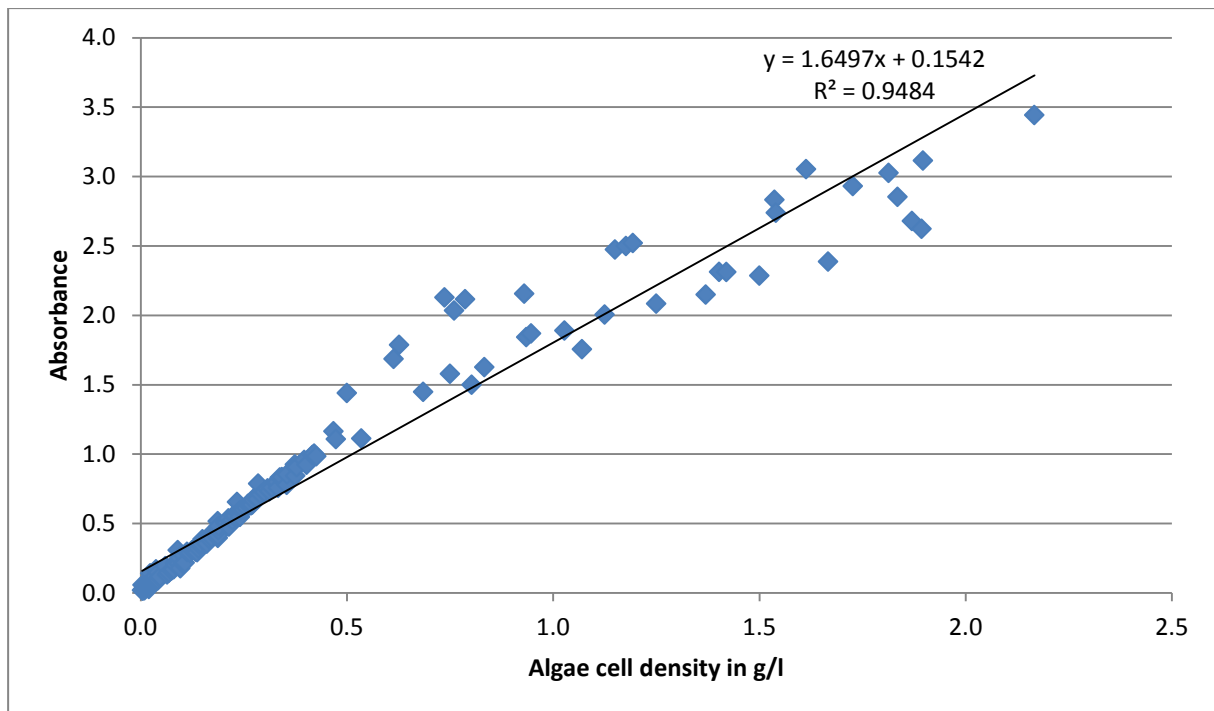


Figure 4.2: Correlation curve of absorbance vs. cell density

It is seen that, at an absorbance over 1.0000 units, the sensitivity of the curve is reduced and that the reading errors become substantial. This area has a correlation function of  $y = 1.1276x + 0.8492$  and has a  $R^2$  value of 0.8285 which is not very accurate. It was therefore decided to use absorbance values of less than 1.0000 units and make dilutions as previously mentioned.

As is seen from Figure 4.3, the sensitivity of the correlation curve is higher when working at lower concentrations. The  $R^2$  value of 0.9906 could be considered to be very good, especially when 122 was used, and it would therefore be the only option to use in conjunction with Beer-Lambert's law to obtain the culture density.

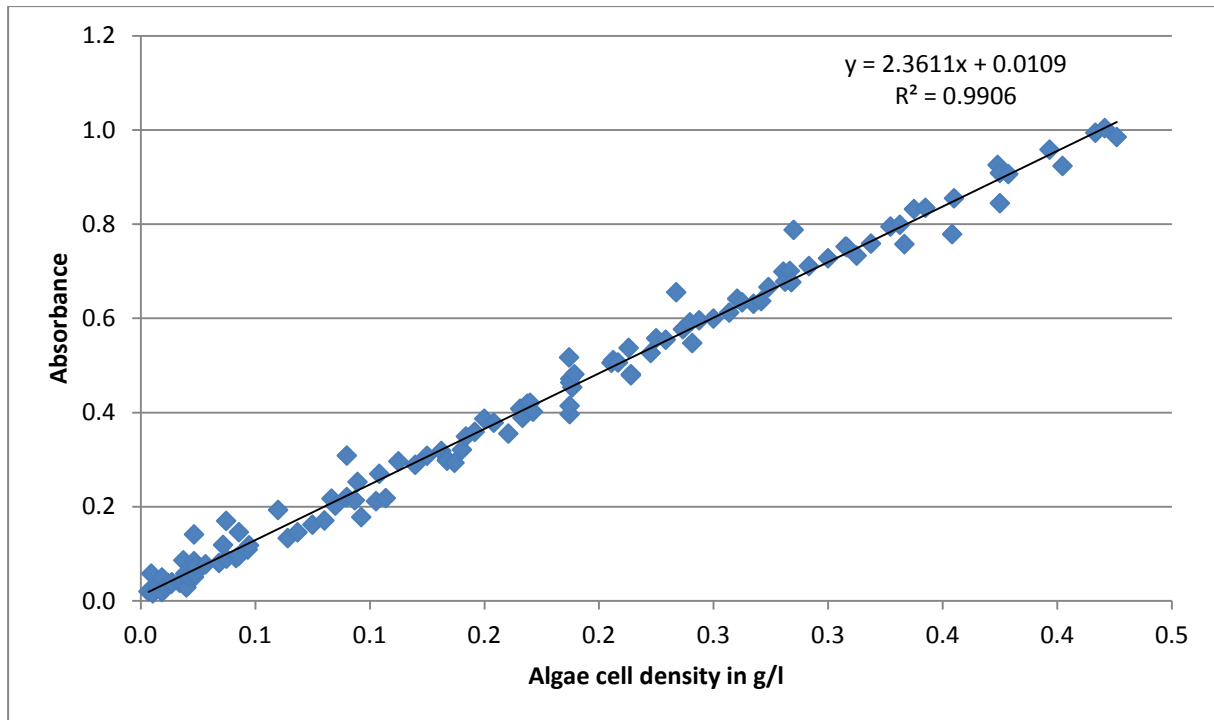


Figure 4.3: Correlation curve used for determination of algae culture density

#### 4.6. Method for testing biomass density

The density of the biomass culture was measured, which allowed the researcher to calculate the volumetric growth, the specific growth and the aerial growth, by taking samples of the suspension. The samples were analysed using a UV-Vis spectrometer at 690 nm. By applying Beer Lambert's law to the correlation curve (Figure 4.3), the density of the biomass was determined.

#### 4.7. Method for testing pH

The pH measurements were taken using a Hanna (Woonsocket, Rhode Island, USA) HI 8424 pH meter (Figure 4.4). The pH meter was used in conjunction with an automatic temperature control, which adjusted the pH to the changes in temperature of the culture. A Hanna HI1286 pH probe was used. It is compatible with algae and other salts found in the culture. The pH meter was calibrated before each usage by using a pH buffer of 4.01 (HI7004L) and 7.0 (HI7007L). After use, the probe was stored in a storage solution (HI70300L) to ensure accurate results.



Figure 4.4: Hanna pH meter used for pH testing

Before sample readings were taken, the probe was rinsed using reverse osmosis water and dried before placing it in the sample. The probe was used to stir the solution as recommended by the pH meter's user manual and left to obtain a reading. The reading was taken only after the hourglass image had disappeared. After the readings had been taken, the probe was rinsed and dried before placing it in the storage solution.

pH readings were taken at the start of each run followed by every six hours. During the addition of pure carbon dioxide, pH readings were taken before and after the addition of carbon dioxide to determine the change in pH caused by the carbon dioxide.

#### 4.8. Performance evaluation

The performance was evaluated by determining the volumetric growth rate, the aerial growth rate and the specific growth rate. The volumetric growth rate was obtained from the difference between the initial and final concentration of biomass in the reactor using Equation 4.2..

$$P_R = \frac{dm_x}{V_R dt_c} \quad [\text{Equation 4.2}]$$

The aerial growth rate was calculated using Equation 4.3 (Converti et al., 2009).



$$P_G = \frac{dm_x}{A_g dt} \quad [\text{Equation 4.3}]$$

where  $dm_x$  is the change in biomass weight in grams over a given time period  $dt$ , with  $A_g$  representing the area required by the reactor.

The specific growth rate was calculated using Equation 4.4 (Converti et al., 2009).

$$\mu = \frac{1}{t} \ln \left( \frac{X_m}{X_0} \right) \quad [\text{Equation 4.4}]$$

where  $X_m$  represents the end concentration of biomass and  $X_0$  the initial concentration of biomass, with  $t$  representing the duration of the run.

The volumetric growth rate is the production of biomass in a given volume of the reactor. This allows the productivity of a reactor to be compared to other reactor design as it does not discriminate against the shape of the reactor. The aerial growth rate determines the productivity per area used by the reactor. Discrimination is created as a horizontal reactor would occupy a smaller area than a vertical reactor of the same volume. The aerial growth is to compare the production of algae biomass to other energy crops which are produced on a specific area of land. The specific growth rate determines the effective productivity of the algae culture as it uses the ratio between the initial and final concentration of biomass in the culture. The volumetric growth rate would be suggested as it allows for the best comparison in productivity although the other growth rates will be calculated.

#### **4.9. Agitation evaluation**

To evaluate the rate of agitation in the reactors used for testing purposes, the reactors were placed under testing condition and filled with water. Food colourant was used to determine the agitation rate to obtain a homogenous system. The duration it took from the addition of two drops of food colourant to the point where a homogenous system was obtain and repeated.

## 5. RESULTS AND DISCUSSION

Initial testing was done in a laboratory to keep the temperature and the light availability constant, while establishing the importance of the variables, because these are two of the most important factors in algae production. The reactors were placed equidistantly from the light source, while the temperature remained at between 15 and 18 °C during the testing period. It must be noted that testing using an outside environment is difficult because changes in temperature, light intensity and light duration are experienced constantly. This affects the results directly and make a factorial comparison very difficult.

### 5.1. pH

A stable pH in the algae culture is one of the most important factors to consider because drastic changes in the pH could cause production problems. These difficulties could include cell damage, changes in the cell composition or contamination of the culture by other species. As seen in the literature review, the main reason for changes in the pH is the formation of  $\text{H}_2\text{CO}_3$  from the equilibrium reaction between water and  $\text{CO}_2$ . The equilibrium shifts to form carbonic acid very fast, as seen in Figure 5.1, where the two-minute and five-minute  $\text{CO}_2$  show the same change in pH – although the two-minute addition has slightly higher values. Readings were taken directly before and after  $\text{CO}_2$  additions.

The two-minute-addition culture is affected more than the five-minute-addition culture because it repeats the change in pH four times (the graph only shows this two of these for comparison purposes) compared to twice daily. These pH changes of approximately 1.5 units create a drastic change in the culture conditions and this affects the algae negatively. This will be discussed later. However, it does appear that, if sufficient light enters the reactor, the algae can recover from the drastic change in the pH of the culture, but that some damage is done. The fact that the timed addition has a higher pH than the continuous addition which is basically buffered shows that all the  $\text{CO}_2$  that was in the culture had been used and that it could have stopped the growth because no  $\text{CO}_2$  was available. Rapid depletion of the  $\text{CO}_2$  levels would cause a large problem when high irradiation levels are introduced and a large amount of ROS forms. This could easily kill the algae and cause severe damage to the photobioreactor. The depletion of  $\text{CO}_2$  would

also stop the production of biomass and reduce the efficiency and directly diminish the profitability of the reactor.

Due to the large changes in the pH during the timed addition of CO<sub>2</sub>, this addition of CO<sub>2</sub> was changed into a continuous addition and buffered using NaHCO<sub>3</sub> at 1 g/l as recommended by Grobberlaar, JU (APPENDIX E). Figure 5.1 shows that both the continuous additions of cultures showed a steady pH throughout the reading and eliminated the drastic changes in culture conditions. The addition of 10 % CO<sub>2</sub> (v/v) enriched air provided a pH of approximately 7.25. This is a very good value for the growth of *C. vulgaris*, although it could be approaching the maximum CO<sub>2</sub> tolerance for the specific strain. The 5 % CO<sub>2</sub> (v/v) enriched air provided a pH of approximately 7.5, which is within the desired range – albeit just barely. The pH of 7.5 can easily be managed by reducing the concentration of the buffer because the buffer that was used is slightly basic – which also increased the pH of the system.

The CO<sub>2</sub> solubility in water at the operation temperatures of 15 to 18 °C are between 0.1789 and 0.1970 grams per 100 ml of water. The maximum solubility is at 0 °C at 0.3346 grams per 100 ml of water. The sorption rate of CO<sub>2</sub> is difficult to determine as it is affected by the partial pressure of the gas, the concentration of CO<sub>2</sub> in solution and the interaction time between the gas and the solution. Once equilibrium has been reached while using enriched air, the sorption will be equal to the rate of fixation by the algae (Lange and Dean, 1973).

Although buffered, the continuous addition of CO<sub>2</sub> showed a much smaller difference in the pH, even when double the volume of CO<sub>2</sub> was added. This would suggest that the large air component of the gas mixture creates a balance that removes excess CO<sub>2</sub> and O<sub>2</sub>. As the excess CO<sub>2</sub> is removed in conjunction with the buffer, the system can maintain a constant pH and thereby provide better conditions for the culture's optimal growth.

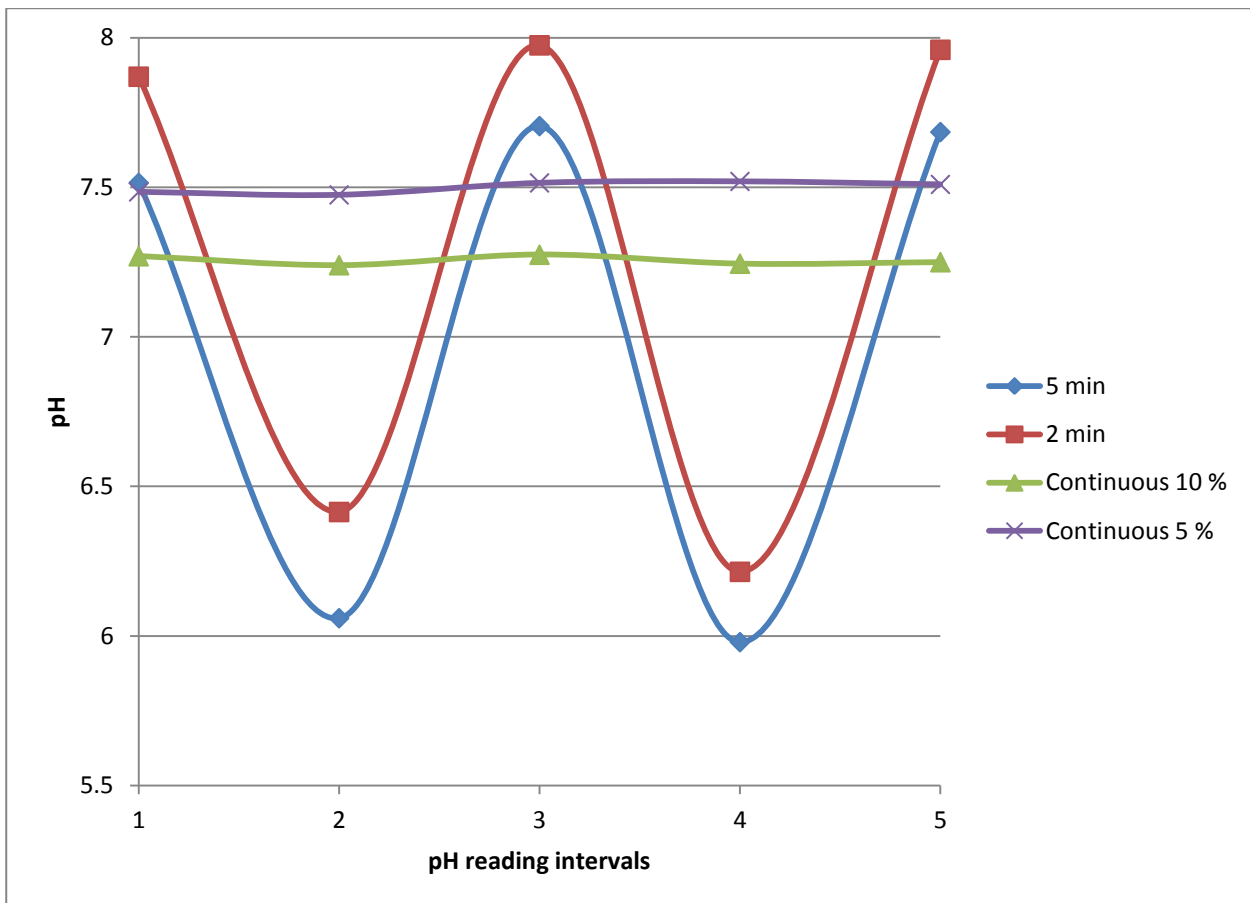


Figure 5.1: pH of the algae culture before and after the addition of CO<sub>2</sub>

When one compares the continuous and timed addition of CO<sub>2</sub>, it is seen that the conditions for the culture are more suitable when a continuous addition was made. In the literature, a timed addition setup is often used, with intermediate additions to control the pH of the culture. The main problem of adding CO<sub>2</sub> to control the pH of the culture is the requirement that the pH of the culture should be measured continuously. This can be achieved easily in a laboratory but on a commercial scale its cost and practical constraints cause a serious problem. A better mass transfer is obtained by adding enriched air continuously.

## 5.2. Agitation inside the reactors

The agitation inside the reactors is of high importance to ensure a good mass transfer and allow for maximum exposure to light. By using food colourant, the rate of agitation can be determined visually.

### 5.2.1. Agitation rate inside the reactor

The mixing rate of the reactors was tested using water and food colourant to measure the time it takes to form a homogeneous system. This gives a good estimate of how the reactor would perform with an algae culture and provides a visual representation of the mixing effect as seen in Figure 5.2. Because the mixing rate was high, the process was repeated several times and the average values used. The RTD-based study was not done in this study and should be included in future studies as it would allow for the comparison with space time and determine dead and bypassing zones.

Table 5.1: Mixing rate using food colourant, showing average time values (in seconds)

Reactor diameter	Gas dispersion unit used	Gas flow rate	Gas flow rate
110 mm		1 litre per min	2.5 litre per minute
	Small	8	7
	Large	8	5
90 mm		0.7 litre per minute	1.5 litre per minute
	Small	6	5
	Large	6	5
50 mm		10 millilitre per minute	20 millilitre per minute
	Small	25	12
	Large	25	12

A homogeneous system is reached very fast, irrespective of the flow rate in most cases. It was noted that, using the 50 mm tubing at 10 ml/min, it took a long time to reach a homogeneous state. The flow rate in the system was in such a state that the mixing occurred mainly on the surface and slowly continued down the length of the tubing. This shows that testing a reactor to ensure a good mixing rate is vitally important in order to ensure that the mass transfer meets the requirements.

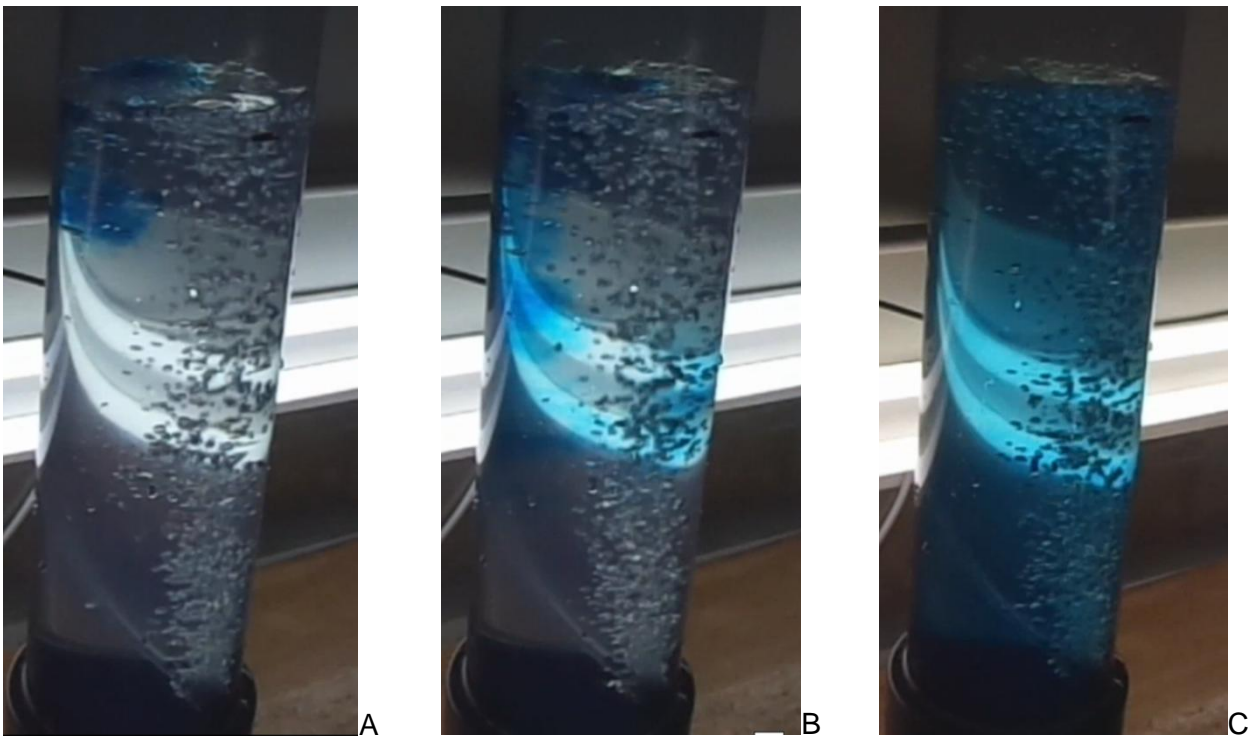


Figure 5.2: Mixing test, showing how the food colourant is mixed through the system to reach a homogenous system

### 5.2.2. The effect of the gas dispersion unit

It was noted that the gas dispersion units used had different effects depending on the diameters of the specific reactor. The 50 mm reactors allowed the smaller units to be positioned lower in the reactor, allowing for a larger mixable volume when compared to the larger units. The larger unit did not fit into the reactor as deeply as the smaller unit and was noted as a mixing dead space was created in the clear tubing area. This is a result of the low flow rate required which caused bubbles to be formed at the top of the dispersion units. Even though a small difference is seen between the mixing times of the two units, the larger dead space that is created in the reactor will allow faster sedimentation to occur in the reactor and could thereby negatively affect the growth of the algae. The sedimentation area could benefit other algae species or especially in the case when a mixotrophic system is used.

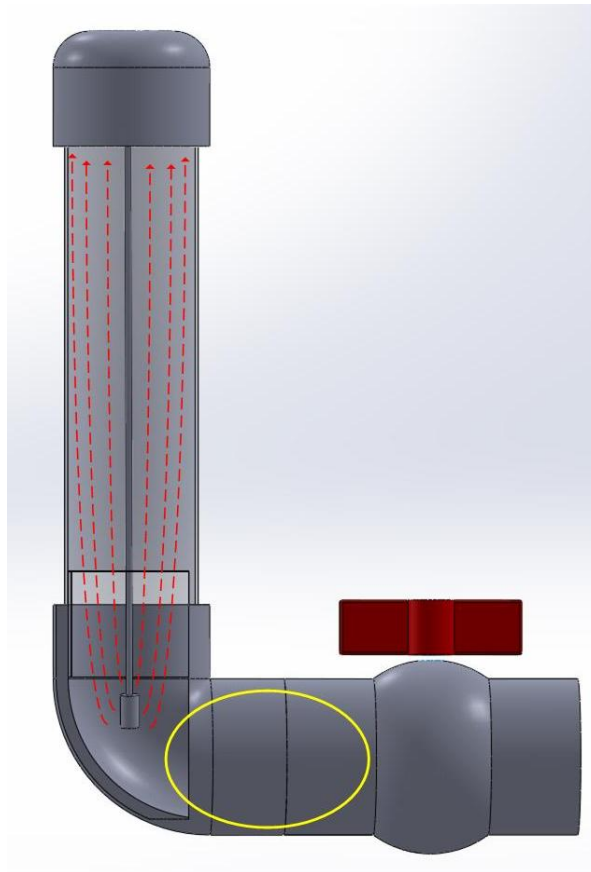


Figure 5.3: A cut-away view showing the gas traveling patterns and the dead zone inside the reactor

The 90 mm tubing did not show any significant visual difference between the different dispersion units. This could be due to the flow rate. Even though the volume per volume per minute flow rate was the same as that in the 110 mm reactors, it was at such a rate that both the units were capable of handling the flow rate with ease. Visually the flow rate did appear to be fairly low, which could have been caused by a sufficient volume per volume flow rate, but an insufficient volume per surface area flow rate or a volume per diameter flow rate. The dead space created by the ball valves increases the need to look at the volume per surface area and the volume per diameter flow rate. The flow rate for the specific volume where bubbles were present was substantially higher than the expected flow rate because a smaller volume was exposed to the bubbles.

The 110 mm tubing showed a clear advantage for the larger gas dispersion unit. Both units fitted into the reactor at the same level. The larger unit created a better overall distribution of bubbles in the reactor, while the smaller unit created a vertical set of bubbles in the reactor. This could be explained by the fact that the larger unit has a larger surface

area on which to create the bubbles and, in this way, it uses less pressure to obtain the same flow rate. The smaller units require a higher pressure than the larger unit. This makes the bubbles expand faster as a result of the lower pressure in the culture, causing them to rise to the surface at a higher rate and reducing the distribution of bubbles in the reactor. This suggests that the smaller unit has surpassed its maximum capacity. In addition, the 110 mm tubing required 56 % more gas flow than the 90 mm tubing.

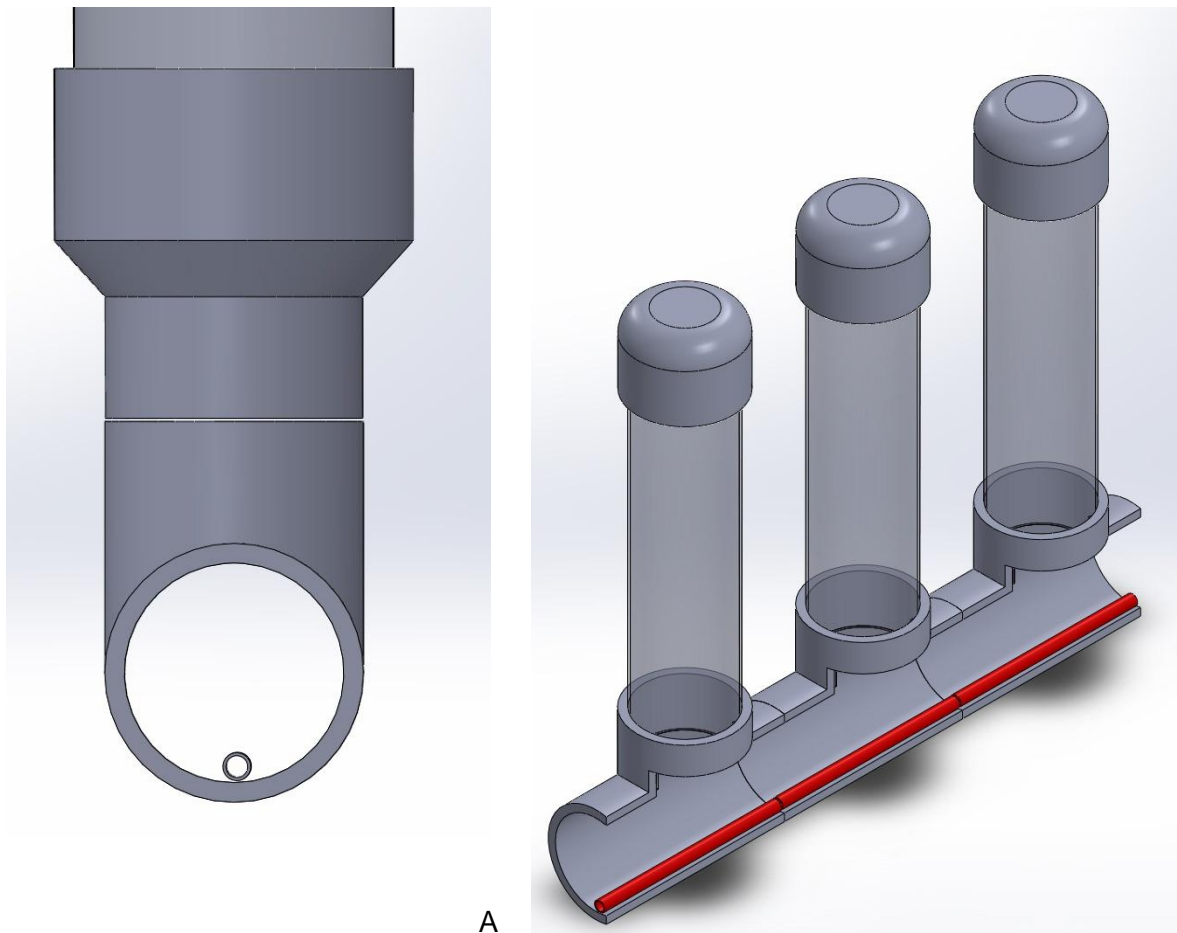


Figure 5.4: Cut-away view of the proposed gas tubing at the bottom of the reactor to eliminate dead zones

The rate of sedimentation was fairly high and unexpected during the initial stages of testing. The sedimentation caused a large amount of algae cells to be deposited in the ball valve region of the reactor. Due to the sedimentation, the algae were mixed twice daily to ensure their maximum exposure to light. Visually it appears that, when the cells are mixed through the agitation of the gas flow rate, a uniform concentration of the culture is achieved. Once the cells get close to the dead space in the bottom of the reactor, they start to move slowly from a higher to a lower concentration up to the point where the concentration of algae exposed to light becomes minimal. The sedimentation of algae is



clearly visible in Figure 5.5 where the reactor on the right-hand side has almost no algae cells in the clear tubing. To ensure maximum growth, it is suggested that some method is added which can continuously or periodically agitate the entire culture to ensure a uniform cell culture throughout the entire reactor. It is suggested that a diffuser hose (Alita Industries, 2011) be placed in the bottom of the reactor (in Figure 5.4), which could be used to add gas continuously or just periodically, eliminating the sedimentation of cells in the reactor, and allowing for better growth.

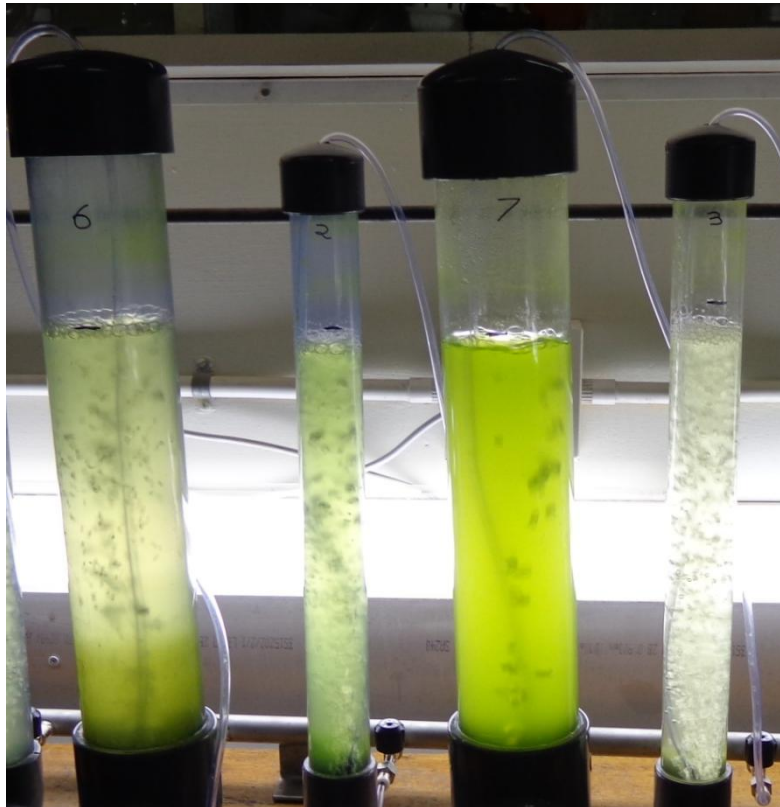


Figure 5.5: The 50 mm and 90 mm reactors used for testing purposes

The rate at which gas is bubbled through the reactor is directly dependant on the diameter and length of the tubing. The 50 mm tubing was operated at a 0.02 vvm flow rate while the 90 mm and 110 mm tubing were operated at a 0.36 vvm flow rate. The difference in flow rates shows that it is important to take the diameter to height ratio of the reactor into consideration. Due to the lower flow rate in the 50 mm tubing and the higher availability of light inside the reactor, it appears that a less enriched air supply could become a limiting factor in production, because there is a limited availability of CO<sub>2</sub>. This creates a massive problem because a higher flow rate would reduce the volume of growth to accommodate the gas volume in the reactor, and increasing the CO<sub>2</sub> enrichment could surpass the

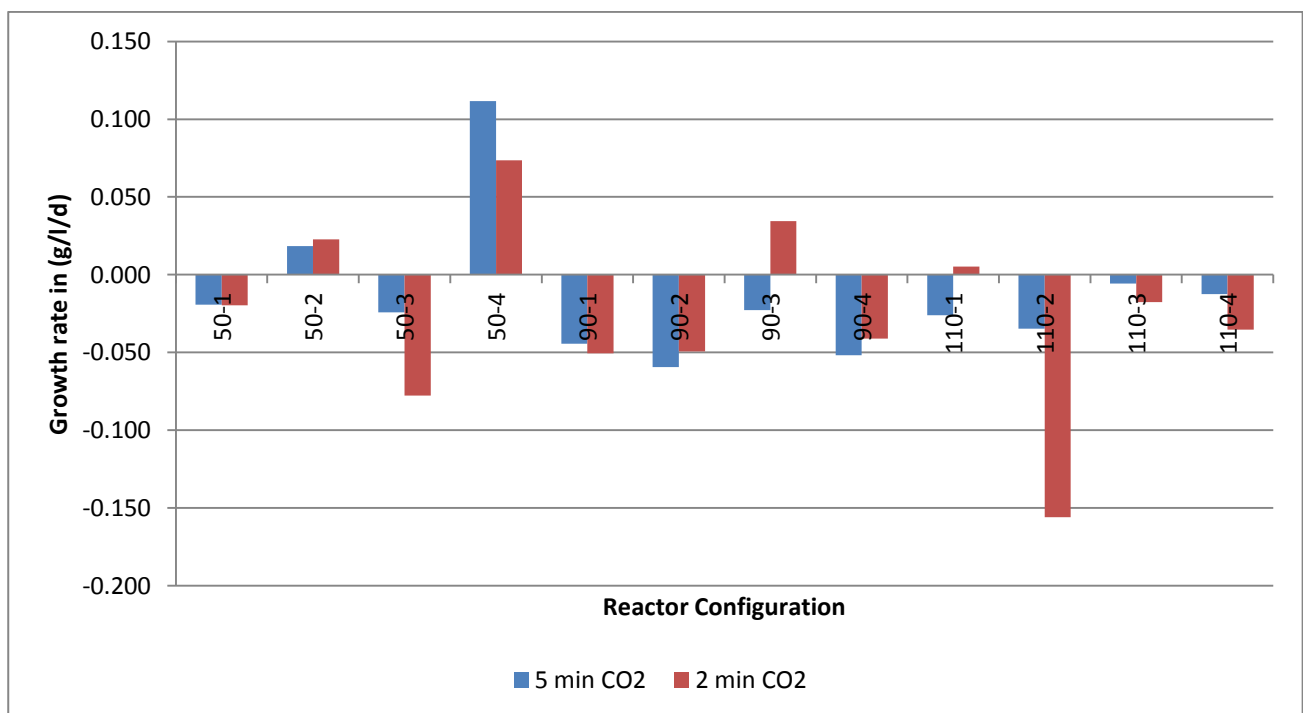
species' threshold for enriched air. The low flow rate would also create pH gradients in the reactor because the CO<sub>2</sub> enrichment would be utilised at the bottom of the reactor and would not be able to supply any CO<sub>2</sub> to the top of the reactor. This would cause the top of the reactor to become unproductive and thereby limit the length of the tubes that could be used. This problem could increase drastically when a high level of illumination is available because it would increase the growth rate and thereby use the available CO<sub>2</sub> at a much higher rate. The best way to overcome the problem would be to add a chemical that reacts with CO<sub>2</sub> (e.g. sodium hydroxide or bicarbonate) and prevents it from exceeding the species' limit and changing the pH as little as possible.

### **5.3. The addition of CO<sub>2</sub> at different rates**

The addition of CO<sub>2</sub> is one of the most important controlling factors in growing algae and the literature reports that it is mainly done using a batch addition process. In the cases where CO<sub>2</sub> is added continuously, the air enrichment is controlled at levels of between 1 and 5 % (v/v). Grobbelaar, JU (APPENDIX E) recommends that a concentration of between 2 and 5 % (v/v) be used for the growth of *C. vulgaris*. Following the literature, we used a timed batch addition of CO<sub>2</sub> initially.

Table 5.2: Factorial design configuration used in figure representations

Reactor configuration	Reactor diameter	Density	Gas Dispersion	Material	Reference
50-1	50 mm	0.5 g/l	Large unit	PVC	Figure 5.6
50-2		1.0 g/l	Small unit	PVC	
50-3		0.5 g/l	Small unit	Acrylic	
50-4		0.5 g/l	Large unit	Acrylic	
90-1	90 mm	0.5 g/l	Small unit	PVC	Figure 5.7
90-2		0.5 g/l	Large unit	PVC	
90-3		1.0 g/l	Large unit	Acrylic	
90-4		0.5 g/l	Small unit	Acrylic	
110-1	110 mm	1.0 g/l	Large unit	PVC	Figure 5.8
110-2		1.0 g/l	Small unit	PVC	
110-3		1.0 g/l	Large unit	Acrylic	
110-4		1.0 g/l	Small unit	Acrylic	

Figure 5.6: Comparison of the volumetric growth rate from the addition of CO<sub>2</sub> at five-minute and two-minute intervals

As stated in the discussion on the changes in the pH, it appears that the smaller reactors that obtain sufficient light could reduce the damage done by the pH changes. It was suggested in the literature that higher density cultures could handle a higher concentration

of CO<sub>2</sub> much better than lower density cultures could. From the results that were obtained, one may conclude that sufficient light is required to recover from large fluctuations in pH and that the density could play a role in the severity of the damage that is done to the culture. In considering Figure 5.6, it appears that the configuration of the density with the gas dispersion unit has a very large effect on the growth rate. The growth also fluctuates severely between configurations in the two-minute additions when compared to the configurations in the five-minute additions, showing that the increase in pH fluctuations causes different effects in different reactors.

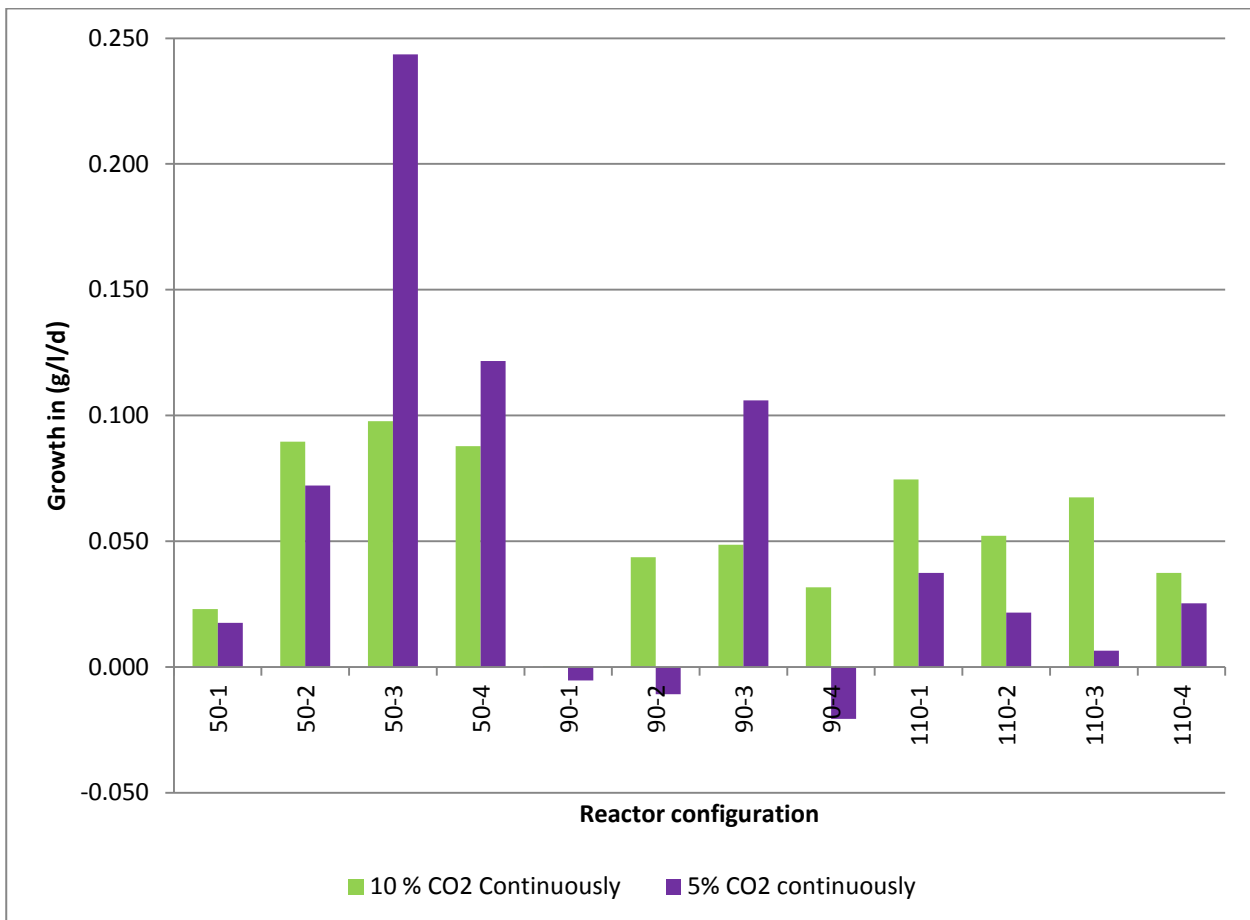


Figure 5.7: Comparison of the continuous addition of CO<sub>2</sub> at levels of 5 and 10% (v/v)

The continuous addition of CO<sub>2</sub>, seen in Figure 5.7, shows a positive biomass production in most of the reactor configurations which are being compared. From the results one can see that the 110 mm tubing (configuration 9–12) at high density performed the best at 10 % enrichment, while the 90 mm tubing at low density (configuration 5,6 and 8)

performed the best at 10 % enrichment. This shows that there could be a correlation between the density of the culture and the gas flow rate (vvm).

The 50 mm tubing (1–4) has a higher growth rate, showing that the light availability inside the reactor does play a large role. The 10 % enrichment shows very little difference in configuration 2-4, while the PVC tubing shows a slight decrease in production at 5 % enrichment. The 50 mm acrylic tubing (3,4) shows a large increase in production at 5 % enrichment which correlates with the suggested range of enriching of between 1 and 5 % for optimal growth. From the results it appears that the 5 % enrichment is only viable on a laboratory-scale reactor and that industrial reactors would require a higher enrichment level to obtain the desired production.

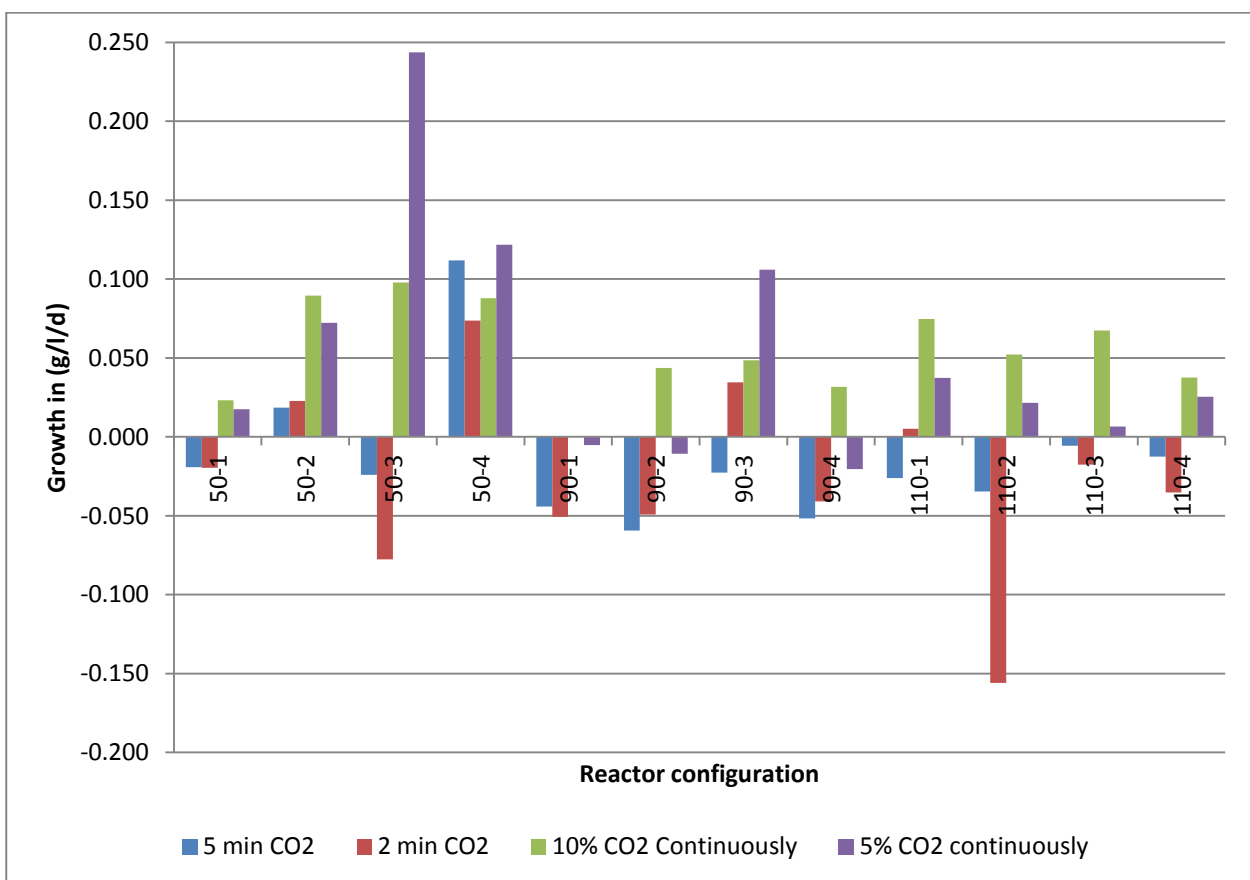


Figure 5.8: A comparison between continuous CO<sub>2</sub> addition and timed batch addition

The continuous addition of CO<sub>2</sub> is beneficial to the production of algae. By comparing the growth data in Figure 5.8, it is clear that the continuous addition gives a better production of biomass in all but one reactor configuration. In reactor configuration 4, the results of all the CO<sub>2</sub> addition methods are very close although the 5 % enrichment still performed best.

When one considers the growth and the consistent pH in Figure 5.1, it is evident that the use of continuous CO<sub>2</sub> addition is the only choice for the production of algae in a PBR.

#### 5.4. Results for the 50 mm diameter reactor

The two 50 mm reactors were tested by using their own factorial design because the flow rate of the gas inside the reactor was only 0.02 vvm which is significantly different from the 0.36 vvm used in the 90 mm and 110 mm reactors. Due to this difference it was decided that the 50 mm reactors would not be compared directly with the other tubing diameters as the effect of the gas flow rate is unknown.

Table 5.3: Factorial design configurations used for illustration purposes in the figures used for comparisons

Configuration 50 mm	Density	Gas Dispersion	Material	Reference
1	0.5 g/l	Small Unit	Effect of construction material evaluation	Figure 5.9
2	1.0 g/l	Small Unit		
3	0.5 g/l	Large Unit		
4	1.0 g/l	Large Unit		
1	0.5 g/l	Effect of gas dispersion unit's size evaluation	PVC	Figure 5.10
2	1.0 g/l		PVC	
3	0.5 g/l		Acrylic	
4	1.0 g/l		Acrylic	
1	Effect of algae density evaluation	Small Unit	PVC	Figure 5.11
2		Large Unit	PVC	
3		Small Unit	Acrylic	
4		Large Unit	Acrylic	

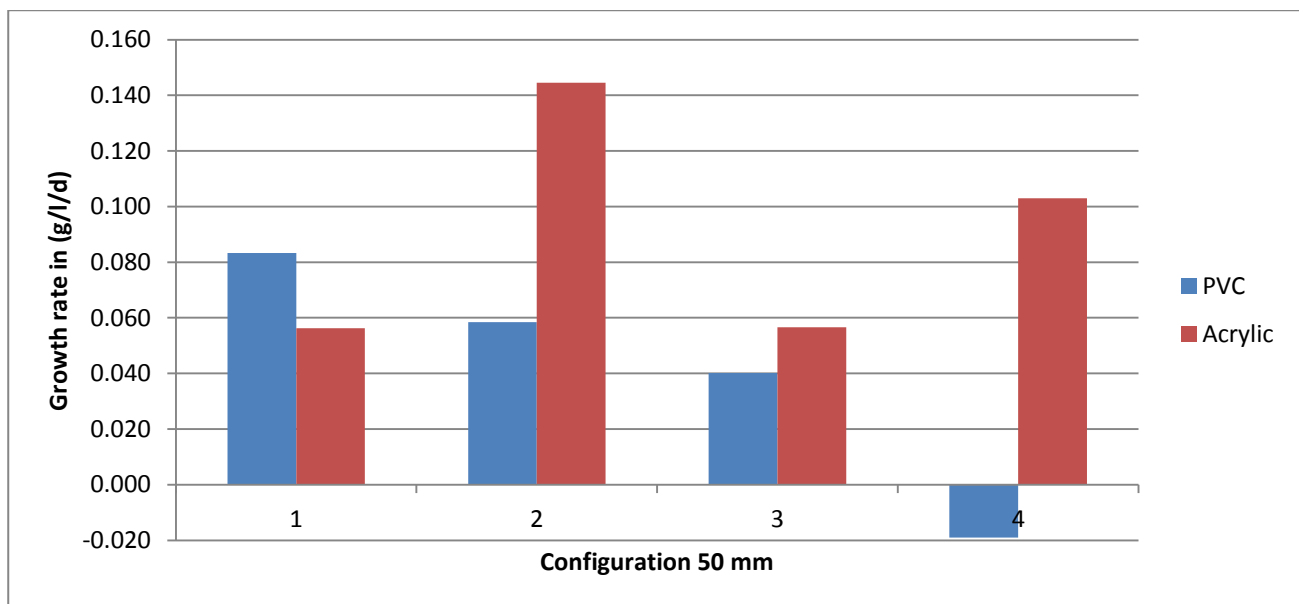


Figure 5.9: Comparison of the volumetric growth rate between PVC and acrylic tubing with a diameter of 50 mm

The results of the 50 mm reactor show that the acrylic tubing has an advantage over the PVC tubing because three-quarters of the configurations have a higher volumetric growth rate. The PVC tubing has a blue tint to it which would absorb a large portion of light compared to the acrylic tubing. The tint in the PVC (seen in Figure 5.5) could be beneficial when over-saturated light conditions are prevalent because this would decrease the amount of photons that enter the reactor and thereby reduce the possibility of cell damage.

In a light-saturated environment, the acrylic tubing would be a better option because it would allow better light penetration. It can be seen in configuration 2 and 4 that the acrylic tubing allows more light photons through for better production when using higher concentrations. In both cases the PVC allowed better production at lower algae concentrations. This shows that the intensity of the light which is blocked by the blue tint is either fairly high or that the specific wavelengths which are blocked have an important influence on the growth of the algae and, by increasing the algae concentration, insufficient light is available for the production of biomass.

At an algae concentration of approximately 0.5 g/l, both materials show a similar growth rate. When considering the cost of the materials, a higher production of biomass is desired because it would increase production for the same capital cost. From the results, it is recommended that testing be done on the site where the PBR will be situated in order to determine which material would provide the best production capacity for the specific

environmental conditions because it is fairly difficult to reproduce all the possibilities in a laboratory environment. The specific conditions under which the materials were tested lead one to the conclusion that the acrylic tubing is the superior choice when constructing a reactor of 50 mm tubing.

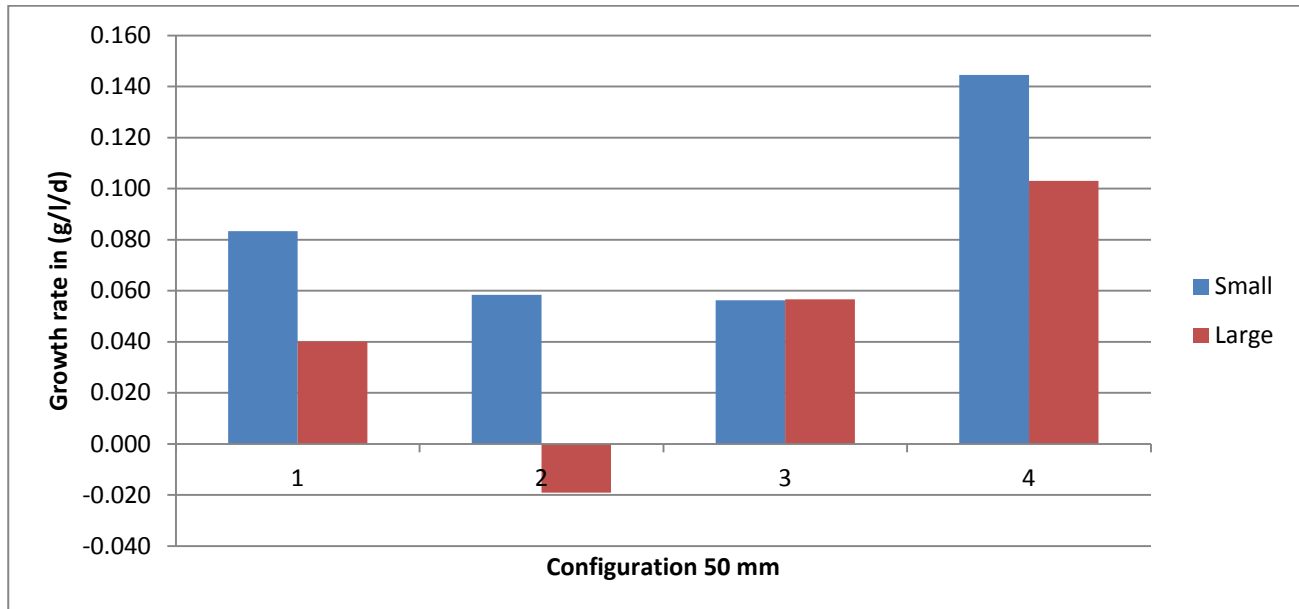


Figure 5.10: Comparison of volumetric growth using different sizes of gas dispersion units in a 50 mm reactor

The results show that in the 50 mm reactor it is beneficial to use a small gas dispersion unit. This is due to the low gas flow rate required for adequate mixing, while having the maximum amount of the culture possible exposed to a light source. In Figure 5.3, the mixing dead zone is shown by a yellow oval. This refers to when a large gas dispersion unit is used in the 50 mm tubing. This dead zone is increased in the vertical tube because the bubble formation does not start low enough and reduces the mixing ability. As the mixable volume is reduced, the ability of the algae to form a sediment at the bottom of the reactor is increased. The increase in the sedimentation rate reduces the time that the algae are exposed to light and thereby directly affects the production.

The increase in the sedimentation rate could be reduced by using longer tubing because this would allow for bubbles to form a lower point in the gas dispersion unit, which would increase the mixable volume and would thus give the same or better results when compared to the small gas distribution unit.



Both of the small gas dispersion units have higher production than the larger units with an increase in algae density, while the large gas dispersion unit shows an increase in production for only the PVC reactor. This could be due to the higher concentration in this unit because it required a longer time period before the algae concentration which had been exposed to light dropped to a level where production was significantly reduced. Because the gas dispersion units were not used to their maximum flow capacity, it seems that the placement of the gas dispersion unit is of vital importance. Therefore it is recommended that a gas dispersion unit should be situated at the bottom of the reactor to prevent the sedimentation – even if it is very low as suggested in Figure 5.4.

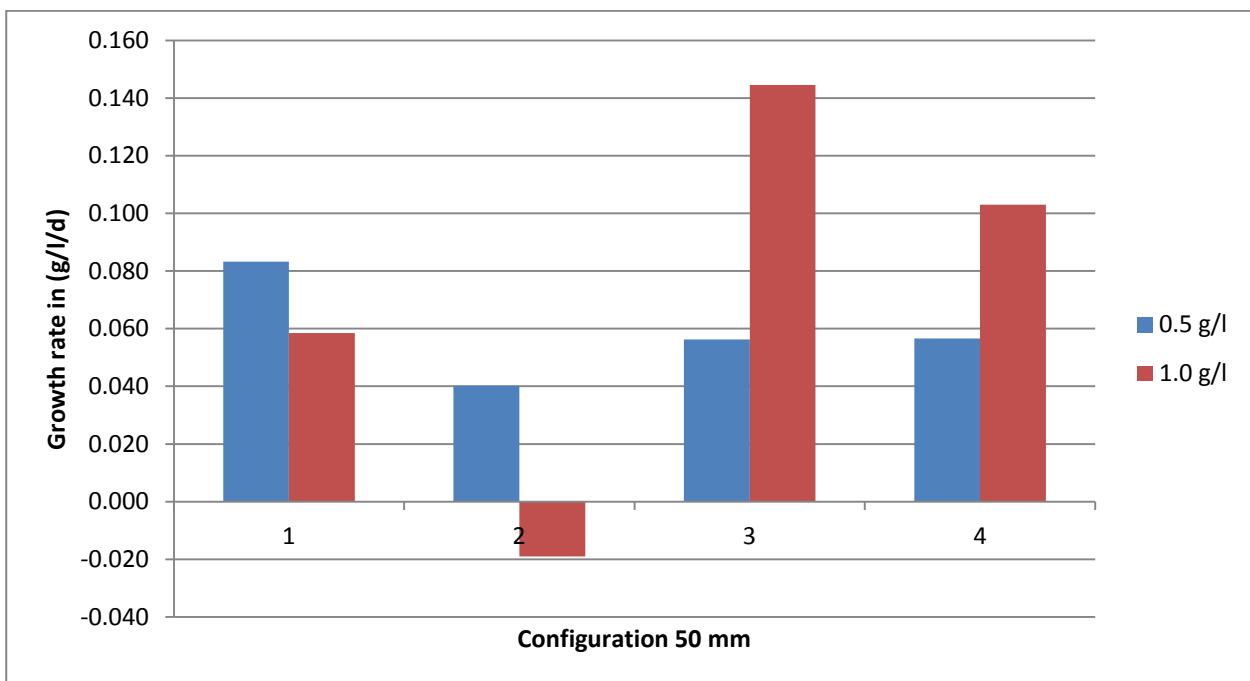


Figure 5.11: Comparison of volumetric growth at different densities in 50 mm reactors

Figure 5.11 indicates that the production of algae at a concentration of 0.5 g/l is similar, irrespective of the material or gas dispersion unit that is used. An increase in algae concentration to 1.0 g/l shows a major difference in the production of biomass. The acrylic tubing has a production of more than double that of the PVC tubing – and in both tubes a clear preference is seen for the smaller gas dispersion unit.

From the results obtained for the growth of *C. vulgaris* using 50 mm tubing, the best combination is the use of acrylic tubing with a small gas distribution unit and a biomass concentration of 1.0 g/l. The growth rate on the combination compares very well with the growth rate shown in Table 2.2 which has a growth rate of 0.17 g/l/d. This shows that the

production rate of the reactor is in a comparable range to the values indicated in the literature.

### 5.5. Results for the 90 mm diameter reactor

The 90 mm reactors have much smaller volume than the 110 mm reactor, which has a similar foot print. The overall results of the 90 mm tubing showed a negative growth. As stated previously, it appears that insufficient mixing of the culture occurred as the 0.36 vvm flow rate was insufficient for the surface area to volume ration. It is thus suggested that the tubing and diameter-to-flow rate be studied to ensure enough mixing and CO<sub>2</sub> addition to the culture is achieved.

Table 5.4: Configurations of graphs produced for 90 mm reactors

<b>Configuration 90 mm</b>	<b>Density</b>	<b>Gas dispersion</b>	<b>Material</b>	<b>Reference</b>
1	0.5 g/l	Small Unit	Effect of construction material evaluation	Figure 5.12
2	1.0 g/l	Small Unit		
3	0.5 g/l	Large Unit		
4	1.0 g/l	Large Unit		
1	0.5 g/l	Effect of gas dispersion unit's size evaluation	PVC	Figure 5.13
2	1.0 g/l		PVC	
3	0.5 g/l		Acrylic	
4	1.0 g/l		Acrylic	
1	Effect of algae density evaluation	Small Unit	PVC	Figure 5.15
2		Large Unit	PVC	
3		Small Unit	Acrylic	
4		Large Unit	Acrylic	

If the gas flow rate had been at an incorrect level for the diameter and length of the tubing, it could have caused a higher rate of sedimentation. This would also suggest that, due to an incorrect flow rate, the mixing dead zone was increased dramatically. Even though the results in Table 5.1 show similar mixing times for the food colourant, the effect that mixing has on the living algae cells is unknown and the culture might not have been mixed sufficiently enough to obtain reliable results.

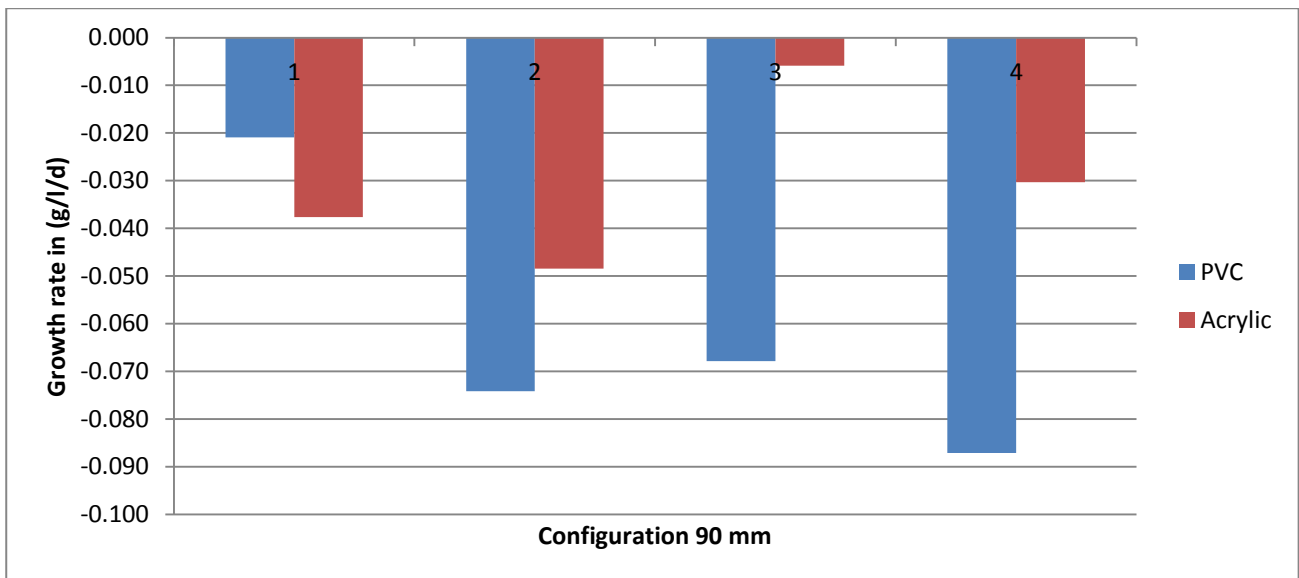


Figure 5.12: Comparison of the materials used in 90 mm reactors

Similar to the 50 mm reactors, the 90 mm reactors show an advantage when the acrylic tubing is used. Only the first configuration in Figure 5.12 performed better using PVC. The negative growth, in conjunction with the incorrect gas, indicated that the flow rate error could be attributed to the lower light penetration in the reactor as well as the blue tint on the PVC tubing which appeared to have had an effect on the growth in the 50 mm reactors.

In configuration rates 3 and 4 in Figure 5.12, a dramatic increase is seen in the acrylic tubing. This would suggest that the blue tint found in the PVC tubing does have a large effect on the growth rate of *C. vulgaris* when the environmental conditions are appropriate.

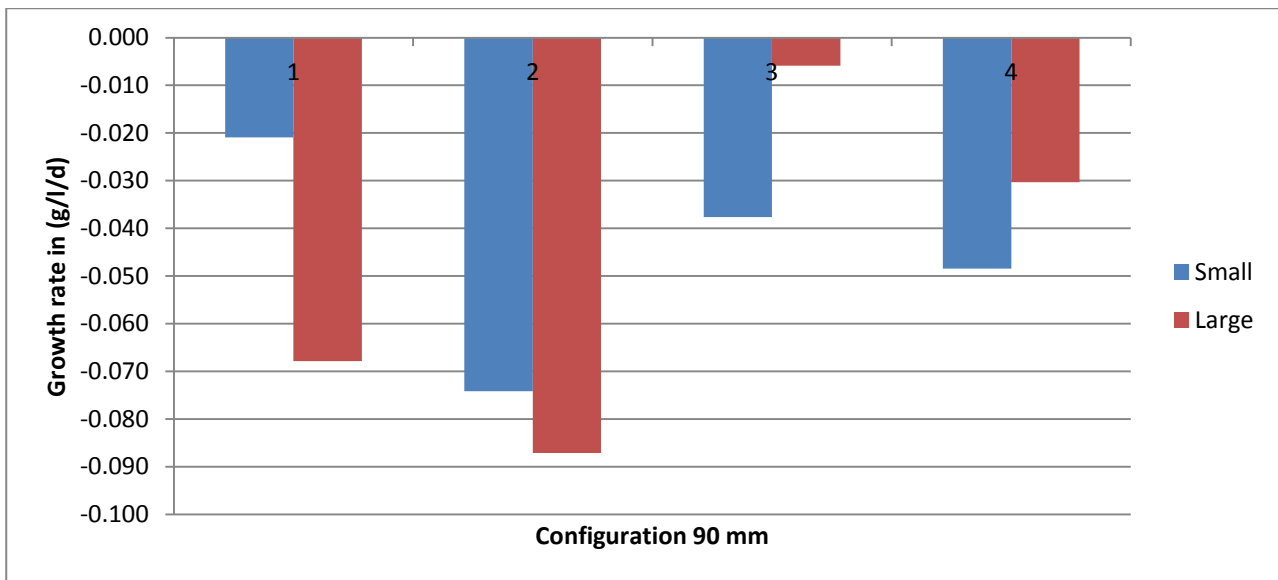


Figure 5.13: Comparison of gas dispersion unit size for 90 mm reactors

The results in Figure 5.13 show the size of the preferred gas dispersion unit for each material. Configurations 1 and 2 (which use the PVC tubing) show a clear preference for the smaller unit, while 3 and 4 show a preference for the large unit.

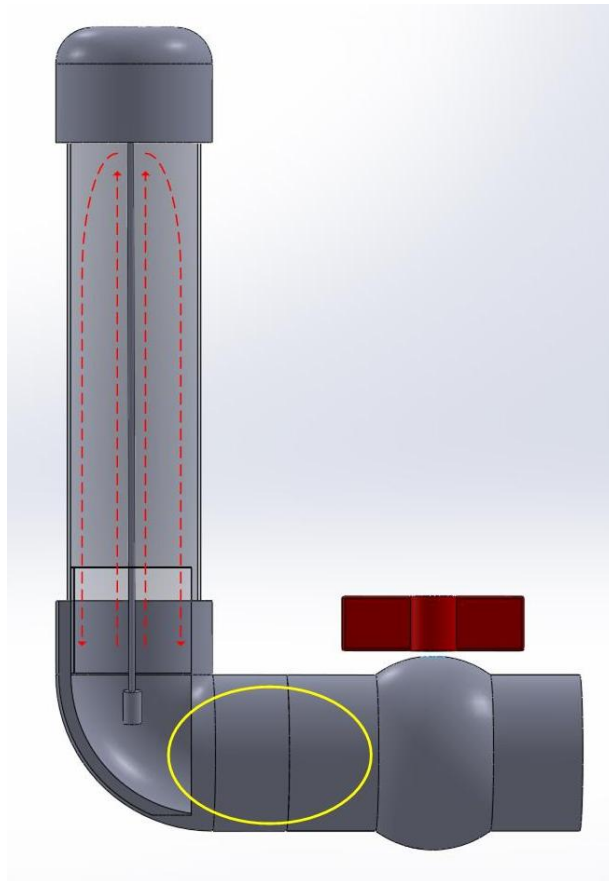


Figure 5.14: Suggested flow pattern in the 90 mm PVC reactors with a small gas dispersion unit

The only possible explanation for this is that the light availability in the reactor is such that the PVC tubing preferred a smaller column of gas that created a circular flow inside the reactor, as seen in Figure 5.14. The acrylic tubing prefers the mixing inside the reactor to be complete (as in Figure 5.3) because it allows for the fast transition between the light and dark areas of the reactor.

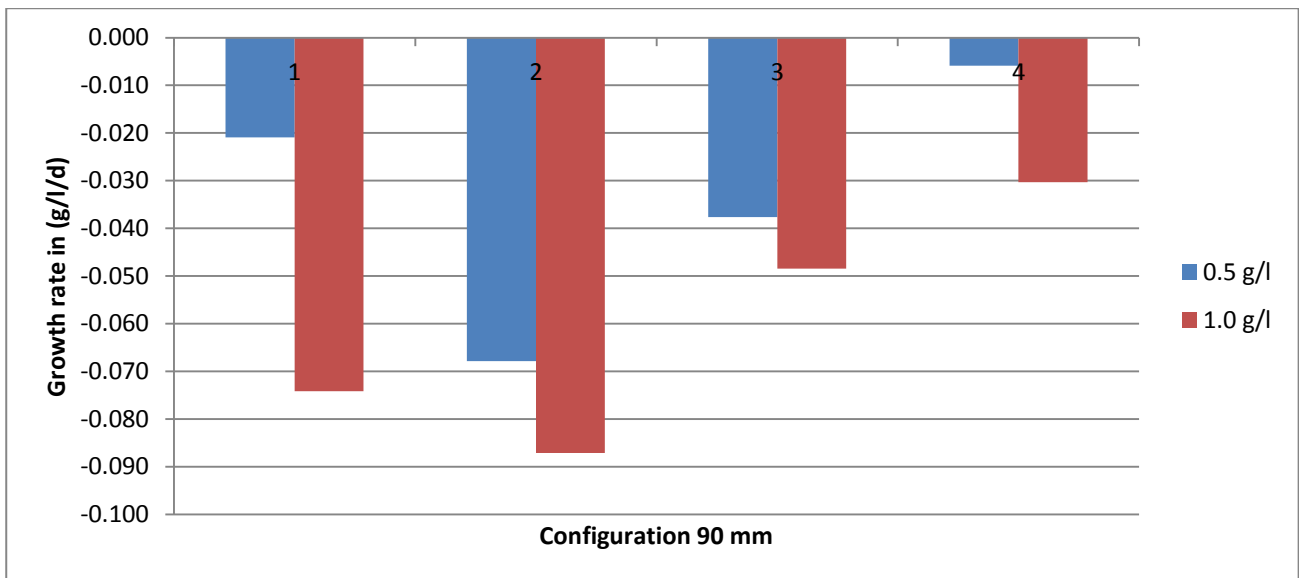


Figure 5.15: Comparison of the growth rate using different culture densities in 90 mm reactors

The 90 mm reactors show a preference for a lower concentration of culture as seen in Figure 5.15. This may be due to the mixing patterns and light availability because the higher concentration of culture does not obtain sufficient light and so the production is lower.

From the overall results for the 90 mm reactors, it can be concluded that the acrylic tubing with a low density and a large air dispersion unit would provide the best results. Nevertheless, this could change if the gas flow rate was increased to provide a better flow rate in comparison to the tubing diameter and length that is used.

## 5.6. Results for the 110 mm diameter reactors

The 110 mm tubing is the tubing with the largest diameter that can be obtained for a fair comparison between it and the acrylic tubing. The PVC tubing continues up to a diameter of 160 mm, as seen in APPENDIX B. The acrylic tubing is available in a many larger diameters but they require the manufacture of special fittings to be used in the building of PBRs and so the capital cost of larger diameter acrylic tubing in PBRs would increase substantially and could make them too expensive for the use in biomass production.

Table 5.5: Configurations of data used in the graphs produced for 110 mm reactors

Configuration 110 mm	Density	Gas dispersion	Material	Reference
1	0.5 g/l	Small Unit	Effect of construction material evaluation	Figure 5.16
2	1.0 g/l	Small Unit		
3	0.5 g/l	Large Unit		
4	1.0 g/l	Large Unit		
1	0.5 g/l	Effect of gas dispersion unit's size evaluation	PVC	Figure 5.17
2	1.0 g/l		PVC	
3	0.5 g/l		Acrylic	
4	1.0 g/l		Acrylic	
1	Effect of algae density evaluation	Small Unit	PVC	Figure 5.18
2		Large Unit	PVC	
3		Small Unit	Acrylic	
4		Large Unit	Acrylic	

The 110 mm reactors are very close in diameter to the 90 mm reactors but, as seen in the results, the small difference in diameter does allow for a large difference in production. The interaction between the variables and the tubing diameter plays a large role in the productivity of the PBR and the profitability of the system. It is therefore only possible to compare the interactions for the PBRs but ultimately the PBR has to be tested for optimisation purposes because it would be in its finished state when used in a pilot, small-medium scale or large scale plant.

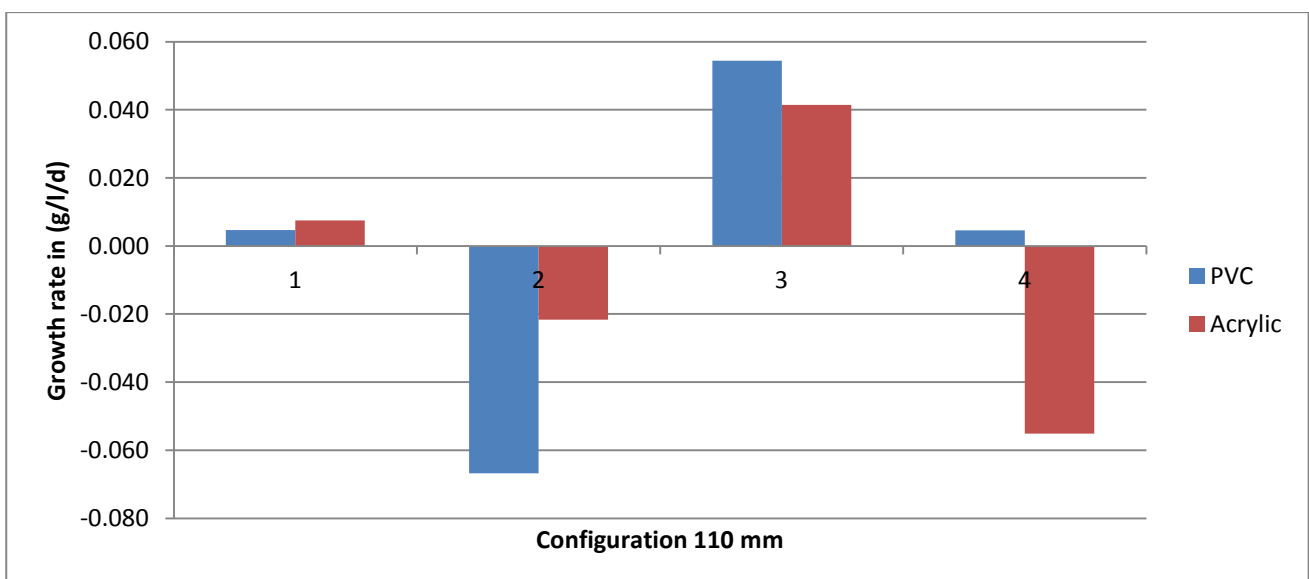


Figure 5.16: Comparison of tubing materials in 110 mm reactors

Figure 5.16 shows that acrylic tubing gives a better result than PVC when a small gas distribution unit is used, while PVC gives a better result when using a large gas distribution unit. When one compares these results to those in Figure 5.12, the opposite is seen because the acrylic preferred the larger unit and a small difference is seen when using the small unit. This shows that the factors are influenced by what is considered to be a constant factor i.e. the gas flow rate. It can be concluded that the tubing diameter to volume-to-gas flow rate ratio is of utmost importance, with the gas distribution unit being of secondary importance as it mixes the culture in a different orientation.

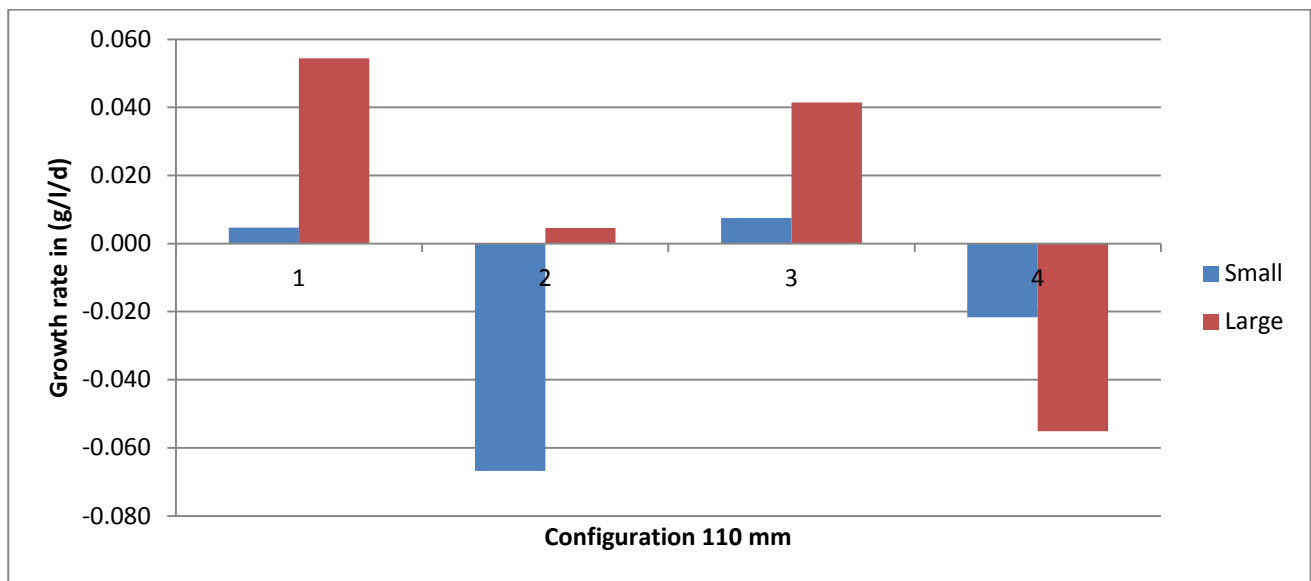


Figure 5.17: Comparison of gas dispersion units in 110 mm reactors

The gas distribution unit plays a larger role as the gas flow rate is increased. Figure 5.17 indicates that a preference is starting to show for the larger gas dispersion unit. In the 4<sup>th</sup> configuration, the smaller unit is preferred. This could be due to preferred circular mixing pattern, similar to what was seen in the 90 mm reactors which were illustrated in Figure 5.14. It was observed that the gas flow rate was at such a level that the large gas dispersion unit produced bubbles from its entire surface, while the small unit produced bubbles from its entire surface in the 90 mm reactors. The small units produced a higher pressure bubble column which travelled at a higher rate to the surface of the culture, almost creating a small fountain. The large unit, on the other, created a large number of small bubbles with large surface areas, with bubbles present in the entire visible area of the column, allowing for better mixing and mass transfer. If higher flow rates were required, the small gas dispersion unit would be under even higher pressure and could



possible start to damage the cells in the culture. Depending on the PBR configuration, the tubing length and the diameter, the large unit which was tested could start to experience the same problems currently experienced by the small unit.

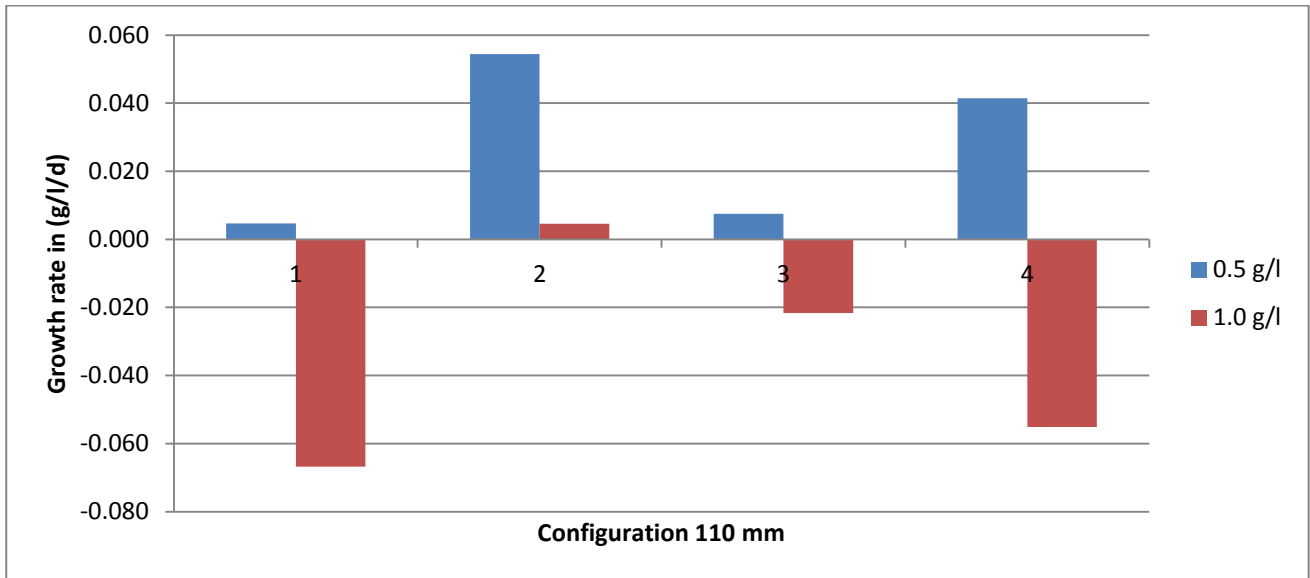


Figure 5.18: Comparison of different culture densities in 110 mm reactors

Both materials in the 110 mm reactors show a preference for lower culture densities. The high tubing diameter would start to influence the availability of the photons inside the reactor. Coupled with the higher density, a smaller percentage of cells would be exposed to light and would therefore have a high production in one area and a high death rate in another area. Figure 2.8 shows how the light intensity inside the reactor changes according to the penetration distance and the side that is illuminated. By increasing the culture density inside the reactor, the profile changes and could become very small at a small penetration distance.

## 5.7. Growth per area

Table 5.6: Configurations used for figure generation in growth rate per area and specific growth rate

Configuration per diameter and material	Density	Gas Dispersion	Reference
1	0.5 g/l	Small unit	Figure 5.19
2	1.0 g/l	Small unit	Figure 5.20
3	0.5 g/l	Large unit	
4	1.0 g/l	Large unit	

The growth per area is calculated to determine the viability of the production of algae biomass on a given surface area in order to compare it with other sources of biomass energy because this is the norm, with most other energy crops being represented in terms of ton per hectare. One problem with using this type of calculation is that it is a relative value and the results would differ if the height were changed. By using larger diameter tubing, a small change made to the area required for the reactor would make a large difference to the volume of the reactor. As the ground area of the reactors with the same tubing diameter is the same, the results would reach the same conclusion as the growth rate per volume which has been discussed – because the volumes were kept the same for the purpose of comparison.

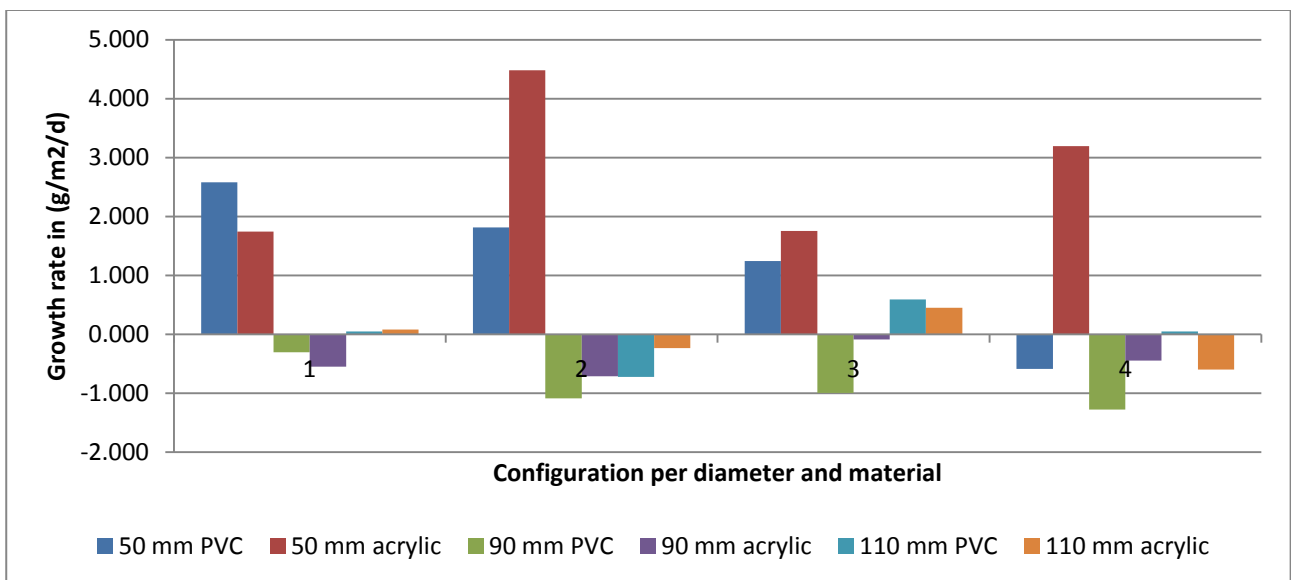


Figure 5.19: Comparison of all the reactors' growth rate per surface area in  $\text{g/m}^2$

The larger diameter reactors have shown a much lower growth rate per area, while the growth rate per volume of the 50 mm and 110 mm reactors in configuration 3 is very similar. The main reason for the lower growth per surface area is the addition of the valves or base in the case of a module and this adds a large ground area. In this case the ground area is much lower than it would be in a module because the entire space required by the module would be used for calculation purposes.

When one compares Figure 5.19 and Figure 5.20, it is seen that the volumetric growth rate has the exactly same curve shapes but that they are enhanced, especially for the larger diameter reactors. As stated previously, the diameter of the reactor makes a small difference to the area that is required but a large difference to the volume, as the

volumetric growth does not take into consideration any space that is not used for production purposes. The error is increased when calculating the ground area because the area between the reactor and light sources has to be taken into account. This would not be the case if sun light were used. By adding this area, the ground area production of a PBR is significantly reduced and the addition affects the larger diameter reactors more negatively than a smaller diameter reactor because a large area is added to the calculation.

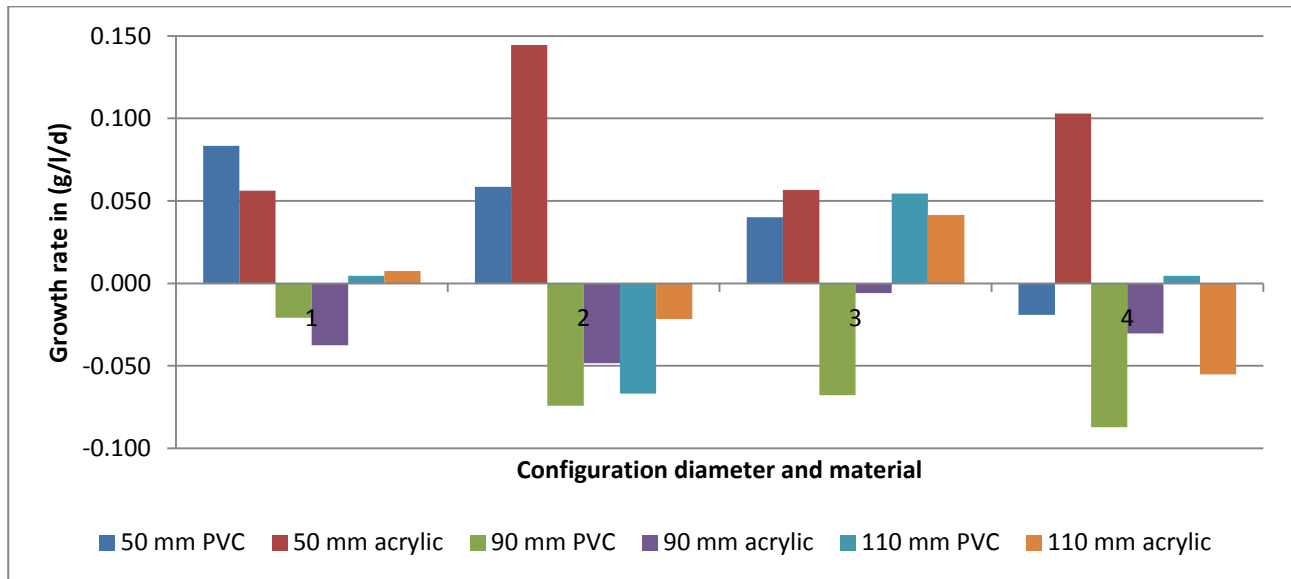


Figure 5.20: Comparison of all the reactors' growth rate per volume in (g/l/d)

The use of growth per area is more suitable for open pond type systems. Because the area they cover is important, a high surface area to volume ratio is sought. PBRs obtain a high surface area to volume ratio by manipulating the surface area and volume of the tubing to obtain the same effect. It is therefore a better choice to compare production per volume when considering PBRs, especially when an area is unproductive as a result of the design of the reactor or the light source that was used. The fact that the depth of an open pond and energy crops remain fairly constant also contributes to a better comparison, while the height of PBRs are changed constantly. This makes comparison unfair because the height can be manipulated to make the results obtained seem more impressive, while the volumetric production remains the same.

## 5.8. Specific growth rate

Table 5.7: Configurations used for figure generation in specific growth rate per diameter

Configuration per diameter	Density	Gas Dispersion	Reference
1	0.5 g/l	Small unit	Figure 5.21
2	1.0 g/l	Small unit	Figure 5.22
3	0.5 g/l	Large unit	Figure 5.23
4	1.0 g/l	Large unit	

The specific growth rate is a measurement of the ratio of production per day because the natural log of the end concentration is divided by the initial concentration. Using the specific growth rate one can determine whether or not the biomass production at a specific concentration is at its best level. The specific growth rate is very important for the costing of the reactor because the costing is directly affected by the growth of the biomass.

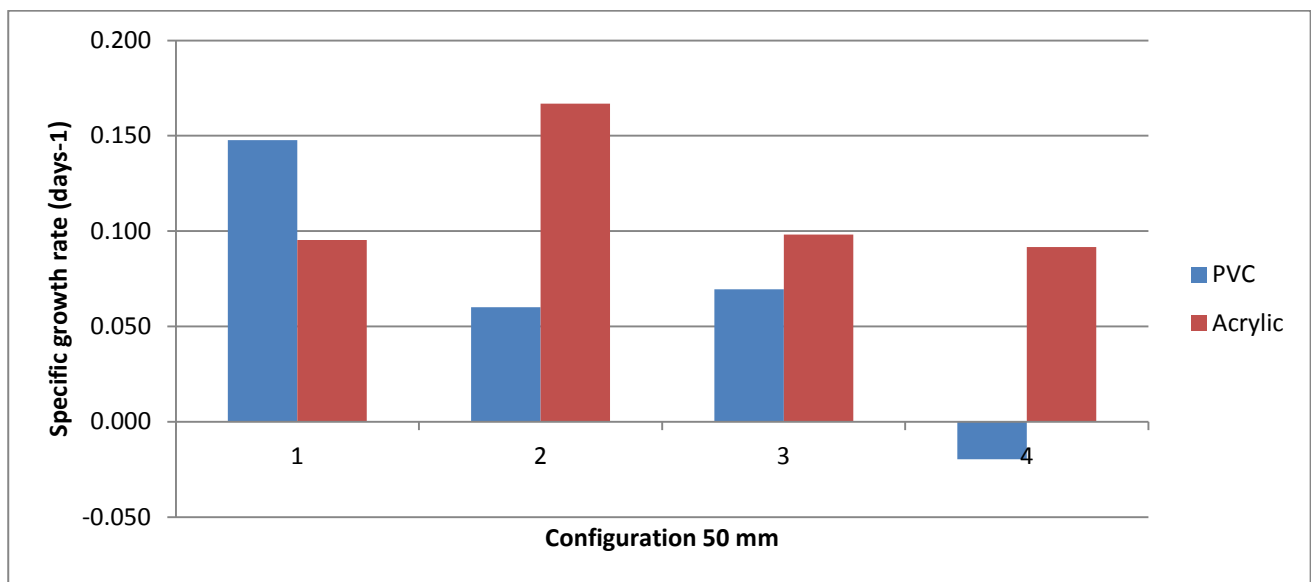


Figure 5.21: Specific growth rate comparison of 50 mm reactor materials

The acrylic curve in Figure 5.21 shows that three of the four configurations have a same specific growth rate, while the acrylic curve in Figure 5.9 shows that only configurations 1 and 3 have a similar growth rate. This shows that the production of biomass is the same in these three cases even though the biomass is at a different concentration in one of the configurations. By using the specific growth rate, it is clear that configuration 2 provides the best production and that configuration 4 has a similar production rate to configuration 1 and 3 when comparing cell productivities. The specific growth rate also allows for a fair comparison between different materials as it removes the culture density from the equation. It is seen that the PVC reactor with configuration 1 and acrylic reactor with

configuration 2 have similar results and would be the best configurations for further research or scale up.

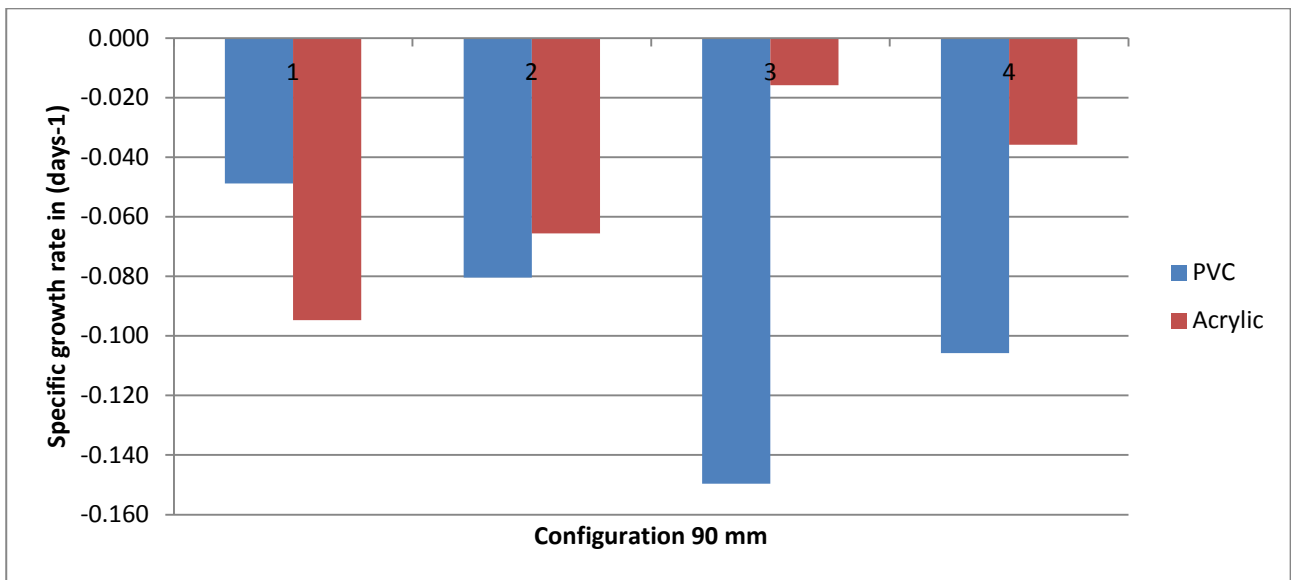


Figure 5.22: Specific growth rate of 90 mm reactors comparing materials

The 90 mm reactors all had a negative growth rate and even though negative production is undesired, the use of specific growth rates shows which configurations are most likely to have a positive production. From Figure 5.22 the reactors that are most likely to have a positive production are configuration 1 of the PVC and configurations 3 and 4 of the acrylic reactors. By using this information, researchers may use these reactors to explore the cause of the negative production and thereby allow changes to be made for a viable plant.

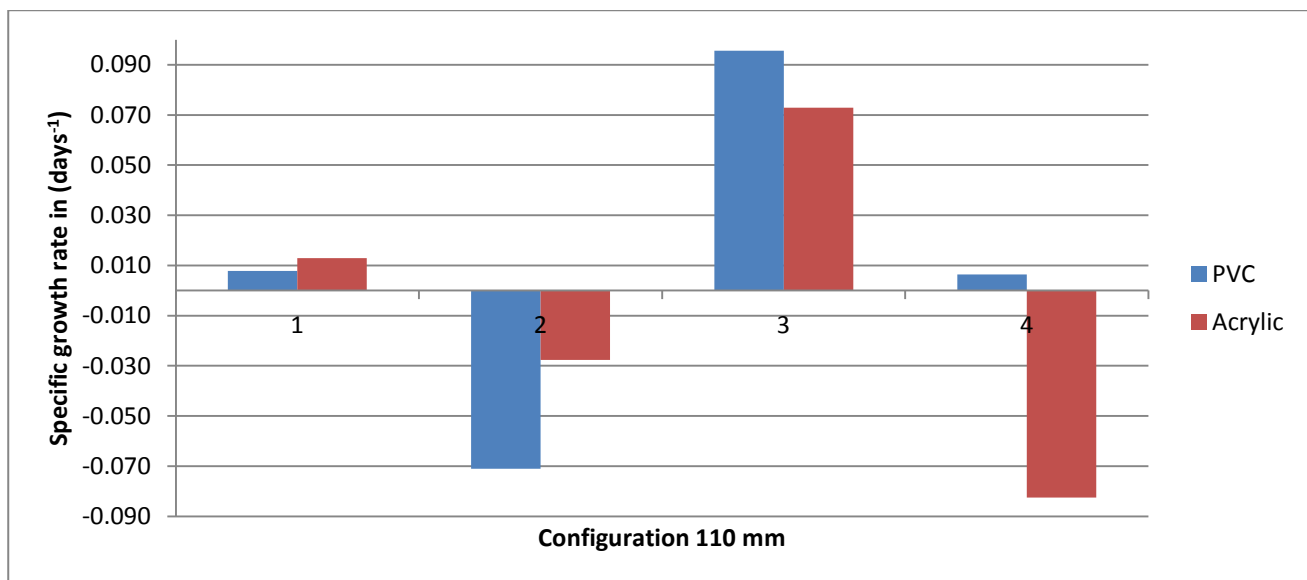


Figure 5.23: Specific growth rate of 110 mm reactors comparing material

When the specific growth rates of the 110 mm reactors are compared, it is seen that the figure allows for the same conclusions that were made from the volumetric growth rate. The change from positive to negative production with an increase in density allows the specific growth rate to have the same shape as the volumetric growth rate. It is thus only comparable when a completely positive or negative production is obtained.

The specific growth rate would show which reactor has the best production under a given set of conditions. As the specific growth rate shows which of the reactors or in this case which configurations have the best production. The information should be used to obtain the best culture conditions, configuration or design and therefore the best production possible.

## 5.9. Interaction between parameters

The factorial design of testing was done in order to determine which interaction had a significant influence on the results. As mentioned previously, the factorial design was split into two parts due to a difference in the gas flow rate which was considered to have a large impact on the results: firstly the 50 mm reactors; and secondly, the 90 mm and 110 mm reactors.

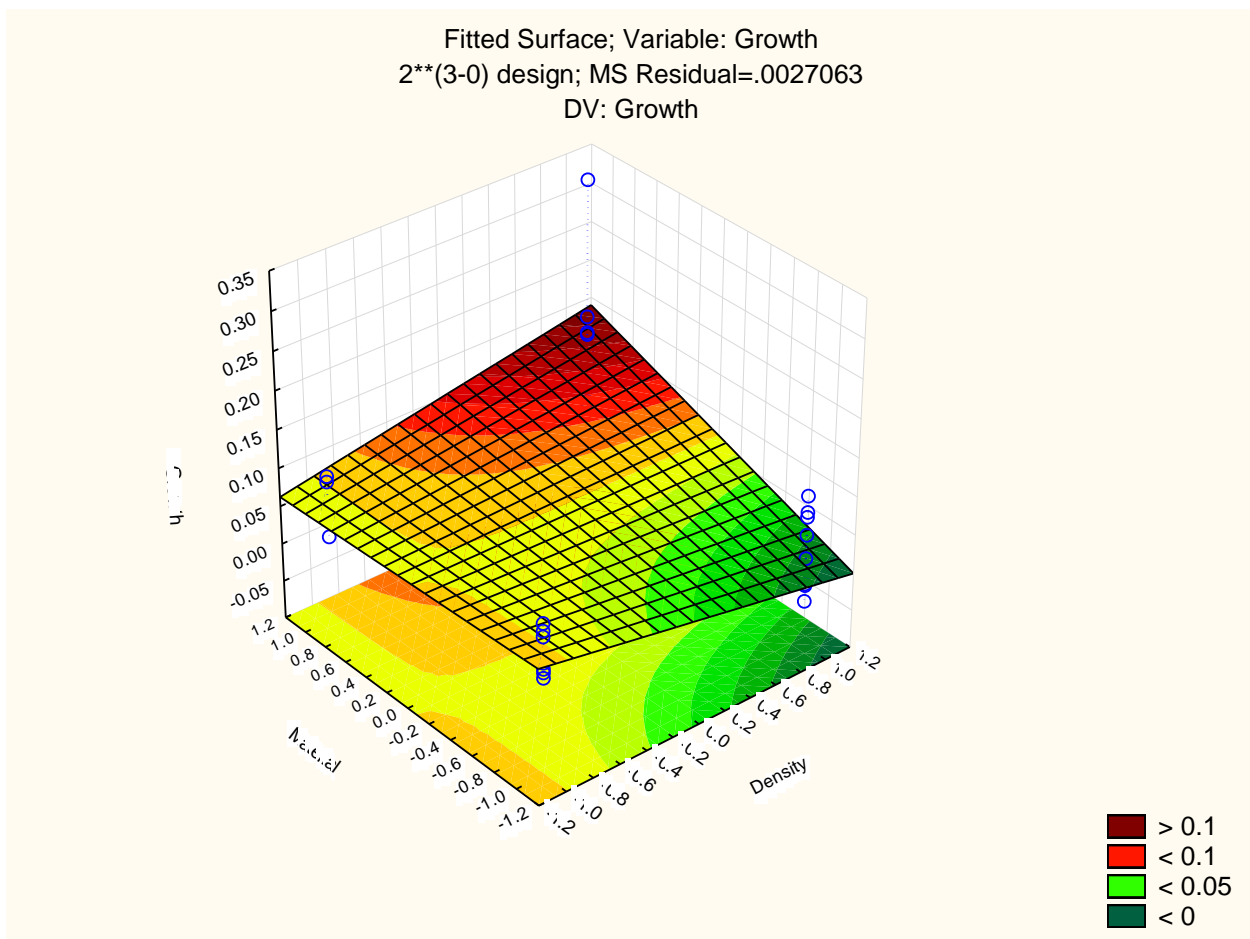


Figure 5.24: Visual representation of the interaction between the culture density and materials used in the 50 mm reactor

Figure 5.24 shows a visual representation of the interactions between two variables i.e. culture density and materials that were used. The bigger the interaction between the two variables are, the larger the slope of the graph. The highest part (reddest) shows the most favourable interaction while the lowest part (greenest) shows the least favourable interactions.

The results of the analysis differed to an extent and were mainly caused by the biological nature of algae, which caused slight difference in production rates. As a result of these differences, the  $R^2$  values on both factorial analyses are low and would therefore need a large volume of results to increase these values.

$$y = \beta_0 + \beta_1x_1 + \beta_2x_2 + \beta_3x_3 + \beta_4x_4 + \sum \beta_{ij}x_i + \sum \beta_{ijk}x_{ijk} + \varepsilon \quad [\text{Equation 5.1}]$$

Equation 4.1 was used to model the response of growth with a varied variable. The  $\beta_0$  represents the intercept with  $\beta_i$  ( $i = 1, 2, 3$  and  $4$ ) representing the regression coefficients

and  $x_i$  the factors.  $B_{ij}$ ,  $\beta_{ijk}$ ,  $x_{ij}$  and  $x_{ijk}$  represent the two-way and three-way interaction's regression coefficients and interaction factors. If a factor is insignificant, its value on the equation will become 0 and is thus not stated in the equation.

Analysis of the 50 mm reactors (Figure 5.25) showed that three parameters had an effect on the results, with a confidence level of 90 %. The material and density-material interactions showed the biggest effect, with the gas dispersion having the smallest effect with a p-value of less than 0.1. As the remaining effects have a p-value of above 0.1, they are considered to be insignificant and are therefore not included in the model.

The visual representation in Figure 5.24 shows that the effect of density has the opposite interaction with the two materials and with Figure 5.25, showing that the interaction is the most important factor for the reactors. The visual representation highlights the interaction and shows why the use of the acrylic tubing at a high density is very important to achieve the best growth rate.



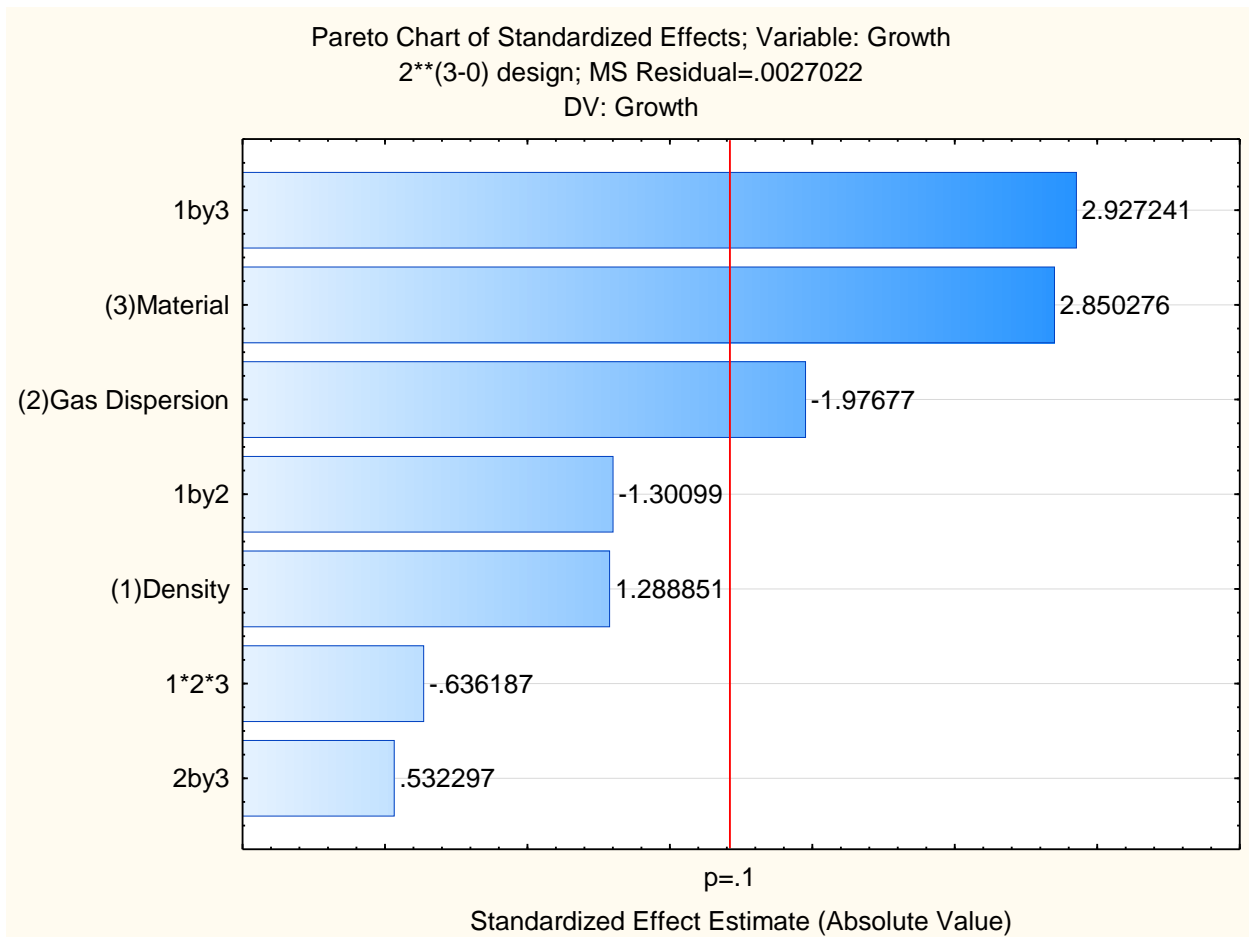


Figure 5.25: Pareto chart of effects for the 50 mm reactors

The density does not have any effect on the overall regression model. As was seen in the results previously discussed, the PVC reactors had a preference for a lower density while the acrylic had a preference for a higher density culture. Due to these contradicting results, the model does not include the density, but rather the interaction between the density and material that was used. The importance of this interaction is seen in Figure 5.25 and it is placed at the top of the list. By refining the model to the significant factors, the following model equations were calculated:

$$y = 0.069679 - 0.018165x_2 + 0.026192x_3 + 0.026899x_{13} \quad [\text{Equation 5.2}]$$

The regression model considers all the data that is available and calculates the significance of the variables. By removing certain variables, e.g. the material that is used, different interactions could be seen and therefore change the results. As the aim of the testing is to see which of these factors has the largest affect, the analysis will not be done to determine the individual effect if some of the variables are reduced. An example of this is the fact that density is considered a significant factor for the 90 mm and 110 mm

reactors, but not for the 50 mm reactors and could become completely insignificant if all the data are analysed.

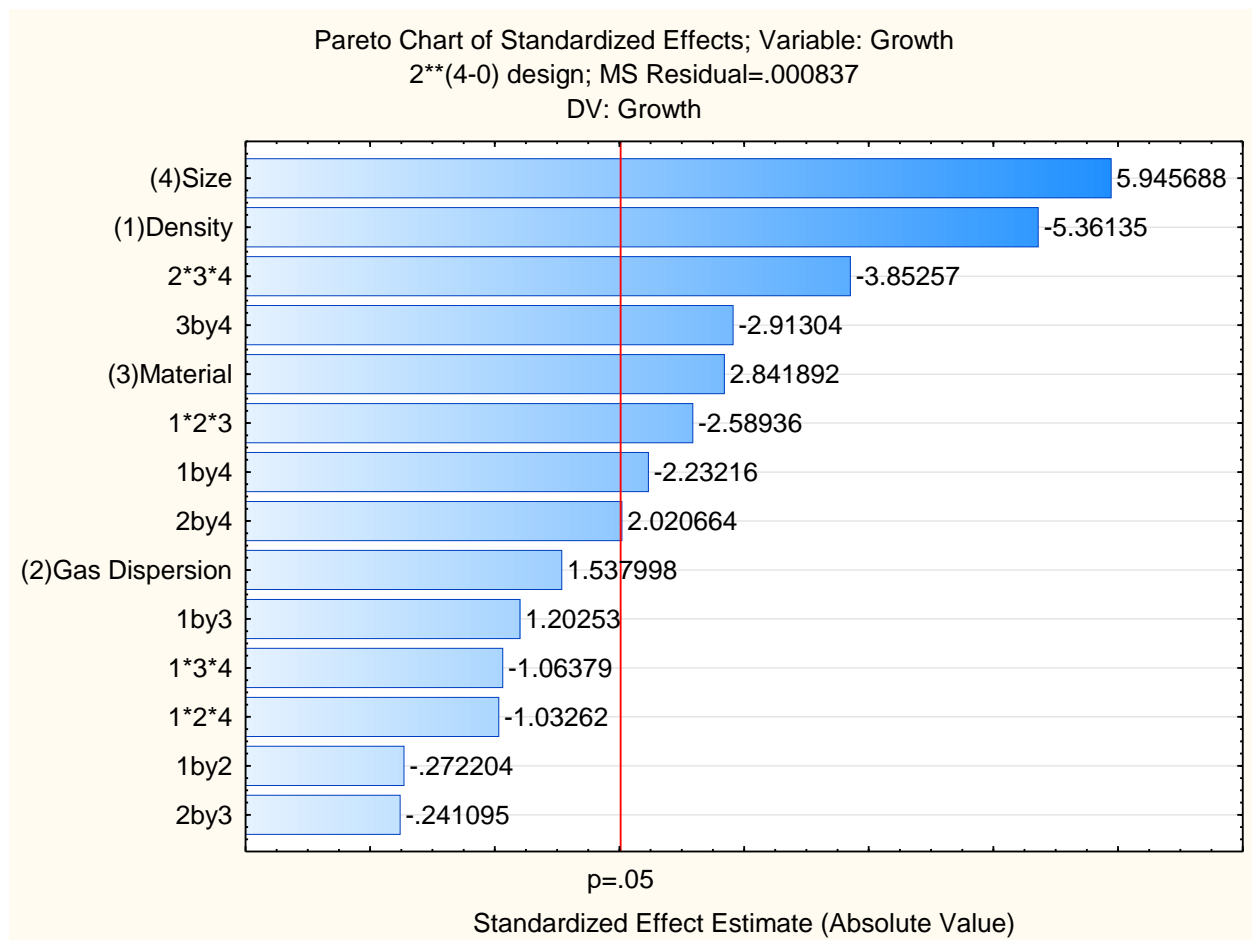


Figure 5.26: Pareto chart of effects for the 90 mm and 110 mm reactors

The 90 mm and 110 mm reactors (Figure 5.26) showed eight significant factors and interactions with a 95 % confidence level and a p-value of 0.05. In contrast to the 50 mm reactors, the gas dispersion unit is not considered a significant factor in the 90 mm and 110 mm reactors, although two of the three-way interactions do consider that the gas dispersion unit's interaction is significant in these interactions. The size or diameter of the reactors is considered to be the most significant interaction. This is due to the large amount of negative growth seen in the 90 mm reactors. The regression model was refined to the significant factors and the following model was obtained:

$$y = -.022695 - .019388x_1 + 0.010277x_3 + 0.021501x_4 - 0.008072x_{14} + 0.007307x_{24} - .010534x_{34} - 0.009364x_{123} - 0.013932x_{234} \quad [\text{Equation 5.3}]$$

For the mathematical model, it is clear that most of the interactions have a negative effect on the growth rate, with very few of the factors or interactions having a positive effect. The two interactions between variables can be found in a visual representation for the 90 mm and 110 mm reactor combination in APPENDIX F.

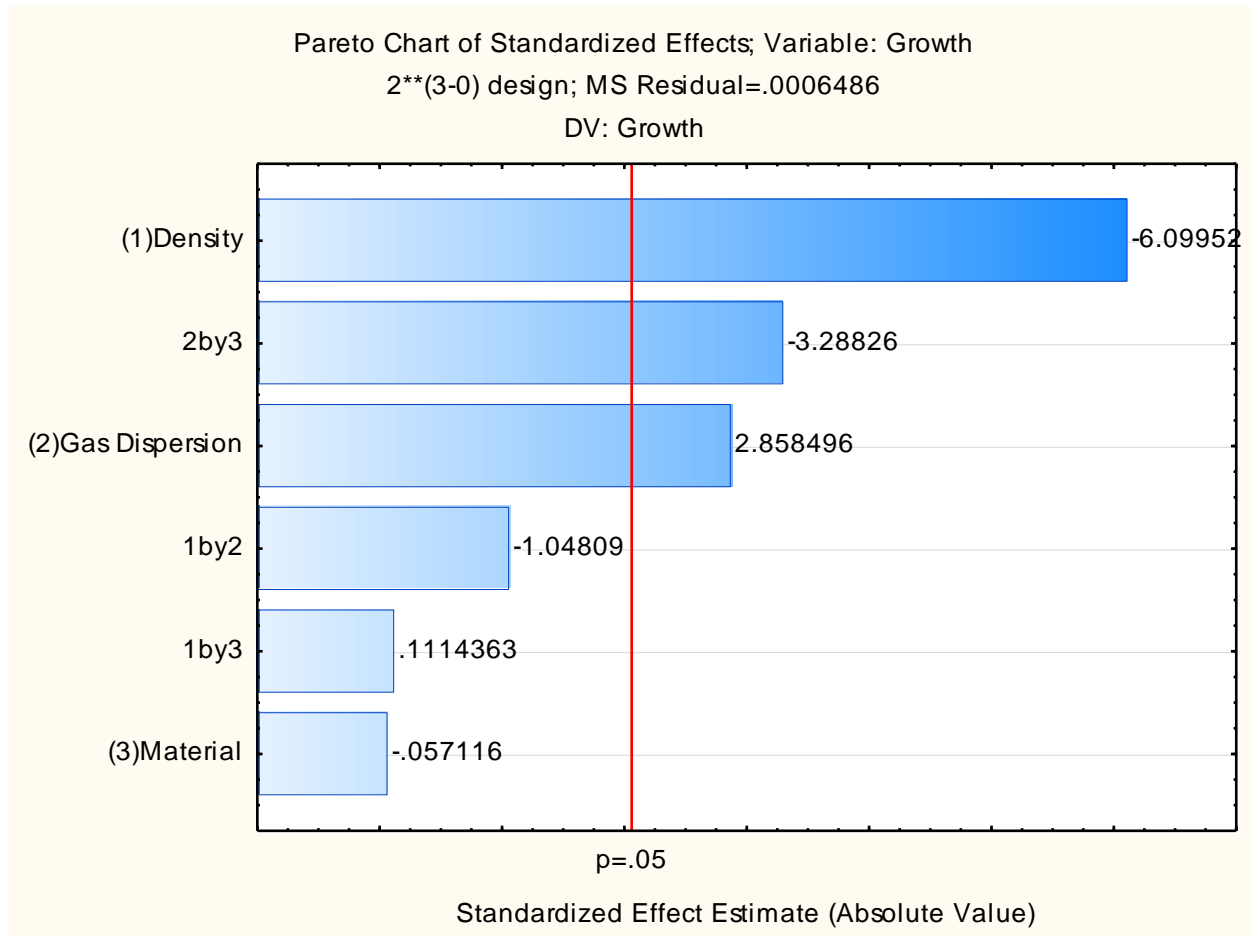


Figure 5.27: Pareto chart of effects of the 110 mm reactors

The 110 mm reactors (Figure 5.27) show three significant factors at a 95 % confidence level and a p-value of 0.05. This includes the density and gas dispersion units. The material was considered to be significant in the other analysis (50 mm, 90 mm and 90-110 mm reactors) while for the specific reactors (110 mm) it shows that the material used for the design is insignificant. The regression model for the 110 mm reactors have a mathematical equation of:

$$y = -0.001194 - 0.027460x_1 + 0.012869x_2 - 0.014804x_{23} \quad [\text{Equation 5.4}]$$

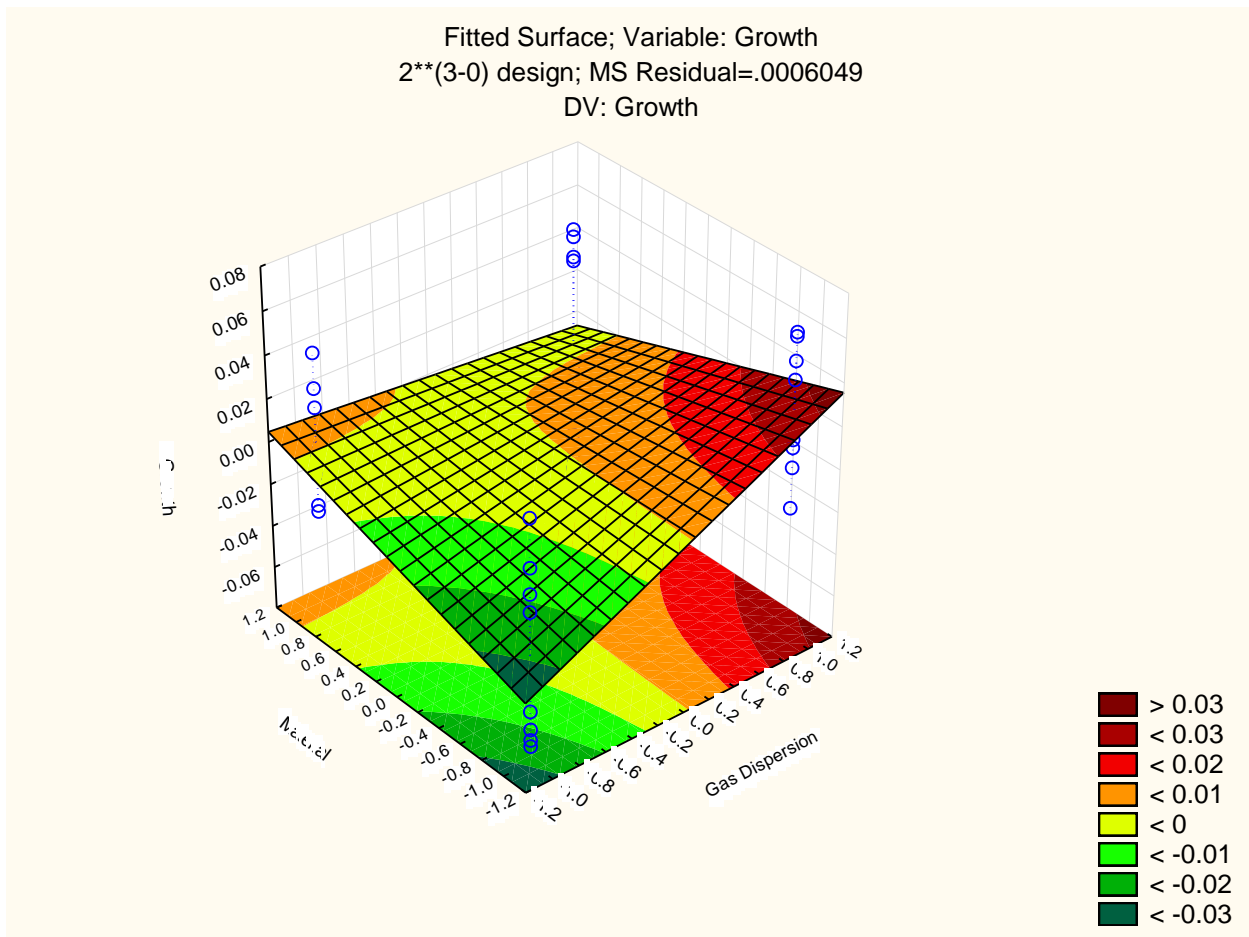


Figure 5.28: Visual representation of the interaction between the gas dispersion unit and materials used in the 110 mm reactor

The 90 mm reactors (Figure 5.29) have three significant factors at a 90 % confidence level and a p-value of 0.1. The 90 mm reactors show the strongest effect for the material that is used, compared to the material used in the 110 mm reactor which has the weakest effect. The analysis of the 90 mm and 110 mm reactors together shows that the materials have a significant effect. In addition, the analysis shows that the addition of a variable could influence the regression model substantially. The regression model for the 110 mm reactors has the following mathematical equation:

$$y = -0.044197 - 0.011316x_1 + 0.020812x_3 + 0.013060x_{23} \quad \text{[Equation 5.5]}$$

The interaction between the gas dispersion units and the materials shows an interesting result as it is placed second on both the 90 mm and 110 mm reactors' Pareto charts. However, in the combined Pareto chart, it is found right at the bottom of the list. This could mean that the interaction has a large effect on the growth of each reactor but when the

reactors are analysed together, the effects are cancelled out as they have opposite effects and suddenly become an insignificant factor.

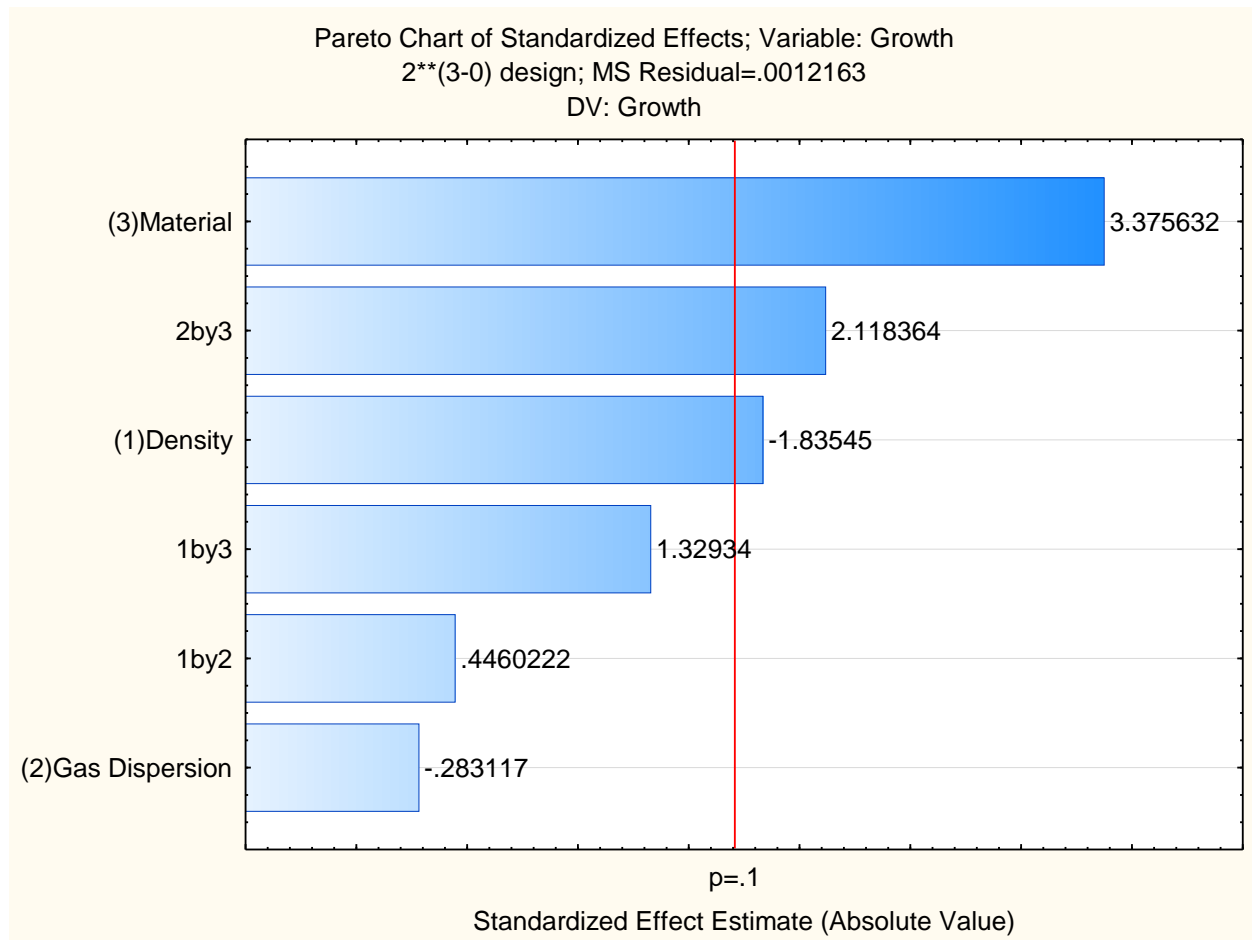


Figure 5.29: Pareto chart of effects of the 90 mm reactor

The interaction between the gas dispersion unit and the material used is represented visually in Figure 5.28 and Figure 5.30. The graphs show that for both the reactors' diameters, the larger unit is preferred although different materials are preferred. Due to the preference for different materials, the combined analysis would be expected to show no interaction between the two variables because they cancel each other out.

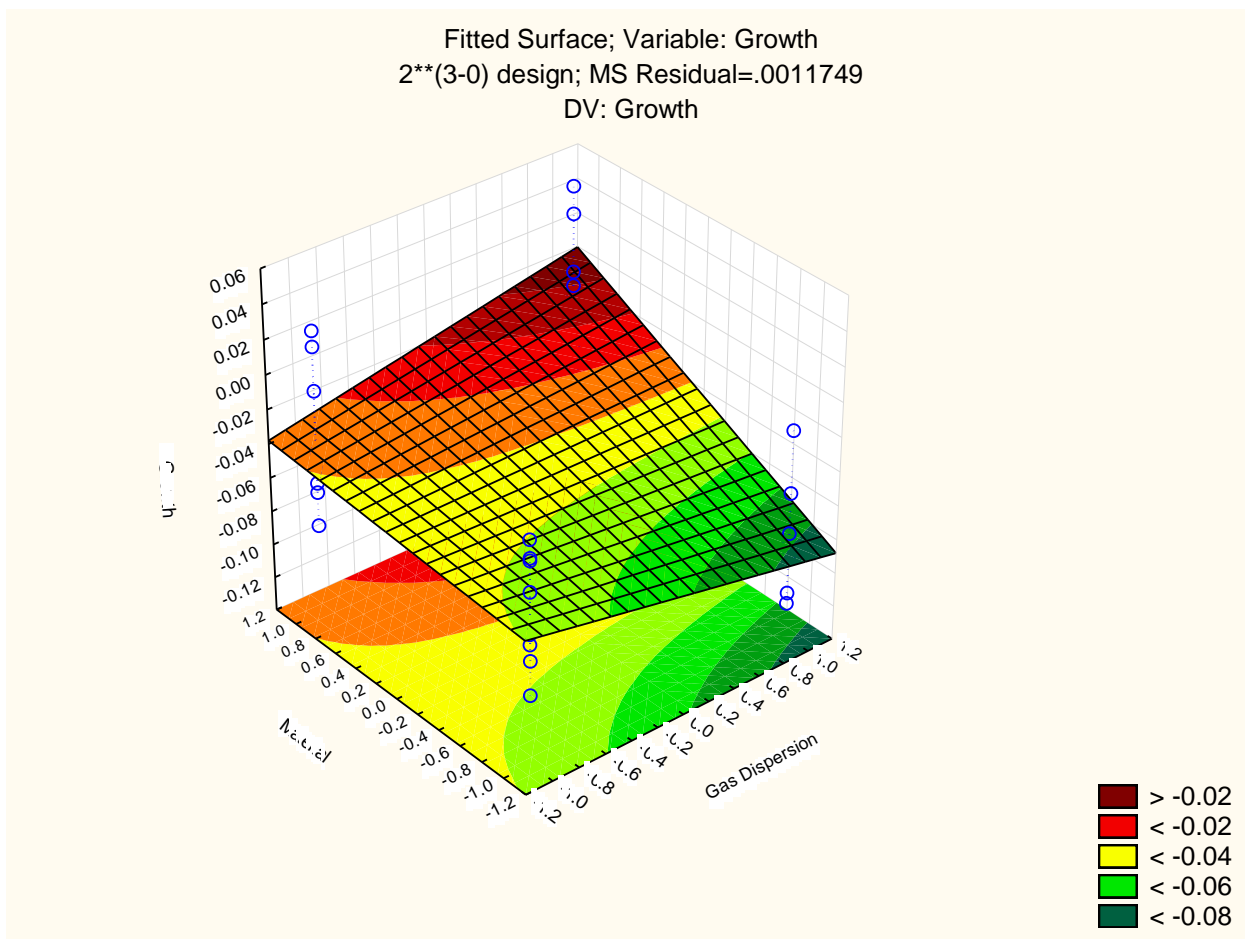


Figure 5.30: Visual representation of the interaction between the gas dispersion unit and the materials used in the 90 mm reactor.

## 6. COSTING

The cost of each reactor's material and the size of the tubing used were calculated with the configuration which provided the best growth. For each calculation the capital cost and the running cost were calculated and the result was used to calculate the profit or loss on a single module or on a plant consisting of 60 modules.

For calculation purposes, all the reactors consisted of a 36 tube module see Figure 3.2, with a vertical tube length of 1.66m. Due to the high capital cost of a reverse osmosis plant and an additional cost which is size dependant, the water cost of normal tap water was used for calculation purposes. The medium was calculated by using the growth rate and the required volume of the medium that had to be replaced after harvesting.

The cost of the electricity was based on the assumption that the air blower would be on for 24 hours per day and that the pump would only be on for the duration of medium replacement at the given flow rate. The CO<sub>2</sub> cost was calculated per bottle required for the enrichment of air at 5 % v/v. The flow rate was taken at 0.36 vvm with continuous CO<sub>2</sub> enrichment occurring 24 hours a day, 365 days a year.

Table 6.1: Growth rates, culture density and reactor volumes used for costing calculations

	<b>50 mm PVC</b>	<b>50 mm acrylic</b>	<b>90 mm PVC</b>	<b>90 mm acrylic</b>	<b>110 mm PVC</b>	<b>110 mm acrylic</b>
Growth rate (g/l/d)	0.0833	0.1030	0.0000	0.0000	0.0248	0.0345
Density (g/l)	0.5	1.0	0.0	0.0	1.0	1.0
Total volume (l)	399	399	1394	1330	2078	2039

The suggested cost of spray drying by Pulse Combustion Systems (2014) is \$2.00 dollar per pound of water. Pulse Combustion Systems (2014) states that the capital cost on their systems ranges between \$300 000 and \$3 million, although second-hand and smaller systems are available. None of the drying costs was included in the costing because the cost of production using the specific PBRs exceeds the value of the product. The value of the algae product was taken as a product that could be purchased by consumers, with the production cost still too high to be profitable.

The capital cost was calculated as the annual loan instalment, based on a 9.25 % interest rate over a five-year period. All the costing was done for the period of a year. A

breakdown of the capital and running costs is available in APPENDIX G. For the 60 module calculations, only the required components were increased as items such as the storage tank and pump could be used on all the reactors.

The cost of medium production is very high and, in addition to the staff salaries, very few staff members were included in the cost analysis, the operating cost of the PBRs is too high. Profitability can only be achieved when using an inorganic medium, if higher production capacities can be achieved in the system. The running cost could be reduced by using waste water and flue gas from a plant, although this would increase the capital cost required.

### 6.1. 50 mm reactor costing

Table 6.2: Profitability of 50 mm diameter PVC reactors

	Description	Price per unit	Quantity	Total
1 module plant				
Price of dried <i>Chlorella</i>	per 100g	R 153.51	121.25	R 18,613.52
Annual operating cost				R 71,112.31
Capital cost				R 5,283.98
Profit/loss annually				R -57,782.77
60 module plant				
Price of dried <i>Chlorella</i>	per 100g	R 153.51	7275.17	R 1,116,811.07
Annual operating cost				R 2,214,738.37
Capital cost				R 196,483.94
Profit/loss annually				R -1,294,411.24

Table 6.3: Profitability of 50 mm diameter acrylic reactors

	Description	Price per unit	Quantity	Total
1 module plant				
Price of dried <i>Chlorella</i>	per 100g	R 153.51	149.98	R 23,024.21
Annual operating cost				R 63,514.59
Capital cost				R 5,503.92
Profit/loss annually				R - 45,994.30
60 module plant				
Price of dried <i>Chlorella</i>	per 100g	R 153.51	8999.10	R 1,381,452.77
Annual operating cost				R 1,758,875.59
Capital cost				R 209,860.37
Profit/loss annually				R - 587,103.19



If one compares the 50 mm reactors, the capital cost of the acrylic reactor is slightly higher than the PVC reactor, but the higher production and operation at a higher culture density make the running cost much lower. The lower running cost is due to the fact that less medium and electricity are required because the pumps run for shorter periods of times. In a larger plant, a bigger pump could reduce the running cost as it would replace the medium at a much faster rate, thus reducing running time.

## 6.2. 90 mm reactor costing

Table 6.4: Profitability of 90 mm diameter PVC reactors

	Description	Price per unit	Quantity	Total
1 module plant				
Price of dried <i>Chlorella</i>	per 100g	R 153.51	0.00	R 0.00
Annual operating cost				R 67,621.95
Capital cost				R 8,340.70
Profit/loss annually				R -75,962.65
60 module plant				
Price of dried <i>Chlorella</i>	per 100g	R 153.51	0	R 0.00
Annual operating cost				R 2,005,317.12
Capital cost				R 379,886.95
Profit/loss annually				R-2,385,204.06

Table 6.5: Profitability of 90 mm diameter reactors acrylic reactors

	Description	Price per unit	Quantity	Total
1 module plant				
Price of dried <i>Chlorella</i>	per 100g	R 153.51	0.00	R 0.00
Annual operating cost				R 66,432.23
Capital cost				R 9,636.74
Profit/loss annually				R -76,068.97
60 module plant				
Price of dried <i>Chlorella</i>	per 100g	R 153.51	0.00	R 0.00
Annual operating cost				R 1,933,933.81
Capital cost				R 457,649.37
Profit/loss annually				R-2,391,583.18

The 90 mm tubing was not calculated as the median growth for all the combinations provided a negative growth. The running cost that is shown consists of the cost of

salaries, electricity and CO<sub>2</sub>, assuming that the plant was running continuously but not producing anything.

### 6.3. 110 mm reactor costing

Table 6.6: Profitability of 110 mm diameter PVC reactors

	Description	Price per unit	Quantity	Total
1 module plant				
Price of dried <i>Chlorella</i>	per 100g	R 153.51	188.22	R 28,894.79
Annual operating cost				R 99,728.46
Capital cost				R 10,650.30
Profit/loss annually				R -81,483.97
60 module plant				
Price of dried <i>Chlorella</i>	per 100g	R 153.51	11293.64	R 1,733,687.29
Annual operating cost				R 3,931,707.65
Capital cost				R 518,462.99
Profit/loss annually				R -2,716,483.36

Table 6.7: Profitability of 110 mm diameter acrylic reactors

	Description	Price per unit	Quantity	Total
1 module plant				
Price of dried <i>Chlorella</i>	per 100g	R 153.51	256.46	R 39,369.70
Annual operating cost				R 105,786.24
Capital cost				R 11,650.18
Profit/loss annually				R -78,066.72
60 module plant				
Price of dried <i>Chlorella</i>	per 100g	R 153.51	15387.81	R 2,362,182.06
Annual operating cost				R 4,295,174.17
Capital cost				R 578,455.78
Profit/loss annually				R -2,511,447.89

Similar to the 50 mm reactors, the acrylic reactor has a higher capital cost, but also a higher running cost. The running cost of the acrylic reactor is higher due to the larger medium replacement required because the ratio of the production and the culture density are much bigger as a result of the same culture density, while the 50 mm PVC reactor has a lower culture density. These higher costs of the acrylic reactor are overturned by the increase in production which is 36 % higher than the production of the PVC reactor, while the cost is only 9.5 % higher when it is compared to the 60 module plant.

## 6.4. Summary

The costing of all the reactors shows that an annual loss is made. The growth rate is important, but it appears that the ratio between the growth rate and culture density is more important for the profitability of a PBR.

The cost of the medium, the CO<sub>2</sub> and the salaries are very high. To ensure that the production of algae is profitable when this type of reactor is used, a different source of nutrients is of high importance as it would reduce the production cost by a fair amount. Because the CO<sub>2</sub> contributes to the largest portion of the running cost per reactor, it would thus be crucial to obtain a low cost or free source of CO<sub>2</sub>. If the cost of the medium and the CO<sub>2</sub> were removed from the calculations, almost all the reactors would turn a profit.

The cost of salaries is very difficult to determine because the work required cannot be calculated. It is advised that a single person should be in charge as many modules as possible. This would reduce the labour cost per module and if the system were properly laid out, one person could take control of more than the 20 modules such as those used in the calculations.

By increasing the production factor and reducing the cost of the medium, the CO<sub>2</sub> and the salaries, the production of algae biomass could become a very profitable business. However, this would also depend on the cost of post-production processing.

## 7. CONCLUSION

Algae can be grown under phototrophic, heterotrophic or mixotrophic conditions. Phototrophic conditions use light and CO<sub>2</sub> as the sources of energy and carbon respectively. Phototrophic conditions are mainly used for the production of algae biomass as they could help to reduce the CO<sub>2</sub> levels in the atmosphere. Heterotrophic conditions refer to the utilisation of organic compounds as sources of energy and carbon, although light could also be used as an energy source. The use of heterotrophic conditions shows a much higher production of algae biomass and oil content, with heterotrophic production yielding 0.08 – 0.15 g/l of biomass daily and 27.0 – 35.0 mg/l of oil daily compared to phototrophic productions' yield of 0.1 g/l of biomass daily and 4 mg/l of oil daily (Chen et al., 2011). Mixotrophic production is a combination of phototrophic and heterotrophic production, but research is limited on this specific condition because the main research is focused on photo- and heterotrophic production.

The design of a PBR is a very complex process as each species of algae has its own requirements which can range from CO<sub>2</sub> tolerance to specific pH requirements. All of these conditions have to be met for the design of an optimal photobioreactor. Several types of bioreactors have been designed, but major challenges have also been experienced. Scaling of the reactor introduced several challenges caused by changes in the culture's environment when pH gradients develop in the reactor, gas flow rates vary too much as the optimal gas flow rate is unknown, and there is insufficient agitation of the culture and all of these effect the cost of producing a large-scale plant is too high.

The required inputs for algae biomass production have to be considered carefully as the algae cells are very sensitive to their environment. Because the phototrophic system was utilised, the surface area to volume ratio of the cultivation reactor was important to ensure that maximum light photons were available to the culture. To ensure that the maximum amount of light penetrates the clear tubing, a thin tubing wall is required. The availability of light inside the reactor is an essential factor because chlorophyll requires adequate light to ensure its production is at an optimal level.

After light, carbon dioxide is considered to be the most crucial factor because it is used in the Calvin cycle to produce the desired products. If the available level of carbon dioxide is too low, the light-harvesting complexes could capture too much energy which would cause

photo-inhibition and, ultimately, reactive oxygen species. This would destroy the cells and reduce production in the reactor. When sufficient carbon dioxide is supplied, the potential of reaching photo-inhibition is limited drastically and the production of biomass would increase to optimal levels. For optimal production, it is crucial to keep the oxygen and carbon dioxide ratios at the correct level. Although algae have developed mechanisms to survive incorrect CO<sub>2</sub> levels. Because incorrect levels of CO<sub>2</sub> could affect their growth rate negatively, this condition should be avoided at all cost.

The nutrient composition used for algae production differs according to the desired products and species that are produced. In addition, the composition of waste water sources may differ and this could cause fluctuations in the growth rate. *Chlorella* biomass is mainly grown in laboratory environments using Bold's Basal medium, which was formulated to achieve an optimal growth rate. The nutrient supply also directly affects the cell composition that is obtained and, by changing the nutrient composition, the production of oils, proteins or carbohydrates could be altered. By using a modified composition of the nutrients, algae production is usually separated into a two-step process: firstly, to produce large quantities of biomass; and secondly, to change the cell composition.

In order to design an optimal photobioreactor, it is necessary to know the effects of each of the variables have on the production abilities of the algae. The literature does not reach consensus in their information on the effect of these variables have on the production of algae biomass. The gas flow rate reported in the literature ranges from 0.05 volume per volume per minute (vvm) Zhang, Miyachi and Kurano (2001) to 0.5 vvm Li et al. (2011). In addition, as these ranges for the gas flow rate are used, irrespective of the reactor design, the determination of an optimal reactor is made almost impossible because a change in the gas flow rate could possibly influence the production of biomass.

It was concluded that a bubble column reactor would be the best base from which to start doing research because it is considered to be the best design available for the production of algae biomass. Testing was done to investigate the effect of several variables on the growth rate of *C. vulgaris* and to determine whether the production would be profitable. The variables that were tested were the materials used for the clear tubing, gas dispersion unit sizes, culture density and tubing diameter.

The results showed that each of these variables has a significant effect on the growth rate of the algae. The effect of the variables differs across configurations that were tested with some variables showing a larger effect on a specific combination and little to no effect on

other combinations. A problem that was found with the bubble column design is the rate of sedimentation which causes a large portion of the algae cells to settle in the dark region of the reactor, resulting in a reduction in the productivity of the reactor. It is suggested that a small pipe be placed at the bottom of the reactor to overcome this specific problem to ensure all the algae cells have light exposure for optimal production from the reactor. Future studies should include the determination of the light profile inside the reactors as this is a very important variable.

To evaluate the interaction of the variables, a regression model was build for each of the reactor diameters tested. It was found that factors considered significant for a specific reactor's diameter could be insignificant for another. When comparing two different reactors' diameters to each other for interaction, the researcher observed that some of the interactions caused them to change from significant to insignificant. Both the 90 mm and 110 mm diameter reactors show a large interaction between the material used and the gas dispersion unit but when analysed together the effect becomes insignificant as the reactors have opposite interactions individually. It appears that the volume per volume flow rate is insufficient in some instances. The norm of using the suggested volume per volume per minute system does not take into consideration the reactor volume to tubing diameter ratio or the length of the tubing. As a bubble column reactor becomes longer, the flow rate of gas has to decrease, otherwise the gas volume inside the reactor would become so large that the culture volume would have to be reduced, making the reactor insufficient. The gas flow rate between the 90 mm and 110 mm reactors was kept constant at 0.36 vvm, but the 90 mm reactor did not obtain sufficient gas flow rate because the diameter of the tubing is very close to each other, but a 56 % difference in volume was found. The 56 % increase in volume provided the 110 mm with a 56% increase in gas flow rate which allowed for sufficient gas flow through the reactor.

A feasibility costing was done to determine whether a bubble column reactor that was run under test conditions would be a viable process. From the costing it was found that the running cost of a plant would exceed its annual income for all the reactors. Given a free or low cost source for both nutrients and CO<sub>2</sub>, an economically viable production is possible, depending on the processing cost of the biomass. By utilising a source of waste water that is nutrient rich, the cost could be reduced drastically, because the addition of only the required compounds needs to be added for optimal growth. Waste CO<sub>2</sub> from flue gas produced in large quantities by power stations and other plants could be used. This would consequently reduce the reactor's carbon footprint and be a free source of CO<sub>2</sub>.

The design of an optimal photobioreactor requires that the effect of the gas flow rate be studied. Firstly, it should be considered how the flow rate affects the algae growth rate with different tubing diameters, lengths and volumes. The gas flow rate would determine whether other variables, for example the CO<sub>2</sub>, could be supplied in sufficient quantities for optimal growth as well as whether the adequate agitation of the culture was achieved. In photobioreactors where gas is used for agitation, the gas flow rate is as influential as the light characteristics and CO<sub>2</sub> availability because it directly affects the production rate.

The building of a photobioreactor is currently not a viable option for the production of algae biomass. The production cost of algae is too high and only viable for a very small niche market looking for high-value products. The production of algae requires a reduction in operating cost and a better understanding of the interaction of different design aspects and materials that are used before algae biomass could become a truly viable and renewable source of products.

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## 9. APPENDIX A



Figure 9.1: Copyright permission for total ion chromatograms



# 10. APPENDIX B

PRETORIA (HEAD OFFICE)  
320 Mitchell Street  
Pretoria West, 0117  
Tel: 086 1100 420



MAIZEY'S (PTY) LTD Reg. No: 1969/017998/07

## PVC - U TRANSPARENT TUBES

13 January 2014

PVC-U SHOWS HIGH MECHANICAL STRENGTHS, TENSILE STRENGTH AND CAN BE USED IN APPLICATIONS RANGING IN TEMPERATURE FROM -15 DEGREES TO + 60 DEGREES. IT CAN ALSO BE EASILY SOLVENT CEMENTED AND WELDED.

**STANDARD LENGTH : 5 000 MM BUT ONLY SOLD IN MULTIPLES OF 1 METERS**

**COLOUR : CLEAR**

<u>PROD. CODE</u>	<u>OD</u>	<u>ID</u>	<u>WALL /THICK</u>	<u>PRICE/M</u>
PVCTC012009I	12	9.2	1.4 MM	25.36
PVCTC020017I	20	17	1.5 MM	37.46
PVCTC025022I	25	22	1.5 MM	51.01
PVCTC025021I	25	21.2	1.9 MM	54.18
PVCTC032028I	32	28.4	1.8 MM	59.94
PVCTC032027I	32	27.2	2.4 MM	70.89
PVCTC040034I	40	34	3.0 MM	114.41
*PVCTC050046I	50	46.4	1.8 MM	100.29
PVCTC050045I	50	45.2	2.4 MM	106.63
*PVCTC050042I	50	42.6	3.7 MM	191.36
PVCTC063057I	63	57	3.0 MM	163.12
PVCTC075071I	75	71.4	1.8 MM	172.91
PVCTC075067I	75	67.8	3.6 MM	286.17
PVCTC090086I	90	86.4	1.8 MM	178.97
*PVCTC110105I	110	105.6	2.2 MM	242.37
PVCTC125120I	125	120	2.5 MM	448.42
PVCTC140134I	140	134.4	2.8 MM	583.01
*PVCTC160153I	160	153.6	3.2 MM	656.79

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**PLEASE NOTE:**

\* (\*) To Be Discontinued  
\* All prices exclude VAT  
\* All prices subject to change without notice

\* (+) Available on Request  
\* Stock subject to confirmation at all times  
\* Terms strictly 30 days

Figure 10.1: Product and price list for clear PVC by Maizey plastics

<b>Technical Data Sheet</b> <b>GEHR PVC-U</b>	
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**I. Physical Properties**

	Test method	Unit	Value
1. Specific gravity	ISO 1183	g/cm <sup>3</sup>	1,36
2. Water absorption	ISO 62	%	0,2
3. Chemical resistance	-	-	DIN 8061
4. Maximum permissible service temp. (no stronger mechanical stress involved)	-	-	-
Upper temperature limit	-	°C	60
Lower temperature limit	-	°C	-15

**II. Mechanical Properties**

	Test method	Unit	Value
1. Tensile strength at yield	ISO 527	MPa	55
2. Elongation at yield	ISO 527	%	3
3. Tensile strength at break	ISO 527	MPa	30
4. Elongation at break	ISO 527	%	> 10
5. Impact strength	ISO 179	kJ/m <sup>2</sup>	no break
6. Notch impact strength	ISO 179	kJ/m <sup>2</sup>	3
7. Ball indentation // Rockwell hardness	ISO 2039-1	MPa	120
8. Shore-D	DIN 53505	-	82
9. Flexural strength	ISO 178	MPa	90
10. Modulus of elasticity	ISO 527	MPa	3000

**III. Thermal Properties**

	Test method	Unit	Value
1. Vicat-softening point	VST/B/50	ISO 306	°C
	VST/A/50	-	°C
2. Heat deflection temperature	HDT/B	ISO 75	°C
	HDT/A	-	°C
3. Coefficient of linear thermal expansion	DIN 53752	K <sup>-1</sup> •10 <sup>-4</sup>	0,8
4. Thermal conductivity at 20 °C	-	W/(m•K)	0,14

**IV. Electrical Properties**

	Test method	Unit	Value
1. Volume resistivity	VDE 0303	Ω•cm	>10 <sup>15</sup>
2. Surface resistivity	-	Ω	≥10 <sup>13</sup>
3. Dielectric constant at 1MHz	-	-	3
4. Dielectric loss factor at 1 MHz	DIN 53483	-	0,01
5. Dielectric strength	VDE 0303	kV/mm	20-40
6. Tracking resistance	IEC 60112	-	KB 600

**V. Additional Data**

	Test method	Unit	Value
1. Bond ability	-	-	+
2. Friction coefficient	DIN 53375	-	0,6
3. Flammability	UL 94	-	V-0
4. UV stabilisation	-	-	Fair

<sup>1)</sup> 65 (solid rod 160 - 200 mm Ø) <sup>5)</sup> 57 (solid rod 220 - 300 mm Ø) <sup>2)</sup> 59 (solid rod 160 - 200 mm Ø) <sup>1)</sup> 51 (solid rod 220 - 300 mm Ø)  
 All values are attributes of the used raw materials.

The physical data contained in this table are typical values. They are obtained on test specimens under specific conditions and represent average values of a large number of tests. The results obtained on this tests specimens cannot be applied to finished parts without reservations, as behaviour is influenced by processing and shaping. Reproduction only with our definite permission.

Figure 10.2: Specifications of clear PVC tubing, provided by Maizey plastics



PRETORIA (HEAD OFFICE)  
320 Mitchell Street  
Pretoria West, 0117  
Tel: 086 1100 420



MAIZEY'S (PTY) LTD Reg. No: 1969/017998/07

"PLEXIGLAS" ACRYLIC TUBE							
							03.02.14
EXTRUDED & CAST							
PRODUCT CODE	OD	ID	PRICE/M	PRODUCT CODE	OD	ID	PRICE/M
AXT005003	5	3	14.65	AXT070062	70	62	241.50
AXT006003.5	6	3.5	18.08	AXT070064	70	64	200.69
AXT006.5004	6.5	4	18.70	AXT080070	80	70	340.59
AXT007005	7	5	18.08	AXT080072	80	72	272.66
AXT008004	8	4	27.73	AXT080074	80	74	217.52
AXT010004	10	4	50.48	AXT090080	90	80	400.42
AXT010006	10	6	24.62	AXT090082	90	82	323.76
AXT010007	10	7	19.32	AXT090084	90	84	254.60
AXT012006	12	6	35.83	AXT100090	100	90	439.99
AXT012008	12	8	26.50	AXT100092	100	92	367.08
AXT012010	12	10	19.32	AXT100094	100	94	284.18
AXT013009	13	9	30.84	*AXT110100	110	100	469.92
AXT013010	13	10	24.62	AXT110104	110	104	300.71
AXT015010	15	10	39.89	AXT120110	120	110	523.51
AXT015011	15	11	33.97	AXT120114	120	114	330.62
AXT015013	15	13	23.07	AXT133127	133	127	361.16
AXT016012	16	12	38.02	AXT133123	133	123	549.39
AXT020014	20	14	66.69	AXT150140	150	140	589.88
AXT020016	20	16	47.99	AXT150142	150	142	462.12
AXT020018	20	18	32.42	AXT150144	150	144	347.15
AXT025019	25	19	80.41	AXT180172	180	172	589.88
AXT025021	25	21	62.00	AXT200190	200	190	809.27
AXT030020	30	20	109.70	AXT200192	200	192	657.19
AXT030022	30	22	91.93	AXT200194	200	194	513.54
AXT030024	30	24	74.49	AXT230220	230	220	1114.65
AXT030026	30	26	53.91	AXT230222	230	222	908.36
AXT040030	40	30	142.41	AXT250240	250	240	1450.89
AXT040032	40	32	122.16	AXT250242	250	242	1182.2778
AXT040034	40	34	99.09	AXT300290	300	290	2267.32
AXT040036	40	36	72.61	AXT300292	300	292	1937.94
*AXT050040	50	40	205.35	AXT400392 (+)	400	392	3345.83
AXT050042	50	42	176.07	AXT400390 (+)	400	390	4011.45
AXT050044	50	44	143.03	AXT450442 (+)	450	442	4051.64
AXT050046	50	46	113.12	AXT450440 (+)	450	440	4814.48
AXT060050	60	50	243.99	AXT500492 (+)	500	492	4803.58
AXT060052	60	52	210.04	AXT500490 (+)	500	490	5779.24
AXT060054	60	54	171.70				
AXT060056	60	56	132.44				
AXT070060	70	60	287.32				

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**PLEASE NOTE:**

- \* (\*) To Be Discontinued
- \* All prices exclude VAT
- \* All prices subject to change without notice

- \* (+) Available on Request
- \* Stock subject to confirmation at all times
- \* Terms strictly 30 days

Figure 10.3: Product and price list for acrylic tubing by Maizey plastics

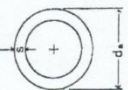


# PERMISSIBLE INTERNAL PRESSURES FOR ACRYLIC TUBES

**NOTICE: 1N/mm<sup>2</sup> = 10kp/cm<sup>2</sup>**

**Permissible internal pressure**

The accompanying table lists the approximate permissible internal pressures of the four types of piping. They can be computed accurately by means of the formula of Mohr:



$$p_{perm} = \frac{2s(d_e - s)}{d_i} \cdot \sigma_{perm}$$

with  $\sigma_{perm}$  the permissible tensile stress in the pipe in the direction of the tangent to the circular circumference.

A high degree of safety must be built into plastics that are subjected to severe stressing. The value of  $\sigma_{perm}$  has therefore been chosen deliberately conservatively.

Material	extruded	cast
$\sigma_{perm}$ (N/mm <sup>2</sup> )	2.5	4

**Example:**

A tube with  $d_e = 80$  mm and wall thickness  $s = 5$  mm.

Substituting these values and the appropriate permitted continuous tensile stress gives the following permissible internal pressure  $p$ :

Material	Extruded	Cast
$p$ (N/mm <sup>2</sup> )	0.29	0.47

These values apply for normal applications at between -40 and +40° C. Negative influences due to liquids, mounting and environment have not been allowed for.

Diameter ext. mm	Diameter int. mm	Wall thickness mm	Weight kg/m	Cast N/mm <sup>2</sup>	Extruded N/mm <sup>2</sup>	Diameter ext. mm	Diameter int. mm	Wall thickness mm	Weight kg/m	Cast N/mm <sup>2</sup>	Extruded N/mm <sup>2</sup>	Diameter ext. mm	Diameter int. mm	Wall thickness mm	Weight kg/m	Cast N/mm <sup>2</sup>	Extruded N/mm <sup>2</sup>														
																		Weight kg/m	Cast N/mm <sup>2</sup>	Extruded N/mm <sup>2</sup>											
5	3	1	0.015	0.8	0.8	32	3	3	0.389	0.35	0.35	96	2	2	0.726	0.25	0.15														
6	3.5	1.25	0.022	0.8	0.8	38	4	4	0.504	0.45	0.45	94	3	3	1.078	0.35	0.2														
6.5	4	1.25	0.024	0.8	0.8	28	5	5	0.611	0.55	0.55	92	4	4	1.423	0.35	0.2														
7	5	1	0.022	0.6	0.6	36	2	2	0.282	0.35	0.25	90	5	5	1.760	0.35	0.25														
8	4	2	0.044	0.9	0.9	34	3	3	0.411	0.55	0.35	104	3	3	1.188	0.35	0.1														
10	6	2	0.059	0.8	0.8	32	4	4	0.534	0.7	0.45	102	4	4	1.571	0.35	0.15														
	4	3	0.078	1.05	1.05	30	5	5	0.648	0.85	0.55	100	5	5	1.945	0.35	0.2														
12	10	1	0.041	0.4	0.4	46	2	2	0.356	0.3	0.2	114	3	3	1.301	0.2	0.1														
	8	2	0.074	0.7	0.7	44	3	3	0.522	0.45	0.3	112	4	4	1.719	0.25	0.15														
13	6	3	0.100	0.9	0.9	42	4	4	0.682	0.6	0.35	110	5	5	2.130	0.3	0.2														
	11	1	0.044	0.35	0.35	40	5	5	0.834	0.7	0.45	127	3	3	1.445	0.2	0.1														
	10	1.5	0.064	0.5	0.5	37	6.5	6.5	1.048	0.55	0.55	125	4	4	1.912	0.25	0.15														
	9	2	0.082	0.85	0.85	56	2	2	0.430	0.15	0.15	123	5	5	2.371	0.3	0.2														
15	11	2	0.096	0.7	0.7	54	3	3	0.634	0.35	0.25	146	2	2	1.097	0.35	0.05														
	10	2.5	0.116	0.7	0.7	53	3.5	3.5	0.730	0.25	0.25	144	3	3	1.634	0.15	0.1														
16	12	2	0.104	0.55	0.55	52	4	4	0.830	0.5	0.3	142	4	4	2.164	0.2	0.15														
20	18	1	0.070	0.2	0.2	50	5	5	1.019	0.6	0.4	140	5	5	2.686	0.25	0.15														
	16	2	0.133	0.45	0.45	59	3	3	0.689	0.35	0.35	174	3	3	1.967	0.05	0.05														
	14	3	0.188	0.65	0.65	57	4	4	0.904	0.45	0.45	173	3.5	3.5	2.289	0.1	0.1														
22	16	3	0.211	0.55	0.55	55	5	5	1.112	0.55	0.55	172	4	4	2.608	0.1	0.1														
25	23	1	0.089	0.2	0.2	64	3	3	0.745	0.3	0.2	170	5	5	3.242	0.1	0.1														
	21	2	0.170	0.6	0.6	62	4	4	0.978	0.45	0.25	194	3	3	2.190	0.1	0.05														
	19	3	0.245	0.85	0.55	60	5	5	1.204	0.55	0.35	192	4	4	2.905	0.15	0.1														
30	26	2	0.207	0.5	0.3	56	7	7	1.634	0.6	0.45	190	5	5	3.613	0.2	0.1														
	24	3	0.300	0.7	0.45	69	3	3	0.900	0.3	0.3	292	4	4	4.387	0.1	0.1														
	22	4	0.385	0.9	0.55	67	4	4	1.052	0.4	0.4	290	5	5	5.465	0.15	0.15														
	20	5	0.463	1.1	0.7	65	5	5	1.297	0.5	0.5	288	6	6	6.536	0.15	0.15														
32	26	3	0.322	0.4	0.4	76	2	2	0.578	0.1	0.1	449	4	4	6.714	0.05	0.05														
	24	4	0.415	0.55	0.55	74	3	3	0.856	0.3	0.2	447	5	5	8.374	0.1	0.1														
						72	4	4	1.126	0.35	0.25	445	6	6	10.026	0.1	0.1														
						70	5	5	1.389	0.45	0.3	441	8	8	13.309	0.15	0.15														
						84	3	3	0.967	0.25	0.15	640	5	5	11.941	0.08	0.08														
						82	4	4	1.275	0.35	0.2	638	6	6	14.313	0.09	0.09														
						80	5	5	1.575	0.45	0.25	634	8	8	19.033	0.12	0.12														
						78	6	6	1.967	0.45	0.3	630	10	10	23.706	0.15	0.15														
CAST																2 000															
EXTRUDED																2 000															

The above table is limited to a statement of the values for tubes and pipes available in the respective material grades and dimensions.

Maximum lengths in mm

Figure 10.4: Specifications of acrylic tubing provided by Maizey plastics



**PRETORIA (HEAD OFFICE)**  
 58 GARDEN STREET,  
 PRETORIA WEST,  
 P O BOX 18502,  
 PRETORIA WEST, 0117  
 TEL (012) 327-2000  
 FAX (012) 327-1987 admin  
 FAX (012) 327-1984 sales

**JOHANNESBURG**  
 6 LOVEDAY STREET SOUTH,  
 WAVEVILLE  
 P O BOX 26106  
 EXCOM, 2023  
 TEL (011) 484-6783  
 FAX (011) 493-1887

**GERMISTON**  
 LANERN ROAD,  
 WADEVILLE  
 P O BOX 14696  
 WILLOWDALE, 1609  
 TEL (011) 824-2683  
 FAX (011) 824-2611

**CAPE TOWN**  
 58 SECTION STREET,  
 PAARDEN ELAND  
 P O BOX 188  
 PAARDEN ELAND, 7420  
 TEL (021) 511-9763  
 FAX (021) 510-1612

**DURBAN**  
 40 BEECHFIELD CRESC.,  
 SPRINGFIELD PARK  
 P O BOX 74587  
 RICHMOND, 4024  
 TEL (031) 579-3765/9  
 FAX (031) 579-3793

**RICHARDS BAY**  
 MCCORD INDUSTRIAL PARK  
 ALUMINA ALLEE  
 ALTON NORTH  
 P O BOX 675  
 RICHARDS BAY, 3801  
 TEL (0351) 973-1578/9  
 FAX (0351) 973-159

**MIDDELBURG**  
 40 HOOB & SLEIGHTKAMP STS,  
 MIDDELBURG  
 MIDDELBURG, 0950  
 TEL (0182) 46-2801  
 FAX (0182) 46-1153

**WELKOM**  
 16 11th STREET,  
 VOORSPOED-OOS  
 P O BOX 1894  
 WELKOM, 5114  
 TEL (057) 5-1184  
 FAX (057) 5-4102



# PHYSICAL PROPERTIES

Mechanical properties	Cast	Extruded	Unit	Test Specs.
Density of the tube	1.18	1.18	g/cm <sup>3</sup>	DIN 53479
Impact strength Standard small test specimen	12	12	KJ/m <sup>2</sup> (cmkgf/cm <sup>2</sup> )	DIN 53453
Notched impact strength Standard small test specimen	2	2	KJ/m <sup>2</sup> (cmkgf/cm <sup>2</sup> )	DIN 53453
Tensile strength (1/1-test specimen 3, v = 5mm/min)	80 (800)	70 (700)	N/mm <sup>2</sup> (kgf/cm <sup>2</sup> )	DIN 53455
Elongation at break (1/1-test specimen 3, v = 5mm/min)	5.5	4.5	%	DIN 53455
Flexural strength (80 x 10 x 4mm test specimen)	115 (1150)	105 (1050)	N/mm <sup>2</sup> (kgf/cm <sup>2</sup> )	DIN 53452
Modulus of elasticity	3300 (33000)	3300 (33000)	N/mm <sup>2</sup> (kgf/cm <sup>2</sup> )	DIN 53457
Modulus in shear	1700 (17000)	1700 (17000)	N/mm <sup>2</sup> (kgf/cm <sup>2</sup> )	DIN 53445
Compressive yield stress	110 (1100)	103 (1030)	N/mm <sup>2</sup> (kgf/cm <sup>2</sup> )	DIN 53454
Indentation hardness	200 (2000)	190 (1900)	N/mm <sup>2</sup> (kgf/cm <sup>2</sup> )	DIN 53456
Thermal properties	Cast	Extruded	Unit	Test Specs.
Coefficient of linear thermal expansion from 0 to 50 °C	70 . 10 <sup>-6</sup>	70 . 10 <sup>-6</sup>	1/K	VDE 0304
Thermal conductivity	0.19	0.16	W/mK (kcal/m h°C)	DIN 52612
Forming temperature (stove temperature)	160	150	°C	-
Max. service temperature	80	75	°C	-
Vicat softening temperature, method B	115	102	°C	DIN 53460
Heat distortion temperature accdg. to ISO 75 a) deflection 1.80 N/mm <sup>2</sup> b) deflection 0.45 N/mm <sup>2</sup>	105 113	90 95	°C	DIN 53461
Electrical properties	Cast	Extruded	Unit	Text Specs.
Volume resistivity dry	>10 <sup>15</sup>	>10 <sup>15</sup>	Ohm cm	DIN 53482
Surface resistance dry	5.10 <sup>13</sup>	5.10 <sup>13</sup>	Ohm	DIN 53482
Dielectric strength (1mm thick specimen, 1 kV/s).	30	30	kV/mm	DIN 53481
Dielectric constant at 50 Hz at 0.1 MHz	3.6 2.7	3.7 2.8	-	DIN 53483
Dielectric loss factor at 50 Hz	0.06	0.06	-	DIN 53483

Information given in this data sheet is accurate to the best of our knowledge and belief, however, Maizey (Pty) Ltd, cannot accept any liability whatsoever in respect of the use of this information nor in the use of any product described herein.

Penrose

M004

Figure 10.5: Specifications of acrylic tubing provided by Maizey plastics, continued



# 11. APPENDIX C

The minimum requirement of CO<sub>2</sub> is 1.83 g for the production of 1g of algae biomass (Chisti, 2007; Posten, 2009). Using a biomass density of 2g/l and a volume of 1200 litre for calculation purposes. Assuming a growth rate of 1 g biomass growth per gram of biomass, the minimum amount of CO<sub>2</sub> required for the reactor is 4 392 g. Because CO<sub>2</sub> has a density of 19.8 kg/m<sup>3</sup> at atmospheric pressure, therefore 2.22 m<sup>3</sup> is required daily. Using a 2 % (v/v) ratio of air and carbon dioxide over a 12-hour day, 111 000 litres of gas are required per day which is equal to 154 l/min. Using a 5% (v/v) of a 12 hour day, 44 000 litres of gas is required per day, which is equal to 62l per min.

The mass balance does not take into account the fact that some CO<sub>2</sub> will not be used and therefore the required CO<sub>2</sub> would increase. As this is calculated according to the minimum requirement, some species may require up to double the quantity of CO<sub>2</sub>.

## 12. APPENDIX D

EG:JM

1:1 Mixture: Mix then autoclave at 15 psi for 15 minutes

The autoclaving of the EG:JM medium is very important because it destroys the yeast extract which could still have some yeast. The yeast or other microorganisms could attack and destroy the algae cells. When using only inorganic salts in a medium, the requirement for autoclaving is reduced drastically, but it is still recommended, especially when a monoculture is required. The major problem with autoclaving is the requirement of large amounts of energy which would increase production cost.

Table 12.1: EG (*Euglena gracilis* medium)

<b>(1) Stock</b>	<b>Per litre</b>
CaCl <sub>2</sub>	1.0 g
<b>Medium</b>	<b>Per litre</b>
Sodium acetate trihydrate (CH <sub>3</sub> COONa.3H <sub>2</sub> O)	1.0 g
"Lab-Lemco" powder (Oxoid L29)*	1.0 g
Tryptone (Oxoid L42)*	2.0 g
Yeast extract (Oxoid L21)*	2.0 g
CaCl <sub>2</sub> stock solution (1)	10.0 ml

Add constituents above and make up to 1 litre with deionised water.

According to CCAP all the \* components were supplied by Unipath Ltd, Wade Road, Basingstoke, Hants RG24 0PW, UK. These were not explored further as the medium was not used. However, it was noted that these compounds are extracts containing minerals and amino acids (Oxoid Limited).

Table 12.2: JM (Jaworski's Medium)

Solution number	Stock	Per 200 ml (in grams)
1	Ca(NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O	4.0
2	KH <sub>2</sub> PO <sub>4</sub>	2.48
3	MgSO <sub>4</sub> ·7H <sub>2</sub> O	10.0
4	NaHCO <sub>3</sub>	3.18
5	EDTAFeNa	0.45
	EDTANa <sub>2</sub>	0.45
6	H <sub>3</sub> BO <sub>3</sub>	0.496
	MnCl <sub>2</sub> ·4H <sub>2</sub> O	0.278
	(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> ·4H <sub>2</sub> O	0.2
7	Cyanocobalamin	0.008
	Thiamine HCL	0.008
	Biotin	0.008
8	NaNO <sub>3</sub>	16.0
9	Na <sub>2</sub> HPO <sub>4</sub> ·12H <sub>2</sub> O	7.2

Add 1 ml of each stock solution (1-9) and make up to 1 litre using deionised water.



Table 12.3: Bold's Basal Medium constituents and formulation

Stock Solution Number	Chemical Name	Chemical Formula	Weight (g)	Distilled Water (ml)
1	Di-potassium hydrogen orthophosphate	$K_2HPO_4$	1.875	250
2	Potassium di-hydrogen orthophosphate	$KH_2PO_4$	4.375	250
3	Magnesium sulphate	$MgSO_4 \cdot 7H_2O$	1.875	250
4	Sodium nitrate	$NaNO_3$	6.250	250
5	Calcium chloride	$CaCl_2 \cdot 2H_2O$	0.625	250
6	Sodium chloride	$NaCl$	0.625	250
7	EDTA tetrasodium salt	$EDTA-Na_4$	5.000	100
	Potassium hydroxide	$KOH$	3.100	
8	Ferrous sulphate	$FeSO_4 \cdot 7H_2O$	0.498	100
	Sulphuric acid conc.	$H_2SO_4$	0.1 mL (weight per mL = 1.84g)	
9	Boric acid	$H_3BO_3$	1.142	100
10	Zinc sulphate	$ZnSO_4 \cdot 7H_2O$	0.353	25
11	Manganese chloride	$MnCl_2 \cdot 4H_2O$	0.058	25
12	Cupric sulphate	$CuSO_4 \cdot 5H_2O$	0.063	25
13	Cobaltous nitrate	$Co(NO_3)_2 \cdot 6H_2O$	0.020	25
14	Sodium molybdate	$Na_2MoO_4 \cdot 2H_2O$	0.048	25

Source: Connon, 2007

To prepare 1 L of medium 10 ml of stock solution 1 – 6 were added, 1 ml of stock solution 7-9 were added and 0.1 ml of stock solution 10 – 14 were added to a beaker and filled to make 1 L. To ensure that the culture remained sterile the medium were autoclaved during the stockpiling stage.

# 13. APPENDIX E



Figure 13.1: Communication with Prof Johan Grobbelaar on CO<sub>2</sub> concentrations and buffer to be used

## 14. APPENDIX F

Due to the large amount of data the median and standard deviation of the values is shown for the pH. The growth results show the actual volumetric growth results with the median and standard deviation, which will be used for graphs. The values were calculated using the median and stdev functions available in Microsoft Excel.

Table 14.1: Median and standard deviation pH values at 5 min CO<sub>2</sub> twice daily

Reading	1	2	3	4	5
Median	7.515	6.06	7.705	5.98	7.685
Std. deviation	0.177	0.237	0.127	0.212	0.123

Table 14.2: Median and standard deviation pH values of 2 min CO<sub>2</sub> four times daily

Reading	1	2	3	4	5	6	7	8	9
Median	7.87	6.42	7.98	6.22	7.96	6.30	7.99	6.34	7.99
Std. Deviation	0.395	0.365	0.119	0.256	0.099	0.262	0.093	0.315	0.128

Table 14.3: Median and standard deviation pH values of continuous CO<sub>2</sub> at 10 % (v/v)

Reading	1	2	3	4	5
Median	7.27	7.24	7.275	7.245	7.25
Std. Deviation	0.0397	0.0284	0.0460	0.0288	0.0353

Table 14.4: Median and standard deviation pH values of continuous CO<sub>2</sub> at 5 % (v/v)

Reading	1	2	3	4	5
Median	7.485	7.475	7.515	7.520	7.510
Std. Deviation	0.0530	0.0529	0.0559	0.0757	0.0530

Table 14.5: Results of 5 min and 2 min addition of CO<sub>2</sub> using the same reactor configurations in g/l/d

Density	Gas Dispersion	Material	Size	5 min CO <sub>2</sub>		Median	2 min CO <sub>2</sub>		Median
-1	1	-1	50 mm	-0.007835	-0.030706	-0.019271	-0.013553	-0.025624	-0.019588
1	-1	-1	50 mm	0.010377	0.026471	0.018424	0.045953	-0.000635	0.022659
-1	-1	1	50 mm	-0.018635	-0.029647	-0.024141	-0.019271	-0.135954	-0.077612
-1	1	1	50 mm	0.157977	0.065436	0.111706	0.164965	-0.017788	0.073589
-1	-1	-1	90 mm	-0.056753	-0.031765	-0.044259	-0.051247	-0.049977	-0.050612
-1	1	-1	90 mm	-0.072847	-0.045741	-0.059294	0.022659	-0.121342	-0.049341
1	1	1	90 mm	-0.034518	-0.011012	-0.022765	0.014188	0.054847	0.034518
-1	-1	1	90 mm	-0.073483	-0.029859	-0.051671	-0.013553	-0.068400	-0.040977
1	1	-1	110 mm	-0.011647	-0.040447	-0.026047	-0.030918	0.041294	0.005188
1	-1	-1	110 mm	-0.030494	-0.038753	-0.034624	-0.137224	-0.174495	-0.155860
1	1	1	110 mm	-0.017577	0.006141	-0.005718	-0.069671	0.034518	-0.017577
1	-1	1	110 mm	0.015882	-0.040871	-0.012494	-0.031553	-0.038965	-0.035259

Table 14.6: Results of continuous CO<sub>2</sub> addition using the same reactor conditions as in Table 14.5 for comparison in g/l/d

Density	Gas Dispersion	Material	Size	10% CO <sub>2</sub> continuously		Median	5% CO <sub>2</sub> continuously		Median
-1	1	-1	50 mm	0.015671	0.030494	0.023082	-0.041506	0.076659	0.017577
1	-1	-1	50 mm	0.158189	0.020965	0.089577	0.046588	0.097836	0.072212
-1	-1	1	50 mm	0.148236	0.047224	0.097730	0.154801	0.332472	0.243636
-1	1	1	50 mm	0.212613	-0.037059	0.087777	0.131295	0.112024	0.121659
-1	-1	-1	90 mm	-0.030282	0.031130	0.000424	-0.063530	0.052941	-0.005294
-1	1	-1	90 mm	0.020330	0.067130	0.043730	-0.113506	0.091906	-0.010800
1	1	1	90 mm	0.090424	0.006777	0.048600	0.048071	0.163907	0.105989
-1	-1	1	90 mm	-0.038753	0.102071	0.031659	-0.061836	0.020753	-0.020541
1	1	-1	110 mm	0.059930	0.089365	0.074647	0.012918	0.061836	0.037377
1	-1	-1	110 mm	0.015882	0.088518	0.052200	-0.005718	0.048918	0.021600
1	1	1	110 mm	0.102918	0.031977	0.067447	-0.029012	0.042141	0.006565
1	-1	1	110 mm	-0.003600	0.078565	0.037483	-0.001906	0.052518	0.025306

Table 14.7: Results of 50 mm diameter reactors from factorial experiments showing the growth rate in g/l/d

Density	Gas Dispersion	Material	Run 1	Run 2	Run 3	Run 4	Median	Standard deviation
-1	-1	-1	0.096989	0.038394	0.079624	0.086952	0.083288	0.025735
1	-1	-1	0.046588	0.097836	0.015656	0.070306	0.058447	0.034933
-1	1	-1	0.046377	0.033887	0.026628	0.059486	0.040132	0.014450
1	1	-1	-0.041506	0.076659	-0.020118	-0.017971	-0.019044	0.052678
-1	-1	1	0.085275	0.053055	0.012652	0.059418	0.056236	0.030057
1	-1	1	0.154801	0.332472	0.041225	0.134259	0.144530	0.121675
-1	1	1	0.041083	0.050873	0.062313	0.092352	0.056593	0.022228
1	1	1	0.131295	0.112024	0.094024	0.076812	0.103024	0.023433

Table 14.8: Results of 90 and 110 mm diameter reactors from factorial experiments, showing the growth rate in g/l/d

Density	Gas Dispersion	Material	Size	Run 1	Run 2	Run 3	Run 4	Median	Standard deviation
-1	-1	-1	-1	-0.021600	-0.020193	-0.009739	-0.039388	-0.020897	0.012302
1	-1	-1	-1	-0.063530	-0.098895	-0.078818	-0.069459	-0.074139	0.015483
-1	1	-1	-1	-0.119859	-0.053943	-0.017509	-0.081742	-0.067842	0.043301
1	1	-1	-1	-0.113506	-0.091906	-0.082377	-0.077671	-0.087142	0.015904
-1	-1	1	-1	-0.087247	-0.067295	-0.007929	0.026308	-0.037612	0.052474
1	-1	1	-1	-0.061836	0.017365	-0.053342	-0.043624	-0.048483	0.035928
-1	1	1	-1	-0.001694	-0.046461	-0.009995	0.032194	-0.005845	0.032297
1	1	1	-1	0.048071	-0.045318	-0.015337	-0.058024	-0.030327	0.047329
-1	-1	-1	1	-0.001271	-0.009529	0.010588	0.032814	0.004659	0.018399
1	-1	-1	1	-0.069247	-0.072212	-0.055694	-0.064328	-0.066788	0.007224
-1	1	-1	1	0.012918	0.061836	0.048918	0.059930	0.054424	0.022714
1	1	-1	1	-0.019116	0.009189	-0.000117	0.040334	0.004536	0.024815
-1	-1	1	1	-0.001906	-0.004024	0.016922	0.025835	0.007508	0.014544
1	-1	1	1	-0.029012	0.042141	-0.031854	-0.014234	-0.021623	0.034464
-1	1	1	1	0.034886	0.036635	0.049547	0.046165	0.041400	0.007154
1	1	1	1	-0.060777	-0.049553	-0.066626	-0.017365	-0.055165	0.021983

Table 14.9: Results of 50 mm diameter reactor showing the growth rate in g/m<sup>2</sup>/d

Density	Gas Dispersion	Material	Run 1	Run 2	Run 3	Run 4	Median	Standard deviation
-1	-1	-1	3.007072	1.190369	2.468688	2.695890	2.582289	0.797883
1	-1	-1	1.444445	3.033334	0.485401	2.179799	1.812122	1.083073
-1	1	-1	1.437879	1.050658	0.825593	1.844339	1.244269	0.448002
1	1	-1	-1.286869	2.376768	-0.623738	-0.557165	-0.590451	1.633245
-1	-1	1	2.643899	1.644927	0.392260	1.842202	1.743564	0.931887
1	-1	1	4.799496	10.308084	1.278166	4.162628	4.481062	3.772445
-1	1	1	1.273738	1.577285	1.931983	2.863314	1.754634	0.689157
1	1	1	4.070708	3.473233	2.915152	2.381502	3.194193	0.726511

Table 14.10: Specific growth rate of 50 mm diameter reactors in (days<sup>-1</sup>)

Density	Gas Dispersion	Material	Run 1	Run 2	Run 3	Run 4	Median	Standard deviation
-1	-1	-1	0.162596	0.071428	0.148655	0.146871	0.147763	0.041244
1	-1	-1	0.046520	0.091169	0.016178	0.073762	0.060141	0.032786
-1	1	-1	0.083525	0.055443	0.048352	0.104891	0.069484	0.026100
1	1	-1	-0.045357	0.082226	-0.020637	-0.018686	-0.019662	0.056545
-1	-1	1	0.145860	0.079106	0.024862	0.111603	0.095355	0.051472
1	-1	1	0.199544	0.328237	0.041633	0.134182	0.166863	0.120462
-1	1	1	0.059290	0.079592	0.116814	0.156407	0.098203	0.042824
1	1	1	0.118845	0.091310	0.091819	0.059115	0.091564	0.024431

Table 14.11: Results of 90 and 110 mm diameter reactors showing the growth rate in g/m<sup>2</sup>/d

Density	Gas Dispersion	Material	Size	Run 1	Run 2	Run 3	Run 4	Median	Standard deviation
-1	-1	-1	-1	-0.316912	-0.296272	-0.142886	-0.577899	-0.306592	0.180486
1	-1	-1	-1	-0.932095	-1.450961	-1.156405	-1.019090	-1.087747	0.227163
-1	1	-1	-1	-1.758552	-0.791444	-0.256894	-1.199295	-0.995370	0.635298
1	1	-1	-1	-1.665343	-1.348430	-1.208616	-1.139576	-1.278523	0.233345
-1	-1	1	-1	-1.280077	-0.987334	-0.116331	0.385979	-0.551833	0.769886
1	-1	1	-1	-0.907239	0.254773	-0.782625	-0.640038	-0.711332	0.527131
-1	1	1	-1	-0.024856	-0.681661	-0.146644	0.472341	-0.085750	0.473861
1	1	1	-1	0.705285	-0.664894	-0.225023	-0.851313	-0.444959	0.694403
-1	-1	-1	1	-0.013761	-0.103207	0.114674	0.355386	0.050457	0.199267
1	-1	-1	1	-0.749970	-0.782079	-0.603187	-0.696691	-0.723331	0.078236
-1	1	-1	1	0.139903	0.669698	0.529796	0.649057	0.589426	0.245995
1	1	-1	1	-0.207030	0.099523	-0.001270	0.436828	0.049127	0.268752
-1	-1	1	1	-0.020641	-0.043576	0.183275	0.279805	0.081317	0.157515
1	-1	1	1	-0.314208	0.456404	-0.344985	-0.154159	-0.234183	0.373255
-1	1	1	1	0.377822	0.396773	0.536614	0.499980	0.448377	0.077484
1	1	1	1	-0.658231	-0.536676	-0.721579	-0.188066	-0.597453	0.238082



Table 14.12: Specific growth rate of 90 and 110 mm reactors in (days<sup>-1</sup>)

Density	Gas Dispersion	Material	Size	Run 1	Run 2	Run 3	Run 4	Median	Standard deviation
-1	-1	-1	-1	-0.04819	-0.04955	-0.03048	-0.1193	-0.048873	0.039251
1	-1	-1	-1	-0.07141	-0.0995	-0.08951	-0.06735	-0.080457	0.015158
-1	1	-1	-1	-0.24349	-0.15179	-0.04518	-0.14737	-0.149581	0.081036
1	1	-1	-1	-0.11737	-0.09607	-0.1002	-0.11128	-0.105740	0.009818
-1	-1	1	-1	-0.18032	-0.16677	-0.02257	0.074029	-0.094673	0.121748
1	-1	1	-1	-0.07027	0.018688	-0.06086	-0.12068	-0.065568	0.057645
-1	1	1	-1	-0.00401	-0.12226	-0.02772	0.087629	-0.015867	0.086237
1	1	1	-1	0.050822	-0.05513	-0.01982	-0.05186	-0.035838	0.049195
-1	-1	-1	1	-0.002218	-0.016179	0.017960	0.053033	0.007871	0.030056
1	-1	-1	1	-0.068544	-0.070375	-0.071585	-0.094265	-0.070980	0.012113
-1	1	-1	1	0.022252	0.100152	0.090990	0.123226	0.095571	0.043440
1	1	-1	1	-0.029981	0.013085	-0.000190	0.056286	0.006448	0.035842
-1	-1	1	1	-0.003971	-0.006386	0.029808	0.057863	0.012919	0.030544
1	-1	1	1	-0.030682	0.044264	-0.052164	-0.024581	-0.027632	0.041748
-1	1	1	1	0.063439	0.067171	0.079896	0.078684	0.072927	0.008232
1	1	1	1	-0.079312	-0.085641	-0.099985	-0.016286	-0.082477	0.037037

Table 14.13: Regression coefficient and 90 % confidence limits for 50 mm reactors

Factor	Regr. Coefficients; Var.:Growth; R-sqr=.42351; Adj:.36174 (Design: 2**(3-0) design (Design: 2**(4-0) design ([No active dataset]) in 50.stw) in 50.stw) 2**(3-0) design; MS Residual=.0027063 DV: Growth					
	Regressn Coeff.	Std.Err.	t(28)	p	-90.% Cnf.Limt	+90.% Cnf.Limt
Mean/Interc.	0.069679	0.009196	7.57688	0.000000	0.054035	0.085323
(2) Gas Dispersion	-0.018165	0.009196	-1.97530	0.058167	-0.033809	-0.002521
(3) Material	0.026192	0.009196	2.84815	0.008149	0.010548	0.041836
1 by 3	0.026899	0.009196	2.92506	0.006756	0.011256	0.042543

Table 14.14: Regression coefficient and 90 % confidence limits for 90 mm reactors

Factor	Regr. Coefficients; Var.:Growth; R-sqr=.41582; Adj:.35323 (Design: 2**(3-0) design ([No active dataset]) in Workbook3) 2**(3-0) design; MS Residual=.0011749 DV: Growth					
	Regressn Coeff.	Std.Err.	t(28)	p	-90.% Cnf.Limt	+90.% Cnf.Limt
Mean/Interc.	-0.044197	0.006059	-7.29401	0.000000	-0.054505	-0.033889
(1) Density	-0.011316	0.006059	-1.86754	0.072328	-0.021624	-0.001008
(3) Material	0.020812	0.006059	3.43464	0.001868	0.010504	0.031119
2 by 3	0.013060	0.006059	2.15539	0.039875	0.002753	0.023368

Table 14.15: Regression coefficient and 95 % confidence limits for 110 mm reactors

Factor	Regr. Coefficients; Var.:Growth; R-sqr=.6827; Adj:.64871 (Design: 2**(3-0) design ([No active dataset]) in Workbook2) 2**(3-0) design; MS Residual=.0006049 DV: Growth					
	Regressn Coeff.	Std.Err.	t(28)	p	-95.% Cnf.Limt	+95.% Cnf.Limt
Mean/Interc.	-0.001194	0.004348	-0.27460	0.785638	-0.010100	0.007712
(1) Density	-0.027460	0.004348	-6.31592	0.000001	-0.036367	-0.018554
(2) Gas Dispersion	0.012869	0.004348	2.95991	0.006202	0.003963	0.021775
2 by 3	-0.014804	0.004348	-3.40492	0.002017	-0.023710	-0.005898

Table 14.16: Regression coefficient and 95 % confidence limits for 90 and 110 mm reactor combination

Factor	Regr. Coefficients; Var.:Growth; R-sqr=.66864; Adj:.62045 (Design: 2**(4-0) design ([No active dataset]) in 90-11.stw) 2**(4-0) design; MS Residual=.0008391 DV: Growth					
	Regressn Coeff.	Std.Err.	t(55)	p	-95.% Cnf.Limt	+95.% Cnf.Limt
Mean/Interc.	-0.022695	0.003621	-6.26778	0.000000	-0.029952	-0.015439
(1) Density	-0.019388	0.003621	-5.35445	0.000002	-0.026645	-0.012132
(3) Material	0.010277	0.003621	2.83824	0.006342	0.003021	0.017534
(4) Size	0.021501	0.003621	5.93804	0.000000	0.014245	0.028758
1 by 4	-0.008072	0.003621	-2.22929	0.029898	-0.015329	-0.000816
2 by 4	0.007307	0.003621	2.01807	0.048473	0.000051	0.014564
3 by 4	-0.010534	0.003621	-2.90929	0.005218	-0.017791	-0.003278
1*2*3	-0.009364	0.003621	-2.58603	0.012387	-0.016621	-0.002107
2*3*4	-0.013932	0.003621	-3.84762	0.000313	-0.021189	-0.006676

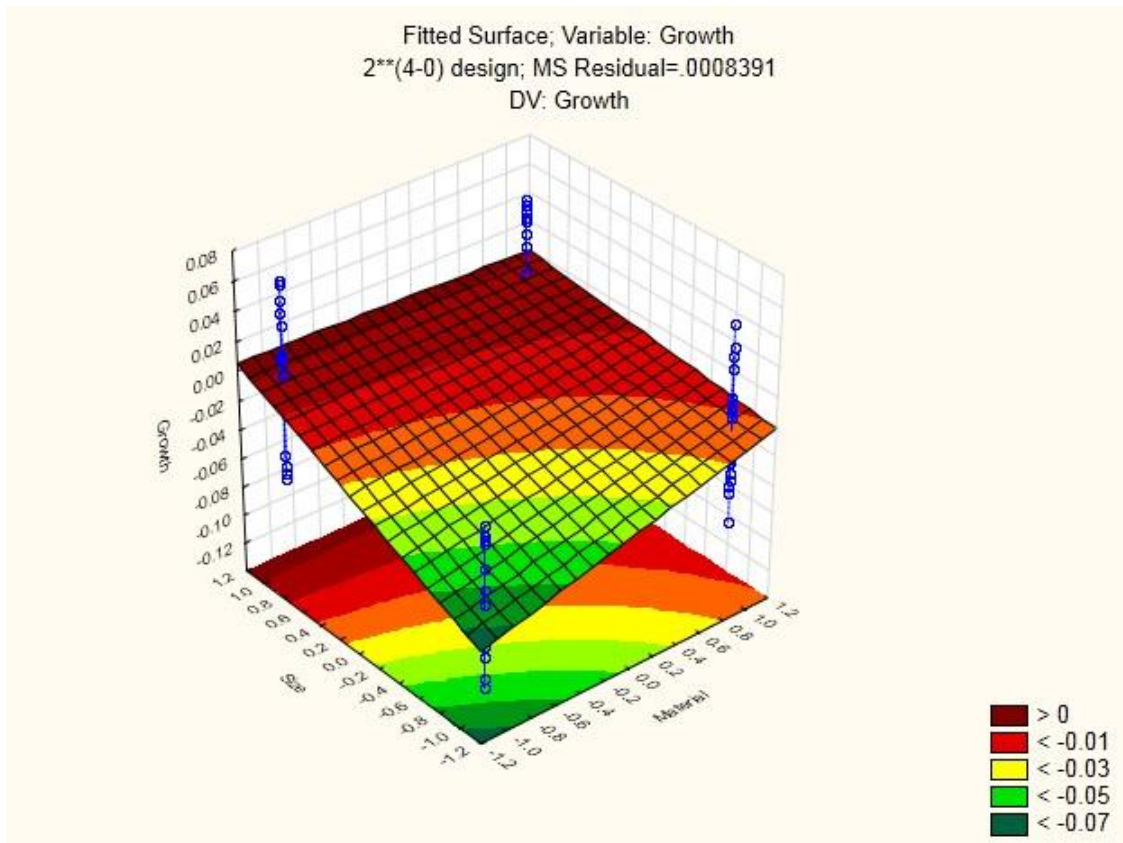


Figure 14.1: Visual representation of the interaction between the reactor size and materials used in the 90 and 110 mm combined analysis.

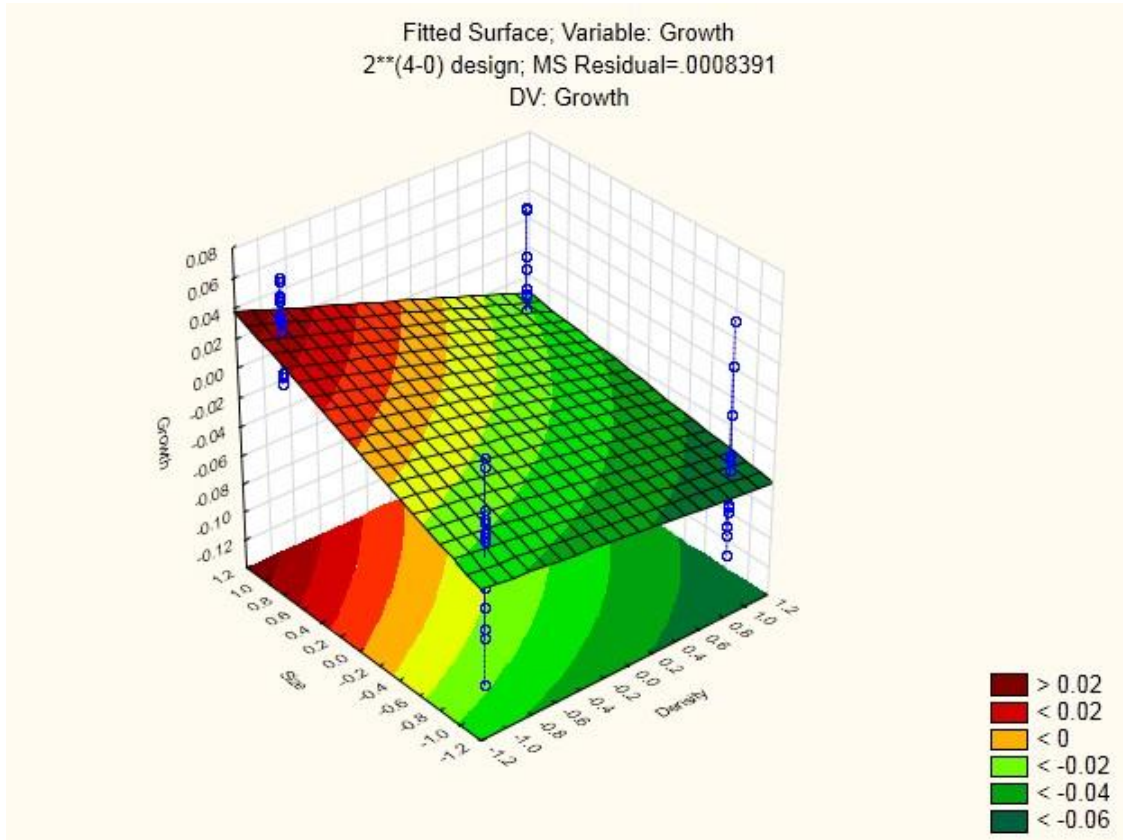


Figure 14.2: Visual representation of the interaction between the reactor size and culture density used in the 90 and 110 mm combined analysis

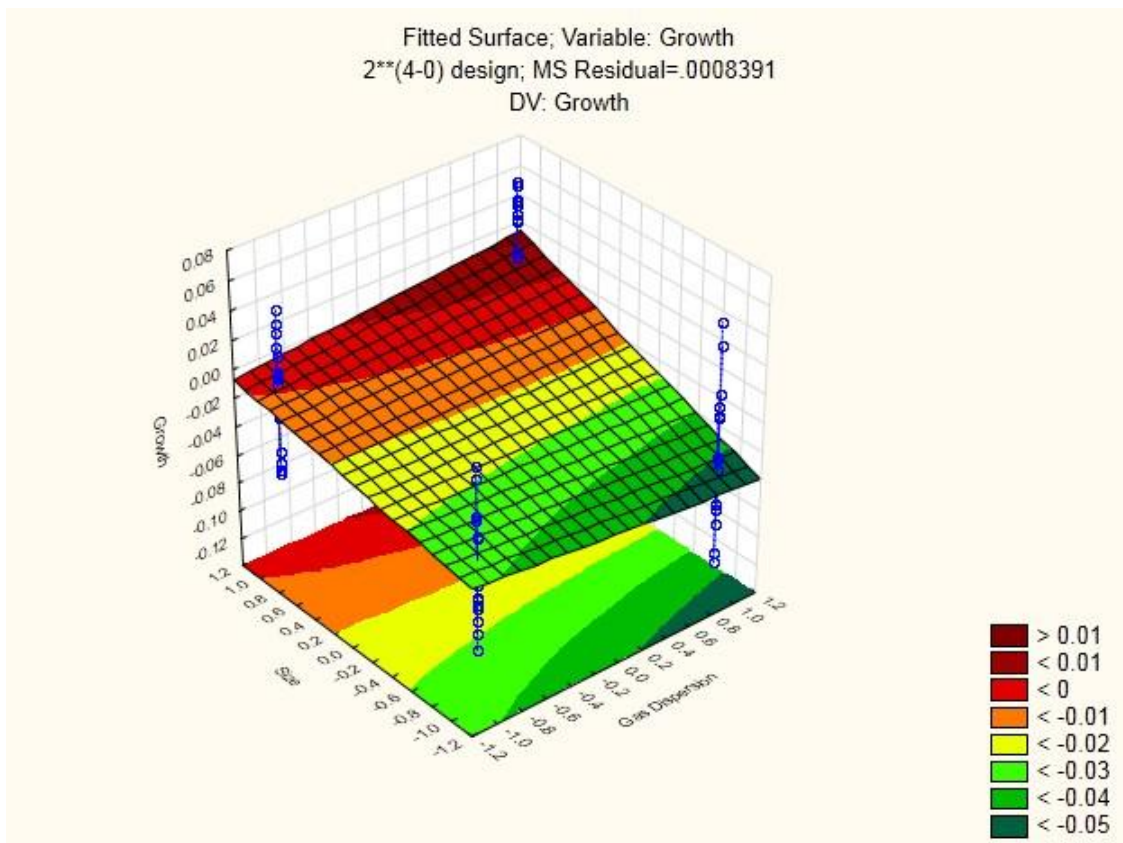


Figure 14.3: Visual representation of the interaction between the reactor size and the gas dispersion unit used in the 90 and 110 mm combined analysis

The interactions seen in Figure 14.1, Figure 14.2 and Figure 14.3 show the effects of the main interactions. These interactions all show that the diameter or size of the tubing is very important. As stated previously, this is due the negative growth seen in the 90 mm reactors.

# 15. APPENDIX G

Name	Formula	SKU-Pack size	Description	Price per unit	Cost to make 1 litre of medium
Di-potassium hydrogen orthophosphate	$K_2HPO_4$	P3786-1KG	ACS reagent, $\geq 98\%$	972.19	0.0729
Potassium di-hydrogen orthophosphate	$KH_2PO_4$	P9791-1KG	molecular biology, $\geq 98.0\%$	851.49	0.1490
Magnesium sulphate	$MgSO_4 \cdot 7H_2O$	230391-1KG	ACS reagent, $\geq 98\%$	796.02	0.0597
Sodium nitrate	$NaNO_3$	S5022-1KG	$\geq 99.0\%$ , plant cell	931.42	0.2329
Calcium chloride	$CaCl_2 \cdot 2H_2O$	C7902-1KG	BioReagent, $\geq 99.0\%$	1004.81	0.0251
Sodium chloride	$NaCl$	S7653-1KG	BioXtra, $\geq 99.5\%$	362.13	0.0091
EDTA tetrasodium salt	EDTA- $Na_4$	03699-1KG	BioUltra, $\geq 99.0\%$	1497.44	0.0749
Potassium hydroxide	KOH	P5958-1KG	BioXtra, $\geq 85\%$	858.02	0.0266
Ferrous sulphate	$FeSO_4 \cdot 7H_2O$	215422-1KG	ACS reagent, $\geq 99.0\%$	758.51	0.0038
Sulphuric acid conc. (1.84g/ml)	$H_2SO_4$	320501-1L	ACS reagent, 95.0-98.0%	316.45	0.0032
Boric acid	$H_3BO_3$	B6768-1KG	BioReagent, $\geq 99.5\%$	649.22	0.0074
Zinc sulphate	$ZnSO_4 \cdot 7H_2O$	Z0251-500G	BioReagent	1109.22	0.0031
Manganese chloride	$MnCl_2 \cdot 4H_2O$	M5005-500G	BioReagent	1089.64	0.0005
Cupric sulphate	$CuSO_4 \cdot 5H_2O$	C8027-500G	BioReagent, $\geq 98\%$	548.09	0.0003
Cobaltous nitrate	$Co(NO_3)_2 \cdot 6H_2O$	239267-500G	ACS reagent, $\geq 98\%$	2128.72	0.0003
Sodium molybdate	$Na_2MoO_4 \cdot 2H_2O$	M1003-500G	$\geq 99.5\%$	2039.00	0.0008

Sub-total	R 0.6695
VAT	R 0.0937
Total:	<u>R 0.7633</u>

Source: Sigma-Aldrich, 2014

Table 15.1: Capital and running cost of a 50 mm PVC reactor

Product number	Description	Price per unit	Quantity	Total
GOD 50	90° Elbow	18.00	2.00	36.00
TID 50	90° Tee	26.00	46.00	1,196.00
CAD 50	End cap	15.00	36.00	540.00
uPVC 50	Pipe class 4 pm	27.00	3.00	81.00
CHD 50	Ball valve	52.00	2.00	104.00
PVCTC050046I	Clear PVC tubing	114.33	60.00	6,859.80
PVC adhesive	1 litre	360.00	1.00	360.00
PVC cleaner	500 millilitre	77.00	2.00	154.00
Gas tubing	6 mm per metre	6.00	80.00	480.00
Gas dispersion units	Large	12.00	36.00	432.00
Pressure pump SM 100	0.75kW 20-90l/min	1,967.00	1.00	1,967.00
Air blower B02A02	0.4 kW 1.3m <sup>3</sup> /min	4,143.00	1.00	4,143.00
Gas flow meters V10420	0-24.68 l/min	2,691.00	1.00	2,691.00
Medium Storage tank	1000 liter tank	2,045.00	1.00	2,045.00
		Sub-total		R 21,088.80
		Vat		R 2,952.43
Capital cost		Total		R 24,041.23
40-RC	CO2 Tec Dry	270.78	27.34	7,404.27
Medium		0.67	20,787.81	13,917.44
Electricity	kW/h	1.64	8,246.22	13,513.08
Salaries	1 person per 20 modules	3,000.00	12.00	36,000.00
Water	kl	13.35	20.79	277.52
		Sub-total		R 71,112.31
		Vat		R 9,955.72
Running cost		Total		R 81,068.03



Table 15.2: Capital and running cost of a 50 mm acrylic reactor

Product number	Description	Price per unit	Quantity	Total
GOD 50	90° Elbow	18.00	2.00	36.00
TID 50	90° Tee	26.00	46.00	1,196.00
CAD 50	End cap	15.00	36.00	540.00
uPVC 50	Pipe class 4 pm	27.00	3.00	81.00
CHD 50	Ball valve	52.00	2.00	104.00
AXT050046	Acrylic Tubing	128.96	60.00	7,737.60
PVC adhesive	1 litre	360.00	1.00	360.00
PVC cleaner	500 millilitre	77.00	2.00	154.00
Gas tubing	6 mm per metre	6.00	80.00	480.00
Gas dispersion units	Large	12.00	36.00	432.00
Pressure pump SM 100	0.75kW 20-90l/min	1,967.00	1.00	1,967.00
Air blower B02A02	0.4 kW 1.3m <sup>3</sup> /min	4,143.00	1.00	4,143.00
Gas flow meters V10420	0-24.68 l/min	2,691.00	1.00	2,691.00
Medium Storage tank	1000 litre tank	2,045.00	1.00	2,045.00
		Sub-total		R 21,966.60
		Vat		R 3,075.32
Capital cost		Total		R 25,041.92
40-RC	CO2 Tec Dry	270.78	27.34	7,404.27
Medium		0.67	13,597.63	9,103.61
Electricity	kW/h	1.64	6,605.96	10,825.18
Salaries	1 person per 20 modules	3,000.00	12.00	36,000.00
Water	kl	13.35	13.60	181.53
		Sub-total		R 63,514.59
		Vat		R 8,892.04
Running cost		Total		R 72,406.64



Table 15.3: Capital and running cost of a 90 mm PVC reactor

Product number	Description	Price per unit	Quantity	Total
GOD 90	90° Elbow	82.00	2.00	164.00
TID 90	90° Tee	110.00	46.00	5,060.00
CAD 90	End cap	66.50	36.00	2,394.00
uPVC 90	Pipe class 4 pm	52.20	3.00	156.60
CHD 90	Ball valve	243.00	2.00	486.00
PVCTC090086I	Clear PVC tubing	204.03	60.00	12,241.80
PVC adhesive	1 litre	360.00	2.00	720.00
PVC cleaner	500 millilitre	77.00	4.00	308.00
Gas tubing	6 mm per metre	6.00	80.00	480.00
Gas dispersion units	Large	12.00	36.00	432.00
Pressure pump SM 100	0.75kW 20-90l/min	1,967.00	1.00	1,967.00
Air blower B02A02	0.4 kW 1.3m <sup>3</sup> /min	4,143.00	1.00	4,143.00
Gas flow meters V10420	0-24.68 l/min	2,691.00	1.00	2,691.00
Medium Storage tank	1000 litre tank	2,045.00	1.00	2,045.00
		Sub-total		R 33,288.40
		Vat		R 4,660.38
Capital cost		Total		R 37,948.78
40-RC	CO2 Tec Dry	270.78	95.58	25,879.95
Medium		0.67	46,259.21	30,970.54
Electricity	kW/h	1.64	14,056.88	23,035.01
Salaries	1 person per 20 modules	3,000.00	12.00	36,000.00
Water	kl	13.35	46.26	617.56
		Sub-total		R 116,503.06
		Vat		R 16,310.43
Running cost		Total		R 132,813.49

Table 15.4: Capital and running cost of a 90 mm acrylic reactor

Product number	Description	Price per unit	Quantity	Total
GOD 90	90° Elbow	82.00	2.00	164.00
TID 90	90° Tee	110.00	46.00	5,060.00
CAD 90	End cap	66.50	36.00	2,394.00
uPVC 90	Pipe class 4 pm	52.20	3.00	156.60
CHD 90	Ball valve	243.00	2.00	486.00
AXT090084	Acrylic Tubing	290.24	60.00	17,414.40
PVC adhesive	1 litre	360.00	2.00	720.00
PVC cleaner	500 millilitre	77.00	4.00	308.00
Gas tubing	6 mm per metre	6.00	80.00	480.00
Gas dispersion units	Large	12.00	36.00	432.00
Pressure pump SM 100	0.75kW 20-90l/min	1,967.00	1.00	1,967.00
Air blower B02A02	0.4 kW 1.3m <sup>3</sup> /min	4,143.00	1.00	4,143.00
Gas flow meters V10420	0-24.68 l/min	2,691.00	1.00	2,691.00
Medium Storage tank	1000 litre tank	2,045.00	1.00	2,045.00
		Sub-total		R 38,461.00
		Vat		R 5,384.54
Capital cost		Total		R 43,845.54
40-RC	CO2 Tec Dry	270.78	91.18	24,690.23
Medium		0.67	44,132.64	29,546.80
Electricity	kW/h	1.64	13,571.76	22,240.04
Salaries	1 person per 20 modules	3,000.00	12.00	36,000.00
Water	kl	13.35	44.13	589.17
		Sub-total		R 113,066.24
		Vat		R 15,829.27
Running cost		Total		R 128,895.51

Table 15.5: Capital and running cost of a 110 mm PVC reactor

Product number	Description	Price per unit	Quantity	Total
GOD 110	90° Elbow	135.00	2.00	270.00
TID 110	90° Tee	170.00	46.00	7,820.00
CAD 110	End cap	112.00	36.00	4,032.00
uPVC 110	Pipe class 4 pm	73.40	3.00	220.20
CHD 110	Ball valve	400.00	2.00	800.00
PVCTC110105I	Clear PVC tubing	276.30	60.00	16,578.00
PVC adhesive	1 litre	360.00	2.00	720.00
PVC cleaner	500 millilitre	77.00	4.00	308.00
Gas tubing	6 mm per metre	6.00	80.00	480.00
Gas dispersion units	Large	12.00	36.00	432.00
Pressure pump SM 100	0.75kW 20-90l/min	1,967.00	1.00	1,967.00
Air blower B02A02	0.4 kW 1.3m <sup>3</sup> /min	4,143.00	1.00	4,143.00
Gas flow meters V10420	0-24.68 l/min	2,691.00	1.00	2,691.00
Medium Storage tank	1000 litre tank	2,045.00	1.00	2,045.00
		Sub-total		R 42,506.20
		Vat		R 5,950.87
Capital cost		Total		R 48,457.07
40-RC	CO2 Tec Dry	270.78	142.47	38,578.48
Medium		0.67	18,366.97	12,296.68
Electricity	kW/h	1.64	7,693.96	12,608.10
Salaries	1 person per 20 modules	3,000.00	12.00	36,000.00
Water	kl	13.35	18.37	245.20
		Sub-total		R 99,728.46
		Vat		R 13,961.98
Running cost		Total		R 113,690.45

Table 15.6: Capital and running cost of a 90 mm acrylic reactor

Product number	Description	Price per unit	Quantity	Total
GOD 110	90° Elbow	135.00	2.00	270.00
TID 110	90° Tee	170.00	46.00	7,820.00
CAD 110	End cap	112.00	36.00	4,032.00
uPVC 110	Pipe class 4 pm	73.40	3.00	220.20
CHD 110	Ball valve	400.00	2.00	800.00
AXT110104	Acrylic Tubing	342.81	60.00	20,568.60
PVC adhesive	1 litre	360.00	2.00	720.00
PVC cleaner	500 millilitre	77.00	4.00	308.00
Gas tubing	6 mm per metre	6.00	80.00	480.00
Gas dispersion units	Large	12.00	36.00	432.00
Pressure pump SM 100	0.75kW 20-90l/min	1,967.00	1.00	1,967.00
Air blower B02A02	0.4 kW 1.3m <sup>3</sup> /min	4,143.00	1.00	4,143.00
Gas flow meters V10420	0-24.68 l/min	2,691.00	1.00	2,691.00
Medium Storage tank	1000 litre tank	2,045.00	1.00	2,045.00
		Sub-total		R 46,496.80
		Vat		R 6,509.55
Capital cost		Total		R 53,006.35
40-RC	CO2 Tec Dry	270.78	139.77	37,847.15
Medium		0.67	24,791.92	16,598.19
Electricity	kW/h	1.64	9,159.66	15,009.93
Salaries	1 person per 20 modules	3,000.00	12.00	36,000.00
Water	kl	13.35	24.79	330.97
		Sub-total		R 105,786.24
		Vat		R 14,810.07
Running cost		Total		R 120,596.31

# Gosair

INSTRUMENTS (PTY) LTD

437 GREENHILLS INDUSTRIAL ESTATE  
SAM GREEN STREET  
TUNNEY, EXT 6  
GERMISTON  
P.O. BOX 12648  
CHLOORKOP  
1624  
TEL: (+27 11) 822-6709  
FAX: (+27 11) 822-1514

To: UNIVERSITY of STELLENBOSCH

From: Eugene van Niekerk

Tel No:

Date: 9-Jul-14

Fax No:

E-MAIL: [15476421@sun.ac.za](mailto:15476421@sun.ac.za)

Attention: RIAAN HAGENDIJK

Page: 1 of 1

Subject: QUOTE GI 16393 - WE OFFER AS FOLLOWS

ITEM	DESCRIPTION	SIZE	UNIT	QTY	PRICE	TOTAL
	<b>OPTION 1 65mm SCALE</b>					0.00
1	VA10420 VARIABLE AREA GLASS					0.00
	FLOW METER ALUMINIUM FITTINGS					0.00
	RANGE: 0 - 24680 ml/min (24,68 LPM)		EACH	1	2691.00	2691.00
	<b>OPTION 2 150mm SCALE</b>					0.00
2	VA20439 VARIABLE AREA GLASS					0.00
	FLOW METER ALUMINIUM FITTINGS					0.00
	RANGE: 0 - 23564 ml/min (23,56 LPM)		EACH	1	4533.00	4533.00
						0.00
						0.00
						0.00
SUB TOTAL						7224.00
VAT 14%						1011.36
TOTAL						8235.36

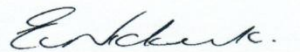
PRICES ARE NETT, NETT &amp; EXCLUDES FREIGHT COSTS TO STELLENBOSCH

DELIVERY: +- 21 WORKING DAYS FROM DATE OF FULL PAYMENT RECEIVED IN ADVANCE

QUOTE IS VALID FOR 7 WORKING DAYS

GOSAIR FILTER SYSTEMS (PTY) LTD STANDARD TRADING TERMS &amp; CONDITIONS APPLY

Regards,



Eugene van Niekerk

Figure 15.1: Quotation for variable flow meters from Gosair instruments




 <p><b>UNIVERSITEIT • STELLENBOSCH • UNIVERSITY</b> jou kennisvennoot • your knowledge partner</p>		<p><b>PURCHASE ORDER</b> *</p> <p><b>AANKOOPBESTELLING</b></p>		Bestelnommer Order number <b>231509181</b>	
		Datum Date 05/09/2014	Bladsy Page 1 van / of 1	ADRESSEER / LEWER GOEDERE AAN: ADDRESS / DELIVER GOODS TO:	
<b>AAN: TO:</b>  <b>AFROX LIMITED</b> P O Box 51 Eppindust Western Cape 7475  Krediteurskode : 10219		<b>STUUR FAKTURE EN STATE AAN:</b> FORWARD INVOICES AND STATEMENTS TO:  Bedryfshoof Universiteit van Stellenbosch Private Bag X1 MATIELAND 7602  Chief Operating Officer Stellenbosch University Private Bag X1 MATIELAND 7602  Geen betaling sal geskied tensy bestelnommer op fakture aangedui word nite. No payment will be made unless order numbers are quoted on invoices.  KONTRAK / CONTRACT : KWOTASIE / QUOTATION : DATUM / DATE : 05/09/2014 GEKWOTEER DEUR : QUOTED BY:  BTW nr. / VAT no.: 4920118959		Persoon/ Person: FS LAYMAN  LOKAAL: C101 PROSESINGENIEURSWESE PROSESINGENIEURSWESE BANGHOEKWEG STELLENBOSCH  Tel.: (021)8084062	
Aantal Quantity 2.00	Eenheid Unit CO2 TEC CYL 31,3KG: 40 - RC ACCOUNT: 1091565	Beskrywing Description	Itemkode Item Code	Eenheidsprys Unit Price 308.69	Bedrag Amount 617.38
<b>TOTAAL (BTW ingesl.):</b> TOTAL (VAT incl.):				<b>R 617.38</b>	US960

Figure 15.2: Afrox CO<sub>2</sub> (40 - RC) cylinder cost

# March 2014

## PIPE AND FITTING PRICE LIST EXCLUDING VAT



Corner La Belle Road and Winelands Close  
Stikland, Bellville  
P O Box 56, Soneike, 7583  
Tel: 021 946 1980 Fax: 021 948 9238  
email: sales@effast.co.za

S.	33°	53'45
E	18°	40'20

## ***Poly Pipe Systems***

Troika Industrial Park, Essex Street  
Meadowdale, Edenvale  
P O Box 2886, Primrose, 1416  
Tel: 011 974 6912 Fax: 011 974 6933  
email: admin@polypipesystems.co.za

S.	26°	09'09
E	28°	10'57

## Natal Plastic Supplies

67 Hannah Road, Congella, Durban.  
P O Box 17222, Congella 4013  
Tel: 031 205 9215 Fax: 031 205 2902  
email: logan@natalplastics.co.za

S.	29°	52'69
E	30°	59'58



ISO 9002 N° 202

website: [www.effast.co.za](http://www.effast.co.za)

## PE lastics

6 Barry Street, Sidwell, Port Elizabeth  
P O Box 32261, Summerstrand  
Port Elizabeth 6109  
Tel: 041 451 2360 Fax: 041 451 2377  
email: kobus@peplastics.co.za

S.	33°	55'18
E	25°	35'38

Figure 15.3: Booklet front page which acted as source for the PVC fittings prices

Table 15.7: Prices and references form where it was obtains

<b>Product number</b>	<b>Description</b>	<b>Price per unit</b>	
Pressure pump SM 100	0.75kW 20-90l/min	1,967.00	(Stewards and Lloyds, 2014)
Air blower B02A02	0.4 kW 1.3m <sup>3</sup> /min	4,143.00	(Ecotao Enterprises, 2014)
Medium storage tank	1000 litre tank	2,045.00	(Water Rhapsody, 2014)
Electricity	kW/h	1.64	(City of Cape Town, 2014)
Water	kl	13.35	(City of Cape Town, 2013)
Price of dried Chlorella	per 100 g	153.51	(Chlorella Africa, 2014)
Gas tubing	6 mm per metre	6.00	(Seal Water tech, 2014)
Gas flow meter			Figure 15.1
Afrox	CO <sub>2</sub>		Figure 15.2
Fittings	PVC fitting		Figure 15.3
Clear tubing	PVC and acrylic		APPENDIX