ALGAL FLUE GAS SEQUESTRATION AND WASTEWATER TREATMENT: AN INDUSTRIAL EXPERIMENT

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Algal Flue Gas Sequestration and Wastewater Treatment: An Industrial Experiment

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Sammanfattning

CJP Solutions och Waste Handling and Management (WHAM) är två företag i Melbourne, Australien, som utvecklar en process för återvinning av bioslam från Melbourne Waters reningsverk. Slammet är förorenat med metaller från industriellt avloppsvatten som har behandlats tillsammans med det kommunala avloppsvattnet. Företagen sökte en hållbar biologisk process inkluderande alger för att rena och fånga in rökgaser från pyrolisering och förbränning av det förorenade slammet. I det här examensarbetet har en teknisk lösning utformats, tillverkats och experimentellt prövats på plats under tjugotvå veckor i Melbourne, med mål att: mäta gas- och vattenreningsprestanda.


Omfattande mätningar av vatten- och gasvärden under experimenten visade att biomassatillväxten uppgick till $P_k = 0.43 \, \text{gL}^{-1}\text{d}^{-1}$ och $P_b = 0.26 \, \text{gL}^{-1}\text{d}^{-1}$ för kolonn- respektive bassängodlingen. Kväveupptag i algerna från avloppsvattnet uppgick till $P_{\text{Nk}} = 54.6 \, \text{mgL}^{-1}\text{d}^{-1}$ respektive $P_{\text{Nb}} = 19.9 \, \text{mgL}^{-1}\text{d}^{-1}$. Verkningsgraden för algernas upptag av rökgaser uppgick i kolonnerna till $\eta_{\text{CO}2} = 44 \%$, $\eta_{\text{NOx}} = 70 \%$ $\eta_{\text{SO}2} = 100 \%$. Efter två dagars tillväxt och biologiskt upptag hade metallhalterna i avloppsvattnet minskat med $c_{\text{Cu}} = 85 \%$, $c_{\text{Zn}} = 93 \%$, efter fyra dagar $c_{\text{Ni}} = 50 \%$.

Med vissa förbättringar av den nuvarande konstruktionen anses en bassängreaktor med en markarea på 78 Ha räcka för att fånga in 100 \% av mängden CO$_2$ som släpps ut i avgaserna från AGLs biogas kraftvärmeverk.
Abstract

CJP Solutions in collaboration with Waste Handling and Management (WHAM), two companies based in Melbourne, Australia, are currently developing a process to treat and recycle biosolids left over from the wastewater treatment process at Melbourne Water’s Western Treatment Plant. The biosolids are contaminated with heavy metals from industrial wastewater, being treated together with municipal wastewater. The companies are looking for a sustainable solution for sequestering flue gases from pyrolysis of the biosolids, into an algal biomass. In this Master Thesis project, a technical solution has been designed, constructed and tested on site over the course of twenty weeks in Melbourne, the goal being to determine gas and water cleanup performance.

After eight weeks of initial literature review covering CO₂-sequestration and industrial applications of algae cultivation, the microalgae Chlorella vulgaris was chosen as the main strain to be used, due to it being robust and having a high growth rate. In addition to the Chlorella v. culture, a mix consisting of local algae cultures together with Chlorella v. was also cultivated throughout the experiments. The experiments were carried out during three weeks at AGL’s biogas power plant, at the Western Treatment Plant. Untreated exhaust gas was led through a system of cooling, filtration, and compression, into the two separate algal culture systems. One consisted of seven 25 litre plastic column reactors, the other of a 250 litre pond reactor. The systems were mixed through air bubbling, exhaust gas inlet, as well as by a mechanical stirrer in the pond reactor. The algae were grown in partially treated wastewater. Factors determining the system design included simplicity in construction, use of cheap, available materials, as well as a three week design and construction deadline.

Extensive gas and water sampling during the experiments determined a biomass productivity rate of $P_c = 0.43\ \text{gL}^{-1}\text{d}^{-1}$ and $P_p = 0.26\ \text{gL}^{-1}\text{d}^{-1}$ for the column and pond reactors, respectively. Nitrogen uptake from the water amounted to $P_{Nc} = 54.62\ \text{mgL}^{-1}\text{d}^{-1}$ and $P_{Np} = 19.86\ \text{mgL}^{-1}\text{d}^{-1}$. The algal exhaust gas utilization efficiency amounted to $\eta_{CO_2} = 44\ %$, $\eta_{NOx} = 70\ %$, $\eta_{SO2} = 100\ %$. After two days of growth and bioremediation, the levels of heavy metal in the wastewater had decreased by $c_{Cu} = 85\ %$, $c_{Zn} = 93\ %$, and after four days $c_{Ni} = 50\ %$.

It is believed that if certain improvements were to be made to the experimental design in this project, a pond system occupying approximately 78 hectares would be sufficient to sequester 100% of the CO₂ released from AGL Biogas Power Plant.
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1 INTRODUCTION

1.1 Background

CJP Solutions in collaboration with Waste Handling and Management (WHAM), two companies based in south eastern Melbourne, are currently developing a process to treat and recycle biosolids left over from the water treatment process at Melbourne Water’s Western Treatment Plant. The biosolids, emanating from digested municipal and industrial wastewater sludge, are described as roughly 50% sand and 50% organic matter, some of which is contaminated with heavy metals.

The process being developed for the treatment of the biosolids is based on pyrolysis converting the biosolids into syngas, suitable for combustion in AGL and Melbourne Water’s existing infrastructure, whilst also extracting the heavy metal contaminants. Part of this treatment process will involve feeding the combustion flue gases into an algae cultivation step to clean the flue gases, and produce biomass suitable for further energy production. In general the proposed process looks as follows, see Figure 1.

For the development of the process mentioned above, CJP Solutions, WHAM and Melbourne Water are interested in finding a sustainable technical solution for the sequestration of flue gases into an algal biomass, which is investigated in this report.
1.2 Aims
The purpose of this project is to design and test a combined flue gas sequestration and waste water treatment process using photosynthetic micro- or macroalgae. The algae shall be grown in partially treated mixed industrial and municipal wastewater to capture primarily CO$_2$, but also other flue gas constituents such as NO$_x$ and SO$_x$, from a biogas combustion process, whilst also aiming to improve the waste water quality. The flue gas stream is exhaust from one of AGL’s gas engines currently running on biogas produced in Melbourne Water’s anaerobic digester. The highly eutrophicated wastewater is sourced from the wastewater treatment process following the anaerobic digester.

The primary goals are to ascertain the growth rate of the selected algae and the level of gas sequestration, whilst also monitoring nutrient and heavy metal paths and balances. This is to be done by means of an industrial experiment.

The project also aims to give a basic idea as to the land area requirements for the realization of such a process on a larger scale.

1.3 Delimitations
The main area of focus for this project is the design of the system from the stage where engine exhaust gases are captured, up until the stage where the biomass is grown. Harvesting and further processing of the biomass will be briefly discussed, as this relates to certain decisions to be made in the design process, but other than that, these topics are left outside the scope of this project.

External expertise will be used for consultation within the fields of microbiology and process chemistry. All laboratory work will be carried out by Australian Laboratory Services (ALS) in Scoresby, Melbourne. Basic economic factors will be taken into account in parts of the design process, but other than that no economic assessments will be performed. Simplifications and assumptions will have to be made in the scale-up estimates, due to the short time frame.

1.4 Method
The method of work within this project will follow the outlines set by the Master of Science Thesis project guidelines set by KTH. More specifically this includes composing a Planning Report, an eight week Frame of Reference or literature study within the subject, followed by a peer reviewed thesis report and presentation.

The basic order of work will start with the Frame of Reference, followed by the design and construction of the experimental equipment. Thereafter an experiment will be performed to determine the growth rate of the selected algae and to measure the algae’s uptake of substances from the given growth medium. The experiment will be performed onsite at the AGL Biogas Power Plant located in Melbourne Water’s Western Treatment Plant, Werribee.

The results from the experiment will be used to answer the questions posed in the aims. The method is covered in detail under section 3 Method and Materials.
2.1 General background: World energy and CO$_2$ problems

The Executive Director of the International Energy Agency (IEA) stated that “if the world continues on the basis of today’s energy policies, the climate change impacts will be severe. Energy, which accounts for two thirds of today’s greenhouse-gas emissions, is at the heart of the problem – and so must form the core of the solution” (International Energy Agency, 2009). According to the IEA the world’s primary energy need is projected to grow by 55% between 2005 and 2030, and during this period fossil fuels will continue to be the main source of primary energy (World Energy Outlook, IEA 2007). At current rates of consumption, coal reserves are predicted to last over 200 years (Khan et al., 2009) and will most likely account for half of the world’s baseline electricity generation by 2015. Regarding oil consumption, the majority of predictions state that peak oil production will be reached around the year 2010 or up until the beginning of the 2020’s (Almeida & Silva, 2009). The carbon dioxide released from burning these fossil fuels results in global warming. The use of fossil fuels is not considered sustainable, and is questionable from economic, ecological and environmental aspects (Naik et al., 2010), yet civilization as we know it depends on their use.

Electricity only accounts for roughly one third of the current global energy demand. The major renewable energy technologies such as photovoltaic, solar thermal, geothermal, wind and wave power are all designed to produce electricity. As such, these renewable technologies do not address the problems of sustainability that accompany liquid fossil fuels, which make up the reaming two thirds of the global energy market. Biofuels are considered to be the only economically viable and sustainable option to secure the future fuel supply market. There seems to be a consensus in the scientific community that the most promising biofuel source of the future is microalgae, due to a number of advantages over conventional crops (Brennan & Owende, 2010) (Mata et al., 2010) (Khan et al., 2009) (Stephens et al., 2010). The reason these fuels have not replaced fossil fuels is due to the cost of their production.

There are a number of ways to reduce the cost of production of algal fuels. A closed-loop systems approach with innovative uses of resources can lead to both more sustainable and cheaper production. Nutrient rich waste water can be used when growing the algae. Using a biorefinery approach in cultivating the algae makes use of as much of the biomass as possible. Capturing and utilizing carbon dioxide from flue gases, rather than emitting them into the atmosphere, also provides multiple advantages. The latter is in itself an important area of technology as it can help mitigate carbon dioxide emitted from the use of fossil fuels or any other industrial carbon dioxide source (Campbell et al., 2009) (Stewart & Hessami, 2005).

Besides negatively impacting ecosystems on a global level, greenhouse gases also carry economic costs due to national and international management control measures such as carbon emissions taxes and carbon dioxide emission quotas such as the Kyoto Protocol (United Nations, 1998).

A technology that can turn carbon dioxide-rich flue gases into a useful product rather than simply waste exhaust could serve as a means of avoiding such emission restrictions and costs. This possibility could also encourage the development of even more efficient production systems of biofuels.
### 2.2 Why algae for CO₂ sequestration?

A transition into sustainable and CO₂ neutral systems requires new technology both to put an end to our dependency on fossil fuels, and to manage and mitigate current CO₂ emissions. Although cleaner production opportunities should always be a first hand choice, energy supply will continue to be heavily fossil loaded, and there are at present technologies in use, and in development, that can mitigate and remediate CO₂ emissions. In order to avoid further irreparable environmental damage, sequestration of carbon from the atmosphere and from industries is essential.

Many options that have been proposed and that are in use for capturing CO₂ can be seen as economically, socially and environmentally short-sighted. A common approach is taking measures to offset any immediate effects, often by simple relocation of the emissions. Injection of flue gases into oceanic or geological sinks are examples of such “end-of-pipe” solutions (Stewart & Hessami, 2005) (Packer, 2009).

In conjunction with fossil fuel combustion processes, scrubbing techniques can be used to separate CO₂ from the flue gases. The solvent monoethanolamine (MEA) can be used in scrubbers to absorb the CO₂. The MEA-CO₂ solution is then reheated in a stripper, which releases the CO₂ in an almost pure form. The CO₂ can then be used for other industrial processes, while the MEA-solvent can be recycled and reused in the scrubber. This technique and scrubbing technologies in general, require large investments and space for equipment. The process also requires a lot of energy, negatively affecting both the net energy balance of the system, as well as the operating costs (Stewart & Hessami, 2005).

Carbon dioxide can also be separated mechanically through a molecular sieve. Demonstrations of such filters have been performed with separation of CO₂ from methane, from air, and from a mixture of gases. The method works by separating molecules based on their weight and size, and the molecules filtered out can be desorbed and released from the sieve quite effectively through applying a low voltage across the sieve. The method is cost effective and produces minimal waste, according to Stewart and Hessami (2005).

In another physical adsorption technique reviewed in the same study, the adsorbent zeolite was used. The material proved effective at removing CO₂ at normal pressure using a technique referred to as temperature swing. However, when using this technique for power plant flue gas filtration, the adsorbent was impaired by the SO₃ contents of the gas.

With these techniques it is possible to separate and purify the carbon dioxide into a highly concentrated form, consisting of around 90% pure CO₂. The next step in the CO₂ management is disposing of the gas in an environmentally and economically sound way. Techniques currently in use include direct injection of gas into the oceans, and injection of the gas into geologic reservoirs such as coal seams and oil reservoirs. These solutions are however only temporary, as they only delay the release of CO₂ into the atmosphere. They also carry a risk of leakage, negative environmental impact on the oceans, as well as high investment costs that give little or no economical return (Stewart & Hessami, 2005). The sustainability of these methods is questionable also in the light of more dynamic options that could possibly return the CO₂ into the natural carbon cycle. The concept of fixating the carbon into biomass through photosynthesis presents a way of sequestering CO₂ in a sustainable manner.

Factors determining the most efficient way of biologically fixating carbon dioxide include plant growth rates and photosynthetic efficiency, resource requirements such as land, nutrients, and water, resistance to environmental stress, as well as the possibility of acquiring useful end products from cultivation. The CO₂ uptake of any plant is proportional to the plant’s growth rate,
which in turn is proportional to several factors, of which the photosynthetic efficiency is the most important.

As will be discussed further in the following chapter, algae have proved to have a photosynthetic efficiency higher than all other plant types. Algae also generally have a simple metabolism and reproduction system, enabling them to grow under harsh and varied conditions. The high photosynthetic efficiency equates to a lower land area demand for cultivation when comparing with terrestrial crops. Also, algae do not need arable land to be cultivated, nor do they require fresh water. There is an ongoing discussion about the economic viability of the various types of biofuels produced through biofixation of CO₂ (Packer, 2009).

Turning CO₂ emissions into a fuel via photosynthesis would in this manner be a way of recycling the carbon, and thereby reducing the demand for virgin resources. The impact on the carbon cycle and global environment would hence be reduced. However, true sequestration of CO₂ would require not only recycling, but reducing the amounts of globally available CO₂. This would require sequestration into materials that do not release the CO₂ back into the atmosphere. Packer (2009) and Bird (2010) report that while this is technically feasible through materials such as biochar and biocrude, which can be produced through pyrolysis (Stephens et al., 2010), it is extremely unlikely in the present global economic market. Biochar could be used as a fuel in its own right, and can also be used as a soil ameliorant, improving the nutrient- and water holding capacities of many soils (Bird et al., 2010).

Algae cultivation can yield a broad range of useful end products, apart from biofuels. The sequestration of CO₂ into algal biomass can become profitable also through the production of high value products such as pigments and high-grade lipids, which are extractable from several species of algae (Stephens et al., 2010). Brennan and Owende (2009) also mention high value products such as animal feed supplements being extractable from the microalgae species *Chlorella, Scenedesmus* and *Spirulina*.

When comparisons of profit are made in the literature between fossil fuels and biofuels including algal biodiesel, several factors that negatively affect the pricing of the fossil products are often disregarded. Embodied costs such as those for initial exploration, price of pipelines, and transport, as well as the economic value of environmental impacts are rarely – if ever – mentioned, according to Campbell et al. (2010).

Australia has a climate that is well suited for growing algae, with high levels of sunlight, favourable temperatures and large areas that can be used for algae cultivation without negative impacts on biodiversity (Campbell et al., 2010). This combined with the fact that there are plentiful sources of CO₂ emissions, based on the amount of energy produced through coal and natural gas, means that water access and nutrition are the remaining factors required for large scale algae production for carbon sequestration, as discussed in the following chapters. Using wastewater as a growth medium provides multiple advantages (see section 2.5 Sustainability aspects of algae cultivation), such as the removal of nitrates, phosphates and even some heavy metals from the wastewater whilst providing nutrients for the algae (Mata et al., 2010) (de-Bashan & Bashan, 2010). For this reason a water treatment facility such as Melbourne Water’s Western Treatment Plant is a suitable place to implement this form of sustainable new technology.


2.3 Melbourne Water

Melbourne Water is a state owned authority that wholesales the fresh water supply and sewage water treatment for the three water retailers that supply households and industries in Melbourne. The services include managing fresh water reservoirs and catchments, distribution systems and treatment facilities. The company's activities are regulated through several policies concerning the water quality, public health, as well as environmental issues and sustainability aspects of the water management. These policies are formulated together with the company’s key stakeholders, which include “customers, government, regulators, other water authorities, land developers, the community and suppliers” (Melbourne Water, 2009).

The Western Treatment Plant (WTP) occupies roughly 11,000 hectares in Werribee and treats 52% of Melbourne’s sewage which is roughly 475 million litres per day. Approximately 49% of this sewage is derived from homes, 14% from commercial sources, 15% from industrial trade waste and infiltration and other inflows account for the remaining 22%. Upon arrival at the plant the sewage is fed through a number of ponds which together make up an augmented lagoon system where micro-organisms are used to consume and settle the organic matter in the sewage. The first ponds in the treatment process have large rubber covers which capture the gases, predominantly methane, produced during anaerobic digestion. This biogas is pumped a short distance to a power station run by AGL, where they are subsequently scrubbed and combusted in large reciprocating engines for heat and electricity generation.

The AGL power station can produce up to 10 MW of electricity. The composition of the flue gases from this combustion process can be found in Stack Emission Sampling & Analysis (MGT Environmental, 2010). The ponds following the anaerobic digesters use aerators, powered by the biogas produced in the anaerobic digesters, to provide stirring and oxygen for the activated sludge process. It takes up to 35 days for the sewage to pass through the different ponds in the lagoon system. Thereafter, part of the treated effluent is recycled for agricultural use and the remaining 340 million litres per day is discharged to Port Phillip Bay under an EPA license (Melbourne Water, 2006b) (Melbourne Water, 2006).

A by-product from this treatment process is a digested sewage sludge which, when dried to roughly 50% water is denoted biosolids. The composition of these biosolids varies depending upon the source of the sewage, which has varied over the last 100 years, but generally contains organic matter, nitrogen, phosphorous, potassium, calcium, magnesium, sulphur, and in some cases also heavy metals, organic pollutants and pathogens. The preferred option for the management of biosolids is agricultural use in the form of land spreading as they can be a good supplement to fertilizer (Williams, 2000). However, this is not always an option when the sewage is sourced from a largely industrial area as heavy metal levels are often too high, as is the case with some of the biosolids at the WTP.

Approximately 45,000 tonnes of biosolids are produced annually at the WTP and there is a stockpile of 1.2 million dry tonnes stored in different locations onsite. For the stored biosolids and the biosolids that will be produced in the future, Melbourne Water has identified energy recovery as the preferred beneficial use option due to the biosolids calorific content. For this reason, Melbourne Water is interested in the development of decontamination and beneficial use technologies including on-site treatment, mine site remediation and other non-agricultural land applications (Melbourne Water, 2006). The process mentioned in section 1.1 of this report is one such research and development project.
2.4 Algae basics

Most sources of energy on Earth can be said to originate from energy transmitted from the sun. Oil and petroleum fields around the world were originally large amounts of organic matter, such as algae, that under specific conditions were transformed into fossil deposits through slow chemical reactions. The amount of naturally occurring absorption of sunlight into biological systems today makes out 0.1-0.5% of the total amount of globally absorbed sunlight. This small percentage of biological absorption takes place through a conversion of radiated energy into biomass, as organisms use the solar energy to bind atmospheric carbon dioxide into organic matter (Packer, 2009). The process is one that has evolved over the ages, adapting with organisms to widely ranging living conditions.

The main points of absorption of carbon in the global carbon cycle are the oceans. As active carbon sinks, the oceans continuously absorb one fourth of the anthropogenic carbon dioxide, although this number is decreasing as the oceans become saturated (Columbia University, 2009). The most common underlying chemical reaction for carbon uptake amongst algae is photosynthesis. In most species of algae photosynthesis is oxygenic, meaning that the process creates oxygen and uses water as an electron donor. The oxygenic photosynthetic reaction is the following:

\[
2n\ CO_2 + 2n\ H_2O + \text{photons} \rightarrow 2(CH_2O)n + 2n\ O_2
\]  

Algae belong to the bottom of the food chain, and apart from serving as a primary food source in many ecosystems they are also the principle producers of oxygen on earth (Khan et al., 2009). Algae are sorted into two distinct groups: microalgae and macroalgae, the latter being larger and more complex organisms, often generally referred to as seaweed. Microalgae are microscopic organisms that can exist unicellularly or together in cellular conglomerations. Algae can grow and reproduce in the following ways:

- **Photoautotrophically:** Conversion of solar energy into chemical energy and organic matter through the photosynthetic reaction.
- **Heterotrophically:** The algae use organic compounds as energy sources, and do not require sunlight to grow.
- **Mixotrophically:** The algae use both organic compounds and also synthesises compounds through photosynthesis.
- **Photoheterotrophically:** not easily distinguished from mixotrophic growth, though it can be said that the solar energy is used to convert organic compounds into a carbon source for the algal growth (Mata et al., 2010).

The metabolism of algae is thus driven by either light, organic compounds, or a combination of the two. The metabolic process determines the composition of the organisms. Heterotrophically grown microalgae usually accumulate more lipids than algae that grow photoautotrophically (Huang et al., 2010), but the organic compounds used for heterotrophic growth are produced ultimately from some other photosynthetic process. This means that the growth of heterotrophic algae is less light-efficient than that of photoautotrophic algae (Chisti, 2007). Heterotrophic and mixotrophic algae thrive in waters containing organic particles, such as liquid waste loaded waters. In clean waters, autotrophic algal dominate (Grobbelaar, 1990).

Microalgae generally have a high photosynthetic efficiency compared to most other plants. They often reach light-to-biomass conversion efficiencies of 1-4%, as opposed to ~1% conversion rates which is normal in other plants and food crops, with sugarcane being an exception at 8%.
(Stephens et al., 2010). Aresta et al., (2005) state that aquatic biomass on average have an efficiency of 6-12% compared to an average in terrestrial photosynthetic efficiencies of around 2%. These numbers all describe the naturally occurring efficiencies, and could be improved through various means. The theoretical maximum level of photosynthetic efficiency in oxygenic photosynthesis is appreciated to be 8-12% (Stephens et al., 2010) (Bolton & Hall, 1991). The photosynthetic efficiency is one of the factors that determine how good an agent the organism is in fixating carbon dioxide into biomass, since, as the chemical reaction in equation (1) shows, an increased organic growth implies an increased uptake of carbon dioxide via photon absorption.

For large scale production of algae, the photoautotrophic species have the advantage of requiring less input in forms of nutrients and management, when compared to heterotrophic algae. They are in this manner more easily grown.

Microalgae are known to have a fast reproduction rate and commonly double their biomass within 24 hours. Under suitable conditions microalgae grow exponentially, reducing the doubling time, often achieving a doubled population within 3.5 hours (Chisti, 2007). Achieving optimal growth for any given species of algae requires several factors to be in order. These factors will be discussed further on. The growth of an algal culture generally goes through five quite well defined stages.

![Graph](image)

**Figure 2.** Algal growth rate in batch culture (solid line) and nutrients depletion (dotted line) *(Mata et al., 2010)*

The first phase (1) is the lag phase, in which algal growth is slow. This is followed by the exponential growth phase (2), which evens out into a linear growth phase (3), followed by a stationary phase (4), followed by decline or death of the algal culture (5). Mata et al. (2010) showed that the nutrients concentration of the growth medium followed an inverse curve, gradually depleting as the algae population increased, as shown in Figure 2. So, the growth rate also depends on the concentrations and composition of the available nutrients. Of these, phosphorous and nitrogen are deemed the most essential substances for algal growth (Stephens et al., 2010). Grobbelaar (2004) formulated an approximation of the general molecular formula of a microalgal cell: \( \text{CO}_{0.48}\text{H}_{1.83}\text{N}_{0.11}\text{P}_{0.01} \). This formula also serves as an estimate for the minimal nutrition requirements for the growth of microalgal biomass.
Also the availability of CO₂ is a determining factor for the growth rate of autotrophic algae. Roughly 50% of a microalgal biomass is made up of carbon, by dry weight. This carbon is derived from CO₂, in accordance with the equation for the photosynthesis. If there is no CO₂ available, there can be no photosynthesis. An abundance of CO₂ can stimulate increased algal growth, as long as the levels in the growth medium do not cause a harmful pH (Stephens et al., 2010) (Chisti, 2007).

The quality and quantity of light reaching the algae also affects the growth rate. Light CO₂ are synthesised into biomass, and hence both resources need to be supplied simultaneously in order to achieve growth. Under natural circumstances (without artificial lighting) the photosynthesis ceases over night. The algae continue respiring, consuming carbon dioxide, but new biomass cannot be created. During the dark hours, biomass can be lost due to respiration. These respiratory losses increase with an increased temperature, and studies show that as much as 25% of the biomass generated during daylight hours can be lost over night (Chisti, 2007).

There is an upper limit also to how much light an algae culture can benefit from. Above a certain point in light intensity, light will inhibit the cells from synthesising. This phenomenon is known as photoinhibition, and occurs at a level of intensity only slightly above the specific optimal level of light intensity for any given algae specie. Photoinhibition results from damage to the photosynthetic apparatus of the algal cell. A unit of measurement called the light saturation constant is used to characterize algae’s light characteristics. The light saturation constant is “the intensity of light at which the specific biomass growth rate is half its maximum value” (Chisti, 2007).

2.5 Sustainability aspects of algae cultivation

Manipulating the algae’s natural living conditions can lead to increased growth rates. This is done at algal production facilities, by managing nutrition levels, light irradiance, CO₂ supply, and other factors. Any measure taken to increase the growth rate must however take into consideration the overall sustainability of the production process. It has been shown for instance that continuously supplying an algal culture with nutrients will affect the overall energy balance of the production system negatively.

Some authors claim that such a method of continuous nutrient supply is likely to consume more energy than the algal growth could be said to produce (Aresta et al., 2005). There is a similar trade-off in efficiency when supplying the algae with artificial lighting, since this will require an energy input. It will also require an economical investment, as well as increased operating costs. This too influences the overall sustainability of the system negatively. An example of such a capital intensive production system can be found in the Japanese NEDO-RITE algae development program (during 1990-2000). Algae were cultivated in optical fibre photobioreactors, with investment costs of around 1000 USD/m², and the system was, according to John R. Benemann, a total failure (Benemann, 2008).

Several authors have concluded that resource management needs to be a high priority in order for any algae production facility to be sustainable and efficient, in means of both energy and economy. Common solutions to reach a sustainable production system include using waste products from other industries as resources for the algal growth. Aresta et al. (2005) showed that huge energy inputs could be avoided by using nutrient rich wastewater from either aquaculture plants or selected municipal waters, rather than using a prefabricated nutrient solution. Packer (2009) also mentions the integration and coupling of industrial processes, such as wastewater but also waste heat, as possibilities for a sustainable process design. Heat can be required for warming the growth medium in early daylight hours, as well as for dewatering the algal solution.
in later stages of refinement. Stephens et al. (2010) point out that an active or passive warming of algae ponds or reactors will reduce the net energy balance of the system, unless waste heat can be used.

Recycling nutrients might not be economical at low productivity levels, according to Stephens, but as production levels increase, the growth media will make up a larger ongoing cost and the incentive to recycle nutrients will grow. A global decrease in phosphorus and increased fertilizer prices are also predicted. This too will serve as an incentive to reduce the resource spills. Biosludge from fermentative methane production can also be used as a nutrient for algae. The residue is rich in substances suitable for increased algae growth and can be recycled through the algae reactors or ponds (Stephens et al., 2010).

The benefits of recirculating waste water through an algae cultivation system are twofold. Firstly the nutrients are supplied in a sustainable way. Secondly, the waste water will be partially cleaned in the process, as the nutrients are absorbed by the algae. This will reduce any eutrophication that the waste water would otherwise have caused if it was released as effluent into a natural ecosystem. Studies of macroalgae in aquaculture residual waters have shown that large amounts of nutrients are absorbed and stored in the plants. Heavy metals such as mercury have also been seen to change forms through bioconversion in algae, representing a possible route to remediation of toxic waters.

Similarly, CO₂ can be used in algae cultivation to stimulate growth, as several studies have shown. Adding CO₂ either in a pure form, compressed or uncompressed, or as part of a flue gas, to algae cultivation, generally leads to increased photosynthetic activity. Studies also show that the NOₓ contents of flue gases generally do not present any problem for the algae cultures. This has been tested with actual flue gases as well as with gas mixtures simulating flue gases (Packer, 2009).

The methods of supplying the algal ponds or reactors with CO₂ vary in efficiency, and in capital and operating costs. When using pipelines the length of the pipelines, the pipe diameters, and the flue gas compression rate affect the efficiency of the gas transport from the CO₂ source to the cultivation location.

In their review of Kadam (1995), Benemann and Oswald (1996) showed that there in cases of long distances is an economic advantage of scrubbing flue gases to produce concentrated CO₂, and then compressing the gas prior to piping it to the algal cultivation location. Benemann asserts that purifying the flue gases is to be preferred when the pipeline distance is significant. For short distances however, a low rate of flue gas compression and large pipe diameters would be most efficient. Using unpurified flue gases is advantageous to purified CO₂ only when the power plant is close to the cultivation location.

2.6 Culture systems

The algal biomass growth in any given culture depends on three variables: the gross productivity *PRD*, respiration losses *RES*, and photoinhibition *INB*. The total biomass productivity *PROD* in the culture can then be expressed as:

\[
PROD = PRD - RES - INB
\]
Out of these variables, photo-inhibition is least understood and hence hardest to model and manage (Grobbelaar, 1990). The variables can be manipulated and managed through various techniques in different culture systems, which will be presented in this chapter.

2.6.1 General

The purpose of the culture system is to provide an environment for the algae culture to grow in. There are a number of different ways to do this. In general, culture systems can be divided into open and closed systems depending on whether the culture grown is open to the surrounding environment, such as in lakes or ponds, or enclosed in a photobioreactor. When comparing and deciding on what culture system to use, factors such as the biology of the algae, access to water, nutrients, energy, land, labour and the climate need to be considered (Borowitzka, 1999).

A major question in deciding which culture system to use is also what the purpose of the production facility is. The economic viability of a system depends on what is aimed at being achieved. Carlsson (2007) for example argues that closed bioreactors are not well suited for wastewater treatment “because the costs for treating wastewater in this system will be too high in relation to the low value added during the production process” (Carlsson et al., 2007). And vice versa; open pond systems are likely to be less suited for producing high value products that require careful management, and that are sold in small amounts. In his review, Carlsson asserts that the cultivation of algae solely for CO$_2$ mitigation or for the sole purpose of producing biofuels is not cost-competitive by 1-2 orders of magnitude.

Factors more specifically related to the growth and well-being of the algae can be classed into abiotic and biotic factors. Abiotic factors include light quality and quantity (considered the most limiting factor in both open and closed systems), temperature (considered the second most limiting factor), nutrients, CO$_2$, dissolved oxygen levels, pH, salinity and the presence of toxic chemicals. Biotic factors include pathogens (such as bacteria, fungi, viruses), competition from other algae and operational factors which include shear stress produced by mixing, dilution rate and harvest frequency (Grobbelaar, 1990), (Mata et al., 2010), (Marinho-Soriano et al., 2008).

Attaining high biomass yields in algal production facilities is imperative. For this reason, nutrients and CO$_2$ are often supplied in excess, making temperature and light energy the only non-fixed variables affecting biomass growth (Grobbelaar, 1990). Chisti (2007) mentions that CO$_2$ feeding systems regulated by pH sensors minimize CO$_2$ losses and also help attain a stable pH level.

Taking these factors into account, it follows that when comparing large scale culture systems, their basic properties such as light utilisation efficiency, ability to control temperature, ability to withstand pathogens and the level of hydrodynamic stress placed upon the algae, should be used (Borowitzka, 1999). Another important factor to take into account is how easy a culture system is to scale up from laboratory/pilot scale to large scale, as the difficulties of growing selected species in substantial volumes is a major handicap in algae cultivation. A general comparison of open versus closed culture systems is presented by Mata et al. (2010) in Table 1.
Table 1. Comparison of open versus closed culture system adapted from Mata et al. (2010).

<table>
<thead>
<tr>
<th>Culture system for microalgae</th>
<th>Closed systems (PBRs)</th>
<th>Open systems (Ponds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contamination risk</td>
<td>Reduced</td>
<td>High</td>
</tr>
<tr>
<td>Process control</td>
<td>Easy</td>
<td>Difficult</td>
</tr>
<tr>
<td>Species control</td>
<td>Easy</td>
<td>Difficult</td>
</tr>
<tr>
<td>Mixing</td>
<td>Uniform</td>
<td>Very poor</td>
</tr>
<tr>
<td>Operating regime</td>
<td>Batch or semi-cont</td>
<td>Batch or semi-cont</td>
</tr>
<tr>
<td>Space required</td>
<td>Matter of productivity</td>
<td>PBRs ~ Ponds</td>
</tr>
<tr>
<td>Area/volume ration</td>
<td>High (20-200 m⁻¹)</td>
<td>Low (5 – 10 m⁻¹)</td>
</tr>
<tr>
<td>Population (algal cell) density</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Investment</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Capital/Operation costs</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Light utilization efficiency</td>
<td>High</td>
<td>Poor</td>
</tr>
<tr>
<td>Temperature control</td>
<td>Ponds 3-10 times lower</td>
<td>PBRs &gt; Ponds</td>
</tr>
<tr>
<td>Productivity</td>
<td>3-5 times more</td>
<td>Low</td>
</tr>
<tr>
<td>Water losses</td>
<td>Depends on cooling design</td>
<td>PBRs ~ Ponds</td>
</tr>
<tr>
<td>Hydrodynamic stress on algae</td>
<td>Low-high</td>
<td>Very low</td>
</tr>
<tr>
<td>Evaporation growth medium</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Gas transfer control</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>CO₂ Losses</td>
<td>Depends on pH, alk, etc</td>
<td>PBRs ~ Ponds</td>
</tr>
<tr>
<td>O₂ inhibition</td>
<td>Greater problem in PBRs</td>
<td>PBRs &gt; Ponds</td>
</tr>
<tr>
<td>Biomass concentration</td>
<td>3-5 times in PBRs</td>
<td>PBRs &gt; Ponds</td>
</tr>
<tr>
<td>Scale-up</td>
<td>Difficult</td>
<td>Difficult</td>
</tr>
</tbody>
</table>

Parameters that can be used when comparing culture systems include (Mata et al., 2010):

- Volumetric productivity $P_v$ [gL⁻¹d⁻¹] which is the productivity per unit reactor volume.
- Areal productivity $P_a$ [gm⁻² d] which is the productivity per unit ground area occupied by the culture system.
- Illuminated surface productivity $P_i$ [gm⁻²d⁻¹] the productivity per unit illuminated surface area of the culture system.
- Culture density $X$ [gL⁻¹]

Packer (2009) arrives at the conclusion that the most useful unit for comparison of productivities of different culture systems would be a “biomass per unit of light energy used or falling over a particular area”.

Culture systems can be operated in batch, continuous or semi-continuous mode. General conclusions drawn by Mata et al. (2010) when comparing continuous growth systems with batch systems are that continuous bioreactors provide a higher degree of control than do batch systems. The biomass concentration in a continuous system can be regulated by varying the dilution rate, which helps maintain an environment promoting exponential and linear growth of the algae culture. Furthermore, the steady-state enabled by a continuous bioreactor also leads to more reliable and easily reproducible results. A trait common to all culture systems is the need of periodical cleaning and disinfection, if monoculture is to be maintained. A possible exception being the cultivation of selective algae strains in unmixed ponds.
2.6.2 OPEN SYSTEMS

The main area of concern and uncertainty in open pond systems is the growth rate of the algae. Whereas closed systems give the benefits of high control over both biotic and abiotic factors, open-air systems run with elevated risks of contamination, and generally fewer possibilities of control and growth management. Common traits for algae that are suitable for growth in open-air systems are therefore high specific environmental tolerances. Commercially operated open-air systems often use the algae strains *Chlorella, Spirulina, or Dunaliella*, which all grow well in extreme climates (Borowitcka, 1999) (Benemann & Oswald, 1996). *Dunaliella* tolerates high levels of salinity as well as iron deprivation, both of which are two major stresses for any photosynthetic organism (Pick, 2006). Growing *Dunaliella* in an appropriate water catchment therefore means that the algae should remain relatively free from contamination of other algae and protozoa.

Similarly, certain *Spirulina* species have a much higher than average tolerance for high levels of bicarbonates, and can therefore be exclusively grown in highly alkaline waters. Non-extremophiles, i.e. algae species that do not have this selective environmental advantage must be grown in closed systems, according to Borowitcka (1999). Khan (2009) reports that open ponds in reality often are inhabited by two to six different algae species.

The *Chlorella* species is highly adaptable to nutrient-rich media, having a very high level of chlorophyll content. It has also been reported to tolerate and even grow well in very CO₂ rich media. These factors together make *Chlorella* a strong candidate for cultivation in outdoor ponds (Brennan & Owende, 2010) (Chinnasamy et al., 2009). Borowitcka (1999) states that *Chlorella* and *Spirulina* should be grown in batch or semi-batch mode with periodic reseeding with new inoculums to prevent contamination of other algae and protozoa (Borowitcka, 1999).

Being open to the environment also entails evaporative losses from the open systems. Khan (2009) reports that water losses through evaporation from open algae cultivation systems amount to quantities comparable to those that would be used to grow land crops.

The algal growth rates, per area or volume of water, in open pond systems are generally lower than those of closed systems. All large commercial algae production systems are however open systems, making up for the lower areal productivity with larger operational areas (Borowitcka, 1999). A typical cell density for an open system is 1 g per litre, according to Stephens et al. (2010).

2.6.3 Unmixed ponds

Unmixed ponds without any artificial energy input require virtually no maintenance or management in order to produce micro- or macro algae (Packer, 2009). The only type of mixing occurring in an unmixed pond is that caused by wind and hydraulic dilution. An example of this type of cultivation is presented in Figure 3.

Without artificial mixing, most processes within a water catchment slow down. The distribution of heat, gas, and metabolites will be less even. Microalgae will not be homogenized and the light uptake will be less even. These factors all lead to a slower biomass growth. Consequently, unmixed ponds have a very low productivity compared with raceway designs, and are for this reason not suitable for CO₂ stimulation, according to Packer (2009).

Other authors have experimented with the irradiance, temperature, and densities of unmixed ponds in attempts to identify the most important variable affecting algal growth. Grobbelaar
(1990) noticed that culture densities above the optimum would result in decreasing biomass yields, under the same conditions of temperature and irradiance. The conclusion was that variations in areal density were the most important factors responsible for productivity differences.

![Figure 3. Algal Farms, Hutt Lagoon WA, Google Earth image (April 18, 2006)](image)

### 2.6.4 Raceway ponds

A lot of research has been carried out on the operation and engineering of raceway design algal culture systems. One key conclusion drawn from early studies was that the raceway designs used CO\(_2\) less efficiently than photobioreactors. Also, raceway ponds have low biomass productivity, according to Chisti (2007), which follows from a low CO\(_2\) system efficiency. Raceways are however low cost, requiring small investment and operational costs. Estimates have shown that even adding plastic covering to the ponds more than doubles the total systems capital and operational costs (Benemann & Oswald, 1996). Raceways can be constructed out of concrete or compacted earth, lined with white plastic or a thin layer of clay. Common depths of raceway algae ponds for algae cultivation are reported to be from 15-20 cm (Khan et al., 2009), to 20-30 cm (Borowitzka, 1999). The choice of depth is a compromise between the need to supply the algae culture with sufficient light, and the need to keep an adequate depth for mixing. The amount of evaporated water will also decrease in relation to the volume with a deeper pond (Borowitzka, 1999).

Supplying CO\(_2\) to shallow open suspensions can also lead to large CO\(_2\) losses to the atmosphere, since CO\(_2\) bubbles need a sufficient residence time in order to be absorbed into the water or algae. An 85\% CO\(_2\) transfer efficiency was reported at a depth of only 20-25 cm, when the carbon dioxide was sparged with a fine bubble diffuser, activated by a pH regulated solenoid (Benemann, 2008). The level of absorption is pH dependent, and controlling CO\(_2\) losses was reported as most difficult at a near neutral pH (Mata et al., 2010).
In an extensive algal systems design study conducted by Benemann & Oswald (1996), paddle wheels were considered the best option for mixing the algae, nutrients, and CO₂ in open ponds.

In one proposed system design, the paddle wheel was placed in a 1.5 meter deep sump in the pond, into which the carbon dioxide was sparged. This depth enabled a take-up of an estimated 95% of the gas. Nutrients, when required, were also fed into the pond at this point. One paddle wheel per raceway was used, keeping a flow rate of 0.10-0.25 m/s, which sufficed to ensure a suitable mixing of nutrients and CO₂ (Campbell et al., 2010).

In another setup pure CO₂ was sparged into the cultures at a constant rate of 2 Lm⁻³h⁻¹ from 1 hour after sunrise to one hour before sunset. To avoid clogging in the spargers during the night, compressed air was passed through them by night (Grobbelaar, 1990). Biomass production rates of 15 gm⁻²d⁻¹ (dry-weight algae) are realistic in open, mixed pond systems. Rates twice as high have been achieved in lab environments, which suggests that higher production rates are possible (Campbell et al., 2010).

The Israeli and USA based company Seambiotic report that the productivity of their raceway paddlewheel mixed ponds amounts to a sequestration rate of 50g CO₂ per square meter, per day. The ponds are depicted in Figure 4 and have a reported depth of 25 cm.

**2.6.5 CLOSED SYSTEMS**

The main principle in closed culture systems is to provide more light to each algal cell by shortening the light path, and to provide a more controlled and protected environment for the algae to grow in (Borowitzka, 1999). Because of the shorter light path, culture densities can be up to 10 gL⁻¹, ten times higher than in open systems (Stephens et al., 2010). This is advantageous when harvesting, as a higher concentration of algal biomass means less energy is required to further concentrate the algal biomass.

Closed systems restrict evaporative water losses from the culture medium, which can be significant in open systems. However, in hot areas closed systems can require additional water for evaporative cooling of the culture medium and to operate heat exchangers (Stephens et al.,
Generally however, closed systems save on water use compared to open systems (Khan et al., 2009). The sampling of gases passing through the system, of particular interest when focus is laid upon CO$_2$ sequestration and the cleaning of flue gases, is easier to manage if the system is closed. This can be of importance if proof of function is required from regulatory authorities.

A number of authors believe that closed systems will be the reactor of choice for future mass production of algae for biofuel purposes (Borowitzka, 1999), (Chisti, 2007), (Khan et al., 2009), other authors however find the notion preposterous due to the higher investment and operational costs (Carlsson et al., 2007). Scalability, an issue for both open and closed systems, seems to be more of an issue for closed culture systems (Chisti, 2007), (LaMonica, 2009). Wall growth can be prevented by a number of different ways such as high turbulence, mechanically through circulation of sand or grit, sending air slugs or close fitting balls through the system or with enzymes (Chisti, 2007). A brief presentation of different closed culture systems are presented in the following sections.

### 2.6.6 Horizontal tubular photobioreactors

Horizontal tubular photobioreactors have a high surface area to volume ratio and use clear tubes made of glass or plastic of diameter $< 60$ mm that are oriented north to south (Brennan & Owende, 2010). The ground beneath the tubes is often painted white or covered with white plastic sheets. The algal culture is pumped through the tubes under a highly turbulent flow to ensure thorough mixing and to avoid biomass sedimentation. The turbulent flow has the added effect of providing a “flashing light” effect to the algal cells as they pass from the light peripheral segment of the tube to the darker centre, which has been proven beneficial to growth as the photosynthetic apparatus is given time to recover (Chisti, 2007).

Airlift devices are often used instead of mechanical pumps due to lower shear stress. The airlift device is often coupled with a gas-liquid separator as this is necessary to remove the dissolved oxygen produced during photosynthesis. Carbon dioxide is introduced at the start of the solar loop and is consumed as the broth moves through the tube. Therefore there is often a dissolved CO$_2$, pH and dissolved oxygen gradient along the length of tube (Brennan & Owende, 2010), which according to Chisti (2007) means that the maximum continuous tube length is seldom greater than $80$ m (Chisti, 2007), (Molina et al., 2001). Some tubular photobioreactors are displayed in Figure 5.

![Figure 5. Tubular reactors at the Biodiesel Expo, Newark, UK 2007 (right) and Australian National NCRIS Photobioreactor Facility (left)](image_url)
A method for designing tubular photobioreactors combining growth based on irradiance levels, oxygen accumulation in the tubes, the hydrodynamics of the airlift device and the flow velocity in the tubes is presented by Molina (2001). The photobioreactor in this paper had problems associated with photoinhibition during periods of intense sunlight.

Scalability is limited with tubular photobioreactors as the light/dark cycling frequencies required, of the order 1 Hz, are difficult to reach without placing unacceptably high shear stress levels on the algal cells due to a higher flow velocity when increasing tube diameter.

A mathematical model that describes the heat transfer process in a horizontal tubular photobioreactor for the growth of *Chlorella sp.* in Turkmenistan is presented in Lyashenko and Redzhepove (1986). The model calculates the temperature in the bioreactor that incorporates a solar collector, a heat exchanger and a gas exchanger, based on a time increment equal to the time it takes the suspension to complete one cycle through the system. See Lyashenko and Redzhepove (1986) for formulas.

Chisti (2007) estimated the cost of producing a kilogram of microalgal biomass to $2.93 for photobioreactors and $3.80 for raceway ponds (Chisti, 2007), there are however more authors that are of the opinion that biomass produced in raceway ponds will incur much lower costs than in photobioreactors (Benemann & Oswald, 1996), (Brennan & Owende, 2010), (Carlsson et al., 2007), (Mata et al., 2010).

**2.6.7 Column photobioreactors**

Column photobioreactors grow the algae culture in vertical columns made of either glass or plastic and can be designed in different ways. Figure 6 shows some different types of column reactors. Mixing is provided by bubbling compressed air through the culture from a sparger located in the bottom of the column. The size of the bubbles and the bubble flow regime is controlled so as to steer the degree of mixing, formulas for calculating such relationships are presented by Kantarci et al. (2005) and Garcia-Ochoa & Gomez (2008). This form of mixing places the algae under relatively low levels of shear stress and oxygen produced during photosynthesis bubbles off easily resulting in low levels of dissolved oxygen. Carbon dioxide is also introduced at the bottom of the column however in much smaller bubbles via a microporous sparger to make them more accessible to the algae enhancing CO₂ mass transfer (Mirón et al., 2002).

![Figure 6. Column photobioreactors, closed systems. Left: Vertical column, homemade. Right: Tilted column reactors at GreenFuel Technologies.](image)
The productivity of column photobioreactors are favourably comparable to other closed photobioreactors yet require less energy input for mixing and are considered to have greater ease for potential scale up (Brennan & Owende, 2010), (Mirón et al., 2002).

Three different types of column reactors, bubble column, draft-tube airlift and a split cylinder, were compared by Mirón (2002). Productivities were found to be virtually the same for all designs during the trial period and the simplest of designs, the bubble column, showed the least signs of photoinhibition (Mirón et al., 2002) during midday sunlight. The bubbling aeration rate provided for mixing was equivalent to a pneumatic energy input of 98 Wm$^{-3}$.

Bubble columns are stated to have a small illumination area and are expensive compared to open ponds (Brennan & Owende, 2010). Wall growth can be limited by tilting the columns somewhat, as shown in Figure 6, as the bubbles clean the surface facing the sun (Merchuk & Wu, 2003). Alternatively walls can be cleaned by sending larger bubbles or “slugs” of air through the column. Stephens et al. (2010) states that further improvements can be made by using “gas transfer membranes, which avoids energy consuming bubbling”, when sparging CO$_2$ into the bioreactors.

2.6.8 Flat plate photobioreactors

A study conducted by Cheng-Wu et al. (2001) showed that flat plate reactors may be the reactor type best suited for mass production of several algae species. Flat plate reactors bare similarities to column reactors, growing the algae in vertical systems. The flat plate reactor used by Richmond and Cheng-Wu was 10 cm wide, and 110 cm high. The geometry of the reactor can be seen in Figure 7.

![Figure 7. Dimensions of a potential large scale flat plate reactor (left). Laboratory flat plate design at Karlsruhe Institute of Technology (right).](image)

The advantages of growing algae in a flat plate reactor such as this one include a shorter oxygen path than that of a horizontal tubular photobioreactor system, which means that the carbon dioxide-oxygen balance will be more even. Turbulent mixing is also more easily introduced in flat plate systems, when comparing to tubular reactors (Richmond & Cheng Wu, 2001). The light paths in flat plate reactors are also reported to be short, which in general leads to a higher volumetric productivity. Flat plate reactors may also be tilted towards the sun, further increasing the irradiance and light quality. The flat geometry of the reactors also enables easier access for removing wall growth.
The angle of reflection at a plane surface is equal to the angle of incidence, which means that a flat plate reactor may have larger light losses due to reflection in comparison with tubular or column reactors.

Stephens (2010) reported that a recent trend can be seen in combining both “bringing light to the algae”, and “bringing algae to the light”, by using fibre optic elements within thin-film reactors, such as flat plate reactors (Stephens et al., 2010).

### 2.6.9 Helical photobioreactors

Helical coil type photobioreactors have been developed to increase the ratio of culture surface area to culture volume, so as to increase the amount of light that can enter the culture whilst improving space requirements. The photobioreactor is similar to the horizontal tubular photobioreactor with the major difference that the tube is wound round a cylinder to create the characteristic helical form, see Figure 8. The helical section can be wound round a vertical or horizontal cylinder, with the vertical configuration more common. Carbon dioxide can be fed either at the top of the spiral and follow the broth downwards, or be fed in from the bottom bubbling up against the flow (Briassoulis et al., 2010), (Watanabe & Hall, 1995), (Soletto et al., 2008).

![Figure 8](image)

*Figure 8.* (top left) Outdoor helical bioreactor for fish food production, (right and bottom left) indoor internally illuminated helical bioreactors.

Temperature and contaminants are relatively easy to control and these systems are stated to be easy to scale up (Briassoulis et al., 2010). The problems associated with the tubular photobioreactors are the same for helical photobioreactors such as cleaning problems, wall growth, oxygen accumulation along the tube and limited length of a single run (Briassoulis et al., 2010).
One way to improve the efficiency of helical bioreactors is to incorporate a double layer of tubing so as to absorb more sunlight. In low latitude regions a conical shape to the helical bioreactor can make the bioreactor receive solar radiation more effectively (Watanabe & Hall, 1995).

2.6.10 Bag photobioreactors

A bioreactor developed by James Cook University and MBD Energy in Queensland for use in algal carbon dioxide sequestration processes for coal fired power plants uses large pillow shaped plastic bags to grow the algae within (James Cook University, 2010). The algae grow in the bottom half of the bag and the flue gases are fed into the upper half of the bag (which is kept under a certain level of pressure to maintain the inflated shape), as can be seen in Figure 9. The shape of the bag provides a large gas-liquid surface area for CO₂ uptake into the algal growth medium whilst also keeping the system closed to control the release of gases out from the system as well as reducing risk for contamination. Temperature control is stated to be one of the major strengths of this culture system (Wilson, 2010). The plastic used has been selected so as to only allow certain wavelengths of light to pass through. This light dilution has been reported to improve productivity (Stephens et al., 2010).

Figure 9. Horizontal bag reactors in research and development use at James Cook University.

2.7 Harvesting and end use for biomass

As the biomass increases in the cultivation system, a portion needs to be harvested to maintain the optimum culture density. Harvesting generally involves removing a portion of the culture broth and replacing that volume with fresh growth medium. The removed and replaced amount, expressed as a part of the entire volume, is known as the dilution rate. Harvesting also involves concentrating the biomass by reducing the water content. This stage is generally considered to be problematic and expensive (Khan et al., 2009) (Wang et al., 2008).
Different methods for harvesting microalgae include flocculation, ultrasonic aggregation, flotation, gravity/centrifugal sedimentation and microscreening/filtering. These processes are reviewed and discussed by Brennan & Owende (2010), Mata et al. (2010) and Wang et al. (2008). There seems to be no single best method for harvesting microalgae, as the required water content in the biomass is governed by how and what product the biomass will be used for. Harvesting macroalgae is generally considered easier than for microalgae, as depending on the size and nature of the macroalgae, the process can be as simple as rising a net installed in the pond or bioreactor (Aresta et al., 2005).

Algal biomass can, depending on species, be used for a broad range of different applications and products such as fine chemicals, fine oils, pigments, aquaculture, animal feed, carbon sequestration and biofuels (Mata et al., 2010). To produce biofuels, processes such as gasification, liquefaction, pyrolysis, direct combustion, anaerobic digestion, alcoholic fermentation, hydrogen production and lipid extraction for biodiesel have been researched. A summary of these different processes can be found in the reviews written by Brennan and Owende (2010), Mata et al. (2010), Naik et al. (2010) and Wang et al. (2008).

A hydrothermal gasification process has been presented by Haiduc et al. (2009) that states to have a thermal efficiency >70% when producing methane, and optionally hydrogen, from algal biomass. The process starts by mechanically reducing the water content of the biomass to roughly 80-85%. The biomass slurry is then liquefied hydrothermally at 400-450°C and 30 MPa, before the organic fraction is passed through a ruthenium-carbon catalyst operating at roughly 400°C, 30 MPa producing methane and CO₂. The concentrated CO₂ is separated from the gas stream and can be returned to the culture system as nutrients, along with the water and nutrients removed during the liquefaction and mechanical dewatering stage (Haiduc et al., 2009).

Basic advantages of this process include very low residence times compared to anaerobic digesters, the ability to process wet biomass avoiding energy intensive biomass drying procedures, gasification temperatures around 400°C as opposed to 800-1000°C for regular gasification processes without water, and no production of char or tars as the gasification is performed above waters critical point of 374°C and 22.1 MPa. The sulphur released from the biomass in the gasification process was “shown to adversely affect the Ru/C catalyst performance” (Haiduc et al., 2009). The lifespan of the catalyst in this article was not reported. The same catalyst when processing liquefied wood is reported to have been stable during a 220 h test (Waldner et al., 2007).

3 METHOD AND MATERIALS

3.1 Method introduction
The method for this project has been planned and refined during the course of the project. To begin with an eight week Frame of Reference literature study was performed aimed at providing relevant background information within the subject of carbon dioxide sequestration through algae. Within the Frame of Reference, focus was also laid upon examining existing culture systems and how to culture algae.

The method thereafter for the design, construction and operation of the experimental equipment used onsite at the AGL Biogas Power Plant in the final Growth Trial, is illustrated in Figure 10. The start of the process was to review the aims set under section 1.2 Aims. Thereafter a selection of potential biomass end use was formed in correlation with the selection of algae. Inoculums of
the selected algae were grown whilst the choice of culture system, design and construction of the trial equipment was performed. The equipment was installed at the AGL Biogas Power Plant and the Growth Trial commenced. The results from the Growth Trial were then used to answer the questions set in the aims.

The choices made in the method process, see Figure 10, are described in more detail in the following sections. A key design goal throughout the process was to build a system that would enable batch growth of the algae. The batch system would then be monitored to identify the conditions which gave the best performance, regarding algal growth and nutrient- and metal uptake. A goal for any future continuous system would be to apply and maintain these optimum growth conditions.

**3.2 Selection of potential biomass end use**

A decision was formed within the early stages of the project regarding the potential end use of the algal biomass. The making of this decision was closely related to the selection of algae, and was based on information gathered in the Frame of Reference.

For the purposes of this project, gasification of the biomass, whereby a combustible gas is produced from the biomass, has been identified as the most applicable end use, for a number of reasons. Firstly, this process is relatively flexible in that it can use any biomass as a feedstock which means some of the limitations and problems associated with the high oil yielding algae strains used for producing biodiesel from algae are not an issue as faster growing and more robust macro- and microalgae strains can be used. Secondly, Melbourne Water and AGL have existing infrastructure for energy production based on the combustion of biogas produced through anaerobic digestion, and potential expansion of that infrastructure to incorporate sustainable natural gas produced from algal biomass through gasification would most likely be easier and more efficient to implement than a completely new process.

**3.3 Selection of algae**

Initially it was outside this project’s scope to select an algal strain. This was because contact had been initiated with an algae research and development company that could provide algal samples for producing inoculums. However, this never eventuated, largely due to the short time frame for this project – 20 weeks – which meant that this delimitation, adopted at the start of the project, had to be altered. Therefore selection of a suitable algal species was performed and specimens to produce inoculums were sourced elsewhere. This selection was closely related to the choice of potential biomass end use, as discussed in the previous section.
For the purposes of this project a microalgae strain called *Chlorella vulgaris*, provided by the CSIRO Australian National Algae Culture Collection, was chosen. Also, as a reserve, four locally collected freshwater algae samples were maintained and inoculums prepared.

*Chlorella* v. was selected for a number of reasons. A primary goal of this project was to sequester as much CO₂ as possible which equates to growing as much biomass as quickly as possible. Species of *Chlorella* are widely known as some of the fastest growing strains of freshwater microalgae (Borowitzka, 1999), (Stephens et al., 2010), (Mata et al., 2010). A number of works have been performed on determining optimal growth parameters for this strain including Chinnasamy et. Al. (2009), Wijanarko et. Al. (2008) and Shaleh (2004) which means this does not need to be performed in this project. From these references it was found that a combination of CO₂ concentration of 6% v/v in the gas stream and a growth medium temperature of 30°C were most conducive to growth. The exhaust gas from the gas engine at AGL has been reported to contain 7.4 % CO₂ (MGT Environmental, 2010) which is close to the optimum presented by Chinnasamy (2010).

The growth rate for *Chlorella* v. reportedly decreases with an increase in temperature but continues to grow even at 50°C at 6% CO₂ level with a measured 379% increase in biomass after 10 days, at these levels. The *Chlorella* v. strain used in Chinnasamy et al. (2009) was originally isolated from the Nehru Vihar Oxidation Pond System in Delhi, India where BOD₅ levels varied between 55-720 mgL⁻¹ at the inlet (Chinnasamy et al., 2009), not entirely dissimilar to the BOD₅ levels in the raw sewage inflow at the WTP with a median reported to be 400 mgL⁻¹ and a maximum of 780 mgL⁻¹ (Melbourne Water, 2006b).

*Chlorella* v. has also been used in numerous trials to bio-remediate different waste water substances such as nitrates (Hu & Sommerfeld, 2004) (Wang et al., 2008), phosphates (Mata et al., 2010) and heavy metals (Das et al., 2008). A review of different trials using single algae strains, multiple algal strains and immobilizing surfaces is presented by de-Bashan (2010) (de-Bashan & Bashan, 2010). *Chlorella* v. is also known to grow under photoautotrophic, heterotrophic and mixotrophic conditions (Mata et al., 2010).

Last, but not least, an isolated axenic culture of *Chlorella* v was readily available for purchase from the CSIRO’s Australian National Algae Culture Collection (CSIRO, 2010). *Chlorella* v. is shown in Figure 11.

*Figure 11. Chlorella vulgaris* upon delivery, (left) *Chlorella vulgaris* cells under 120x magnification in a light microscope (right).
The local algae samples were collected from Alvie Rd creek, Mt Waverley and a freshwater tank in Sorrento. These were considered to be macroalgal, or a cyanobacteria due to their colour and filamentous nature. Aresta (2005) states that microalgae have received more attention than macroalgae for CO$_2$ sequestration largely because more research has been performed on microalgae strains for biofuel – particularly biodiesel – production purposes. Microalgae are also considered easier to grow in ponds or bioreactors. Macroalgae however, require much less stirring when growing and can grow either freely floating in the water or on a solid substrate.

The larger more filamentous nature of the macroalgae make them much easier and less energy intensive to harvest (Aresta et al., 2005). Macroalgae are considered robust and efficient in reducing concentrations of nutrients such as nitrates and phosphates (Marinho-Soriano et al., 2008) (Carlsson et al., 2007). Aresta (2005) and Genifuel (2009) state that biofuels produced through gasification of macroalgae can be more cost effective than gasification of land plants, yet this does not entirely correspond to information presented in (Carlsson et al., 2007). The local algae samples are shown in Figure 12.

Figure 12. The four locally collected algae samples after 1 week of growth.

3.4 Selection of culture system

Performing a comparison of culture systems in order to select one single best system is considered difficult due to the mutually exclusive nature of many optimal operational parameters for algal growth. Examples of this can include light levels within a culture versus optimum culture density, in relation to areal productivity, as well as high levels of mixing required for light access for algal cells versus induced shear stress upon cells. Decisions of which culture systems to develop also depend highly on the priorities of the project.

The choice of culture systems to develop for the growth trials was made based on information gathered in the Frame of Reference as well as on the interests of CJP Solutions and Melbourne Water, regarding potential for realization and scalability as, particularly the latter, has often proven to be the downfall for previous attempts at commercializing similar processes.

Two culture systems were chosen to proceed with, one open system and one closed. For the open system, the raceway pond model was chosen due to its low investment and operational cost, ease of scalability and existing documented performance. The closed system selected was the vertical column reactor due to considered ease of control of mixing, low dissolved oxygen rate, considered ease of scalability, and documented high areal productivity.
3.5 Growth of inoculums

Whilst growing the different algal cultures to volumes suitable for inoculation of the system in the main growth trial at AGL, a number of smaller experiments were performed to determine certain operational parameters. These are presented below.

3.5.1 Inoculation 1: Introduction of algae to Pond 2 water

To start with small volumes of the local algal samples labelled A – D were introduced to the waste water to be used as the growth medium. This water came from Melbourne Water’s 55E lagoon Pond 2, which was readily accessible onsite at the AGL Biogas Power Plant. It was deemed necessary to ascertain whether the algae could survive in the Pond 2 water or if levels of nutrients, contaminants or other microbial activity would prove inhibitory for the algae. Two dilutions of Pond 2 were tested, the lower concentration comprised of 50% Pond 2 water plus 50% potable water, and the high concentration comprised of 100% Pond 2 water. Two samples of each variable were kept, thus four containers for each algae type, resulting in 16 containers in total.

The samples were grown over the course of 5 days starting on the 30th of September 2010 in 500ml glass beakers in a glass greenhouse outdoors without mixing. The samples were photographed each day and visually compared at the end of the growth period, see Figure 13. There were insufficient volumes of Chlorella v. at this stage to include Chlorella v. in this experiment. Instead the Chlorella v. culture was split and expanded in four 500 ml glass beakers with air mixing, in an artificial growth medium at two levels of concentration. The artificial growth medium used was Thrive®, at a concentration of 1.5 gL⁻¹ and 3 gL⁻¹ respectively, as described in appendix Appendix A: Algae Inoculation Documentation. Temperatures ranged from 14-28° C during the growth period.

Figure 13 shows the algae samples on day 1 and day 5. All algae samples grew favourably in the Pond 2 water. Higher growth was observed in the 50 % concentration as these were a richer green colour than the samples growing in the 100 % concentration; a richer green colour indicating a higher optical density and hence a higher cell concentration, equating to more biomass. They also had more oxygen bubbles produced from photosynthesis, trapped on the surface. The algae samples had survived five days in the Pond 2 water.

Figure 13. Local algae cultures A-D on day one (left) and day six (right). Four beakers per culture, algae A in top left beaker and downwards. Beakers were kept without aeration or CO₂.
3.5.2 Inoculation 2: Further performance in Pond 2 water

The cultures grown from Inoculation 1 were expanded on the 6th of October 2010. The beakers containing local algae samples A, B, C and D respectively were combined to produce new corresponding inoculums A2, B2, C2 and D2. These were then each grown in 50% Pond 2 water, 50% potable water one litre growth mediums, since growth observations from inoculation 1 had given indications that the algae grew better in this concentration as compared to the 100% Pond 2 concentration.

The four beakers of *Chlorella v.* were combined to produce an inoculum. Slightly better growth had been observed in the growth medium containing 3 gL⁻¹ of *Thrive* nutrients. The *Chlorella v.* inoculum was then introduced to four separate concentrations of Pond 2 water combined with potable water. The concentrations of Pond 2 water were 25 %, 50 %, 75 % and 100 % Pond 2 water. The goal here was to ascertain whether *Chlorella v.* would survive in Pond 2 water, and if so, at what dilution would it grow best.

Furthermore, equal amounts of algae A, B, C, D and *Chlorella v.* were combined to form a mixed algal culture, hereafter denoted *Algae X*. This was cultured in a one litre, 50% Pond 2 water growth medium. In total, the nine one litre cultures were inoculated into column bubble reactors made from 2L PET bottles mounted on a wooden bench with the bottle necks facing down. The bubble reactor is shown in Figure 14.

![Figure 14. Bubble reactor table](image)

Air mixing was introduced through one-way valves mounted through the bottle caps and was turned off at night. Pure CO₂ was introduced manually to the cultures through the same one-way valves that provided mixing, when the cultures were observed as having a pH > 8.5. The reactors were photographed daily and pH observations recorded. Growth was measured by visually observing change in culture colour.
Figure 15 displays the different cultures on day 1 and day 8 of Inoculation 2. Days 1 - 3 showed signs that *Chlorella v.* growing in the lower concentrations of Pond 2 gave better growth, yet towards the end of day 3 the cultures growing in the higher concentrations of Pond 2 were of an equally royal green colour.

![Figure 15. Day 1 (above) and day 8 (below) of Inoculation 2.](image)

Inoculation 2 ended on the 14th October 2010 at which point the algae cultures A2, B2, C2 and D2 had changed colours from green to a yellow-brown and were considered dead, and were hence disposed of. The *Chlorella v.* and *Algae X* cultures had grown increasingly green but stagnated in colour during the last days of growth. The *Algae X* had far outgrown its individual constituents, labelled A2.1, B2.1, C2.1 and D2.1, see Figure 15, but had not outgrown the *Chlorella v.* samples. A decision was therefore made to proceed with *Algae X* in the final Growth Trial at AGL. It was concluded that all algae samples were to be grown in 100% Pond 2 water.

### 3.5.3 Inoculation 3: Introduction to exhaust sparged Pond 2 water

On the 15th of October 2010 the remaining *Chlorella v.* and *Algae X* cultures where deemed saturated in their growing conditions and therefore further inoculated into larger volumes. The cultures were prepared according to appendix Appendix A: Algae Inoculation Documentation as to test their possibilities of growth in water which had been sparged with exhaust gas from Engine 1 at the AGL Biogas Power Plant.

### 3.5.4 End of inoculum growth period

At the end of Inoculation 3 on the 25th October 2010, it was decided that the *Chlorella v.* and *Algae X* cultures were both fit for the final Growth Trial at the AGL Biogas Power Plant. The cultures had proved resilient and to thrive in high concentrations of Pond 2 water as well as Pond 2 water that had been sparged with exhaust gas. All *Chlorella v.* and *Algae X* cultures were compiled to produce inoculums of volumes around 30 litres, of *Chlorella v.* and *Algae X* respectively, for the start of the Growth Trial at AGL, see section 3.7 Growth Trial at AGL Power Station.
**3.6 Design and construction of trial equipment**

With the choice of algae made, inoculum volumes being increased and decisions made as to which culture systems to use in the growth trial, the design of the experimental equipment for the Growth Trial at AGL could proceed. Important factors considered when designing the equipment, included the use of cheap, simple and readily available materials and components in order to keep costs to a minimum, but more importantly to be able to meet the three week design and construction process deadline. Spill containment was important, as was adhering to the size limits given by the available area onsite at AGL. A schematic figure of the process equipment is provided in Figure 16.

**Figure 16. Experimental equipment process overview**

The exhaust gas was collected from the stack sampling point located approximately half way up the AGL generator 2 chimney, and was accessed from a sampling platform. A flexible stainless steel hose of internal diameter 15 mm was connected to a cooling coil which was deemed necessary when the gas engines were running on full load. Thereafter the gas was pulled through a 20L stainless steel condensate trap to remove the larger portion of the condensate from the flue gas stream. The condensate, believed to be comprising of water, and hydrosulphuric and nitric acids, was regularly removed and had a pH = 3.0. The gas was thereafter passed through a 5µm textile filter to remove particulates before being pumped into a 2 hp air compressor tank. The required suction to draw the gas from the stack sampling point through to the pressure tank, was provided by the air compressor. The flue gas was injected into the algae column reactor and pond (described in the following sections), when required, by means of a pH controller that regulated a solenoid valve for each culture system. Thereby the optimum pH for the culture, set to pH = 7.6, as reported by Rachlin and Grosso (1991), was maintained. The solenoid valves were connected to time clocks that recorded the accumulated time that the valves were open.
## 3.6.1 Column reactor design

The columns were first planned to be made from Perspex tubing but the material cost, around AU $250/m was deemed as too high when considering scalability. Two other options were considered, one involved creating a tubular bag by means of clear 1 mm thick PVC sheeting, and the other involved using 0.2 mm thick extruded polyethylene (PE) tubing. The latter was deemed easier and more time and cost efficient. A 55 m roll of 0.2 mm thick PE tubing of width 220 mm was purchased for $1/m. When filled with water the resulting column diameter was 140 mm. The PE tubing had water tank fittings installed at the bottom end of the column to allow installation of a valve for collection of algal samples and to provide a surface to mount one-way valves for flue gas injection and continuous air mixing, see Figure 17. Below this fitting, the PE tubes were heat sealed. Lacking reliable material data for the particular PE used, especially when having been heat affected through the welding operation, meant a trial and error approach was necessary to determine the load capacity of the column. Several columns with different heat seal geometries were manufactured and hung for a period of two weeks. A maximum water column height was thereafter determined to be 1800 mm, as imperfections in the heat seal, heat from sunlight and creep made some bags fail.

![Figure 17. Column reactor (left) north side, (right) south side.](image)

Air mixing was provided by means of a Schuco Stroke Vac diaphragm air-pump connected to a manifold made from internal diameter 20 mm PVC piping, linking the air pump to the columns. A second identical manifold was used for distributing the flue gas to the different columns. All columns received the same flow of air at 0.1626 m$^3$h$^{-1}$, and flue gas at 0.2088 m$^3$h$^{-1}$. This was measured at the injection points of each individual column. These values were calculated as actual metric gas values from the measured units of Standard Cubic Feet per Hour, SCFH, at ambient temperature conditions. The columns were mounted on a pipe frame pointing east – west. By being rolled around the crossbar, a galvanized steel bar of outer diameter 55 mm, the friction within the rolled section proved sufficient to support the entire column’s weight. The
The crossbar was mounted on two A-frame supports welded together from galvanized steel 50x50x2 mm square pipe profile, bolted to wooden sleepers.

The crossbar had a flat plate of steel welded to one end and was fastened to the top section of the A-frame supports by means of U-bolts. The frame and columns were mounted above a polypropylene tub which was used as a spill catchment container. This also meant that the pond reactor could be placed north of the column reactors, fitting within the allotted area onsite at AGL, without shading the columns. The frame and crossbar had a width of 185 cm, which allowed the hanging of seven manifold-fitted columns above the 170x170 cm tub.

### 3.6.2 Pond reactor design

The conventional proven design for raceway ponds as described by Benemann and Oswald (1996) is still considered the easiest and most effective design for large scale algae production (Campbell et al., 2010). This design involves a pond with wide rounded corners to allow the flow of the algal broth to be guided round the corners into the new direction, see section 2.6.4 Raceway ponds. Sourcing a suitable container with a form similar to these ponds proved difficult in the limited time available for the design and construction process. A decision was therefore made to make use of the most suitable container available and to attempt to mimic the operational conditions in the raceway ponds.

The pond measured 1x1 meter with a culture depth of 0.22 m, as reported by Benemann (2008). The level of mixing was adjusted by means of a diaphragm pump as opposed to the conventional paddle-wheel to a level of light turbulence to avoid sedimentation and provide light and dark cycling for the algae. Mechanical pumps have been reported to induce too much shear stress on the algal cells (Chisti, 2007). Diaphragm pumps are considered to induce only low levels of sheer stress, and are often used in food processing such as pumping yoghurt, which contains bacteria of similar size as microalgal cells (Wilson, 2010).

A paddle-wheel was initially not used for mixing in this pond system as one design criteria was to allow simple enclosure of the culture system to allow for sampling of the gas flow through the system. Gas testings were intended to be performed on the pond by sealing the top off with a glass pane with foam edges, allowing light to enter the reactor, see Figure 18. Flue gas was injected at a rate of $0.43306 \text{m}^3 \text{h}^{-1}$ (30 SCFH) into the bottom of the container via a ring sparger with fourteen 0.2 mm holes when necessary as regulated by the pH-controller. A rotational mixer was added at the start of Phase 2 as this was deemed necessary.

![Figure 18. Pond reactor (left) during Phase 1 (right) Phase 2 with rotational mixer.](image-url)
3.7 Growth Trial at AGL Power Station

The trial equipment was installed at the AGL Biogas Power Plant located within the Western Treatment Plant in Werribee, Melbourne, for a period of 3 weeks commencing on the 25th of October 2010. As explained in section 1.2 Aims, the experiment was designed and conducted to empirically evaluate the growth rate of *Chlorella vulgaris* and its effects on the exhaust gas and water qualities, when grown in Pond 2 water and fed flue gases from the biogas combustion process. The growth medium – Pond 2 water – was collected from the water feed duct upstream of the biogas scrubber. During the Growth Trial, *Algae X* was fed exhaust gas as regulated by the *Chlorella v.* culture pH probe, and sampled and analyzed to a lesser extent.

The growth trial was divided into two phases as described below. During these two periods water samples from the culture systems were collected and delivered for analysis at Australian Laboratory Services (ALS) located in Scoresby, Melbourne. Analysis during the Phase 1 was focused strictly on chlorophyll a and phaeophytin a values, the intention being to ascertain growth performance with the experimental equipment so as to better plan for Phase 2.

During Phase 2 sampling and analyses were expanded, to include further water analysis as well as gas sampling, in order to attain sufficient data to draw conclusions on the questions set forth in the aims of this project. During Phase 1 no sampling was performed on the columns containing *Algae X*. As sampling was expanded in Phase 2, chlorophyll a and phaeophytin analyses were conducted also on *Algae X*, but only at three points during the five day trial. This was due to *Chlorella v.* being selected as the algae of choice for the experimental design, whereas *Algae X* had been kept predominantly as a back-up. The decision to expand chlorophyll sampling to include *Algae X* was based on the apparent well-being of the culture during Phase 1. At this stage little was known of the contents of *Algae X*, although seeing as it had survived better than its individual constituents, the culture must have evolved into a mix that was fit for the prevailing conditions.

The samples of *Chlorella v.* that were collected for analysis during the trials consisted of equal parts of the different *Chlorella v.* columns.

3.7.1 Growth Trial at AGL: Phase 1

Phase 1 commenced on the 26th of October with the inoculation of the pond and column culture systems. The pond and five of the seven column reactors were inoculated with *Chlorella v.* and two of the columns with *Algae X*, further described in Appendix A: Algae Inoculation Documentation. At this point an extensive laboratory analysis of the growth medium – Pond 2 water – was also carried out by ALS.

Chlorophyll a and phaeophytin a samples were collected on five occasions during the eight day growth period. See Appendix B: Sampling Plan During Phase 1.

Operational parameters monitored and recorded during Phase 1 of the Growth Trial were: pH of the cultures, sunlight levels (measured in lux), ambient and culture temperatures, gas injection intervals, and compressor duty cycles. These observations together with the results from the laboratory were used when determining the sampling plan for Phase 2 of the Growth Trial.
3.7.2 Growth Trial at AGL: Phase 2

Phase 2 of the Growth Trial at AGL commenced on the 8th of November 2010 with a new inoculation of the pond and column systems. The pond and five of the seven columns were inoculated anew with *Chlorella v.* from the previous trial. One column was inoculated with a local algae culture collected by Melbourne Water, and was not included within the scope of this project. The seventh column containing *Algae X* was inoculated through dilution of the existing culture.

During this period more rigorous sampling and testing was performed as can be seen in the water sampling plan displayed in Table 2. Table 3 shows the parameters which were measured. Twice daily sampling was performed to measure a group of parameters denoted *Half Suite*, intended to determine the algal growth rates and bioremediation performance of *Chlorella v.* On three occasions, during the five day trial, samples were taken to yield comprehensive data on the quality of the water and algae, in a sweep denoted *Full Suite*. Also, gas sampling was conducted during one day of trials in this phase, to further assess the effect of the algae systems on the gas flows, see section 4.9 Flue gas analysis.

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<td>Full Suite</td>
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<td>AGL-Column</td>
<td>Point 2: Algae Column</td>
<td>Full Suite Half Suite</td>
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<td>Point 3: Algae Pond</td>
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<td>Full Suite* Half Suite</td>
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<td>AGL-Pond</td>
<td>Point 3: Algae Pond</td>
<td>Full Suite*</td>
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*Included filtered heavy metal tests

At the start of Phase 2, the pond had been further equipped with a rotational stirrer to improve the level of mixing and to prevent sedimentation, which had been observed during Phase 1. Operational parameters including pH of the cultures, sunlight levels (measured in lux), ambient and culture temperatures, gas injection intervals, accumulated time of gas injection periods and compressor duty cycles were observed and recorded.
3.7.3 Water analysis methods

The sampling during Phase 2 was aimed at determining the growth rate of the algae and the level of gas sequestration, whilst also monitoring which substances, and at what rate, these were removed from the waste water by the algae. The parameters measured in the test groups Full Suite and Half Suite are shown in Table 3, and discussed in the following sections.

Table 3. Tests included in the test groups Full Suite and Half Suite

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<th>Method Standard:</th>
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<td>APHA 5210 B</td>
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<tr>
<td></td>
<td>COD - Chemical Oxygen Demand</td>
<td>APHA 5220 D</td>
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<tr>
<td></td>
<td>TKN/TP (HL) - Total Kjeldahl Nitrogen as N</td>
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<td></td>
<td>TKN/TP (HL) - Phosphorus, total as P</td>
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<tr>
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<td>VSS - Volatile Suspended Solids (W/V)</td>
<td>APHA 2540 D, E</td>
</tr>
<tr>
<td></td>
<td>Alkalinity - Bicarbonate Alkalinity as CaCO3</td>
<td>APHA 2320 B</td>
</tr>
<tr>
<td></td>
<td>Alkalinity - Carbonate Alkalinity as CaCO3</td>
<td>APHA 2320 B</td>
</tr>
<tr>
<td></td>
<td>Alkalinity - Total Alkalinity as CaCO3</td>
<td>APHA 2320 B</td>
</tr>
<tr>
<td></td>
<td>CO2 (Aggressive) - Carbon dioxide (Aggressive)</td>
<td>VIC-CM058 #</td>
</tr>
<tr>
<td></td>
<td>TCN - Total Nitrogen as N (Calc)</td>
<td>VIC-CM019</td>
</tr>
<tr>
<td></td>
<td>NH3 as N (DA) - Ammonia, as N</td>
<td>VIC-CM003</td>
</tr>
<tr>
<td></td>
<td>NOX as N (DA) - Nitrate + Nitrite, as N</td>
<td>VIC-CM007, 035</td>
</tr>
<tr>
<td></td>
<td>Chl-a - Chlorophyll a</td>
<td>VIC-BM003/ISO 10260</td>
</tr>
<tr>
<td></td>
<td>Chl-a - Phaeophytin a</td>
<td>VIC-BM003/ISO 10260</td>
</tr>
<tr>
<td></td>
<td>Chloride - Chloride, as Cl</td>
<td>VIC-CM031</td>
</tr>
<tr>
<td></td>
<td>Turbidity - Turbidity</td>
<td>APHA 2130 B</td>
</tr>
<tr>
<td></td>
<td>MS Total Metals – Ca, Ch, Cu, Pb , Hg, Ni &amp; Zi</td>
<td>VIC-CM050 A,C</td>
</tr>
<tr>
<td></td>
<td>MS Sol. Metals – Ca, Ch, Cu, Pb , Hg, Ni &amp; Zi</td>
<td>VIC-CM050 A,C</td>
</tr>
<tr>
<td></td>
<td>MAH – Benzene, Toluene</td>
<td>VIC-CM055</td>
</tr>
<tr>
<td></td>
<td>OES Scan - Calcium</td>
<td>VIC-CM050 A,D</td>
</tr>
<tr>
<td></td>
<td>OES Scan - Sodium</td>
<td>VIC-CM050 A,D</td>
</tr>
<tr>
<td></td>
<td>PAH - Total PAH</td>
<td>VIC-CM043</td>
</tr>
<tr>
<td></td>
<td>PHENOLS – Total Phenols</td>
<td>WEC100</td>
</tr>
<tr>
<td></td>
<td>Temp - Temperature (Field)</td>
<td>VIC-OP001</td>
</tr>
<tr>
<td></td>
<td>pH - pH (Field)</td>
<td>VIC-OP001</td>
</tr>
<tr>
<td></td>
<td>DO - Dissolved Oxygen (Field)</td>
<td>VIC-OP001</td>
</tr>
<tr>
<td></td>
<td>pH - pH</td>
<td>APHA 4500-H,B</td>
</tr>
</tbody>
</table>

Chlorophyll a and phaeophytin

Since the cultures were grown in batch systems, the frequent sampling for chlorophyll a gave an appreciation of the algal growth rate and of changes in algal biomass during the trial period, as well as an indication of the health of the culture when chlorophyll a levels where compared to levels of phaeophytin a.
Nitrogen

The combined analyses of Total Kjeldahl Nitrogen (TKN), nitrates, ammonia and Total Calculated Nitrogen (TCN), showed the changes of nitrogen compounds within the culture medium throughout the Growth Trial. Dissolved nitrogen in the form of ammonium $\text{NH}_4^+$ is the most readily available source of nitrogen for algal uptake, together with nitrates (Mata et al., 2010). $\text{NH}_4^+$ was measured through $\text{NH}_3$ tests in the samples prior to and post digestion of organic matter, as conducted in a TKN analysis. The difference between the values of $\text{NH}_3$ and TKN represents the amount of nitrogen having been taken up organically (Lindblom, 2010). In this manner the levels of nitrogen removed through algal uptake $N_{\text{uptake}}$ could be calculated, as shown in equation (3).

$$N_{\text{uptake}} = \text{TKN} - \text{NH}_3 \quad (3)$$

Phosphorus

The compositional change of phosphorus in the pond and columns was measured by means of total phosphorus (TP) and reactive phosphorus analyses. The difference between these values corresponded to the amounts taken up by the algae.

Heavy Metals

Total heavy metal tests were performed on the three Full Suite occasions during Phase 2 to determine levels of cadmium, chromium, copper, lead, mercury, nickel and zinc in the culture medium. On the 2$^{nd}$ and 3$^{rd}$ Full Suite tests, duplicate samples were passed though a Whatman GF/C filter paper and a total heavy metal test, as described above, was performed on the filtered solution. The unfiltered samples gave the total heavy metal content, as all organic matter was digested in the process. The filtered samples gave the heavy metal content in the medium, less that which had been taken up by the algae. The heavy metal uptake performance of the algae was therefore calculated according to equation (4):

$$\text{HMR}_i = \frac{\text{UF} - \text{F}}{\text{UF}} \times 100 \quad \text{[%]} \quad (4)$$

Where HMR$_i$ is the heavy metal reduction for heavy metal $i$, UF is the value for the un-filtered test and F is the value for the filtered test.

TOC, COD, BOD and Suspended Solids

The changes in total organic carbon (TOC) levels were assumed to result from changes in algal biomass. TOC measurements from particulate-rich waters such as algae cultivations have however been reported to carry a high level of uncertainty, due to instrument related issues such as small sampling volumes, fine tubing and bore needles (Lindblom, 2010), (Lundquist, 2010), implying that the TOC data would not serve as a reliable measurement of the biomass carbon increase. To this end, measurements of COD, BOD and Suspended Solids were also used as indications of changes in biomass through a conversion factor derived from municipal wastewater treatment data from Stockholm Water, see Appendix D: and Table 4.
### Table 4. Conversion factors from BOD, COD and suspended solids to TOC

<table>
<thead>
<tr>
<th></th>
<th>COD to TOC</th>
<th>BOD to TOC</th>
<th>Suspended solids to TOC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Divide by</strong></td>
<td>3.522</td>
<td>1.16625</td>
<td>1.28375</td>
</tr>
</tbody>
</table>

### Total Solids

An analysis of total solids was ordered and performed on the Chlorella v. cultures, after the trials had been finished. This analysis was performed on two samples stored by ALS, from the third and fifth day of Phase 2. The test measured the dry weight of a GF/C filter paper through which the growth medium and algae samples respectively, had been passed. The difference between the two gave an indication of the weight of the biomass in the samples.

The total solids measurement was not used as the primary biomass indicator as it could not differentiate between living and dead organic matter (phaeophytin). Also suspended solids and non photosynthetic organisms are measured which can be misleading when the purpose was to determine the photosynthetically active biomass increase. It was used on the two occasions described above to compare with the biomass through chlorophyll a analyses.

### Algae Species Count

The compositions of the Chlorella vulgaris and Algae X cultures were assessed by the laboratory during the Growth Trial by light microscoping Sedgewick-Rafter volumes at 200x and 100x magnification. Analysis results included a culture description from a certified analyst from ALS, and a total count of cells per species and order, per ml. The Algae X sample was analyzed on the first day of Phase 2. The Chlorella vulgaris sample had been stored for one week following the end of Phase 2, prior to having the species count performed.

### 3.7.4 Growth Trial at AGL: Flue gas analysis

In order to measure changes in the exhaust gas composition when passing through the algal cultures, a portable flue gas analyser, Testo Model 350XL was used on the fourth day of Phase 2, the 4th of November 2010. The instrument had been calibrated by the rental company TechRentals two weeks prior to use, and was used to measure the occurrences of CO₂, CO, NO, NO₂, SO, SO₂, etc. See Appendix C: Flue Gas Analyser Technical Data for further information. Gas sampling was performed on three columns containing Chlorella v. and on the column containing Algae X. The exhaust inlet sampling point was the hose leading to the gas manifold, and the outlet sampling point was within the column tubes approximately 10 cm above the water level, see Figure 19. The difference in gas composition between the sampling points was caused by the algae suspensions.
Prior to conducting a given gas sampling, the algae cultures were first allowed to grow without exhaust injection until a pH ≈ 9 was reached within the culture. Once exhaust gas injection was started, the exiting gas was frequently sampled and recorded until the culture had reached a neutral pH again. During this period, sunlight levels, temperature, and pH levels were recorded, see Appendix E: Gas Exhaust Analysis Data. Three measurements were taken from the manifold and the average of these readings were used to represent the composition of the exhaust inlet, as sampling could not be conducted at the inlet and outlet simultaneously as there was only one probe on the instrument. Separately, the ambient air was analysed, as was the flue gas at the stack sampling point. No gas measurements were made on the pond due to a lack of time. Apart from the gas sampling, a recording of the amounts of flue gas continuously supplied into the pond and columns respectively was kept through a time-clock connected to the solenoid valves regulating the gas injection. This provided data on the quantities of exhaust gas needed to stabilize the pH with the given sparger set up.
4 RESULTS & DISCUSSION

4.1 Operational parameters during Growth Trial at AGL

During the Growth Trial at AGL a number of operational parameters were monitored. These included pH of the cultures, sunlight levels, ambient and culture temperatures, gas injection intervals, accumulated time of gas injection periods and the compressors duty cycle. These have been compiled in Appendix F: Operational Parameter Log. During periods of linear growth, the pH was observed to rise at a rate of up to 0.17 pH units/minute. The chemical reaction driving the pH up is the algal uptake of carbon from bicarbonate in the growth medium (Deas & Orlob, 1999). Algae primarily grow through uptake of CO\textsubscript{2} (aq), readily available from the injected exhaust gas. As the amounts of available CO\textsubscript{2} (aq) decrease, the algae increasingly turn to carbon available as bicarbonate (HCO\textsubscript{3}\textsuperscript{−}). The pH levels rise as hydroxide (OH\textsuperscript{−}) is released in this process (Ruttner, 1974).

\[
\text{HCO}_3^- \rightarrow \text{CO}_2 + \text{OH}^- \quad (5)
\]

Light levels varied between 0 Lux during night time and peaked at 143000 Lux during strong sunlight on certain days. The average recorded Lux level was 92900 and 80100 for Phase 1 and Phase 2 respectively. Temperatures in the column reactors varied between 9.9°C and 36.3°C during the course of both growth periods with an average daily temperature of 25.6°C for Phase 1 and 27.4°C for Phase 2. The temperature in the pond varied between 10.8°C and 38.3°C during the both growth periods with an average daily temperature of 25.8°C for Phase 1 and 25.5°C for Phase 2.

4.2 Chlorophyll a, phaeophytin a and health of the cultures

Analyses of chlorophyll a and phaeophytin concentrations in the cultures maintained during Phase 1 and Phase 2 were indicative of the health and photosynthetic activity within the cultures, and correlated to the algal biomass. The ratio of chlorophyll to biomass is however not entirely constant in Chlorella v. nor in Algae X, as will be discussed in the following section, 4.3 Biomass determination.

Phaeophytin a is a breakdown product from the algal photosynthesis. The ratio of phaeophytin a to chlorophyll a increases as the health of the culture declines. This can be caused by a prolonged lack of sunlight, toxicity, disease, lack of nutrients, or by other causes. During rapid growth the levels of phaeophytin are reported to be low in general for microalgae, which also can be seen in the early stages of growth in the pond and column reactors during the Growth Trial.

4.2.1 Chlorella v.

The results of the chlorophyll a and phaeophytin a analyses for the Chlorella v. systems are presented in Figure 20 to Figure 22.

As can be seen in Figure 20, the Phase 1 column cultures of Chlorella v. maintained a stable level of phaeophytin a, and a positive growth curve over the first seven days, which indicates that the culture was healthy and thriving in the experimental setup.
As can be seen in Figure 21, *Chlorella* v. in the column reactors during Phase 2, the level of phaeophytin rises and even surpasses the levels of chlorophyll a as the algal population enters into its final phase – its decline and probable death – after having been stable at a stationary phase on the third and fourth days of growth. The decline was also visually observed, as the *Chlorella* v. cultures turned from a full dark green colour into a dark green with slight yellow hues. This had also been observed on several occasions in earlier stages when cultures were kept in non-continuous systems, with Pond 2 water for prolonged periods (ca more than one week). This phenomena was observed at some stage for all algae types kept during the project; algae A-D, *Algae X*, as well as *Chlorella* v.. However, original first generation cultures of algae A-D which had been collected and kept in their original fresh water, never underwent this decline and were a healthy dark green colour even at the end of Phase 2, 2 ½ months after being collected, in 250 ml unmixed open glass beakers.

The common denominator for algae populations that at some point entered into a decline and death phase was the growth medium – Pond 2 water. Air mixing, carbon dioxide, and exhaust
gas injection were present in all varying combinations. The effects of these variables did increase growth rates, judging by rates of change in pH, visual inspection and through chlorophyll a analysis. Hence, the increased growth rate attributed to these variables, as well as to Pond 2 water, can be the underlying cause of the death of the algae cultures in question.

The stages of microalgal growth in batch systems are well recognized in the literature, beginning with a lag phase, going through an exponential growth phase, followed by linear growth leading to a stationary phase, before the gradual death of the culture. The setup of this industrial experiment was deliberately non-continuous (i.e. batch), since this allowed a study of the effects of the algae culture at different stages of growth. Observing a closed system in this manner allows for determination of optimum growth parameters, which can then be applied and maintained in a continuous cultivation system.

It is likely that the high levels of incident light in conjunction with decreasing amounts of available nutrients in the pond and columns, led to the decline or death of the algal cultures, most notably seen in the Phase 2 columns, Figure 21.

The CSIRO, suppliers of the *Chlorella* v. strain used in this experiment, mention that light levels that during the first three stages of growth are adequate or optimal for growth often become stressful once the algae have adapted to limitations in available light, due to the increased density. This is what leads to a decrease or total stop in growth, as is observed in the stationary phase. Unless the amount of available light is limited at this stage, photoinhibition will set in, leading to the death of the algal culture.

The Phase 1 pond culture, shown in Figure 22, showed a significant decline in health after the 4th day.

![Figure 22. Chlorophyll a and phaeophytin a concentrations in the pond, during Phase 1.](image)

A lesson learned from this cultivation was to improve the level of mixing as sedimentation had gradually increased, and chlorophyll concentrations decreased. For this reason, a horizontal paddle wheel was added prior to Phase 2.
Figure 23. Chlorophyll a and phaeophytin a concentrations in the pond, during Phase 2.

The Phase 2 pond culture, seen in Figure 23, shows a slower growth curve but a more stable level of phaeophytin, indicating a more stable level of health, when compared to the growth curves of the column cultures.

4.2.2 Algae X

Chlorophyll levels in Algae X, sampled only three times during Phase 2, were considerably higher than in the Chlorella v. cultures, indicating a higher biomass concentration. The chlorophyll levels of the Algae X column compared to Chlorella v. are presented in Figure 24.

Figure 24. Chlorophyll a concentrations of Algae X and Chlorella v. columns during Phase 2.

The data suggests that the species mix contained in Algae X had a superior growth rate compared to Chlorella v. at the time of measurement, as both cultures were grown in the same medium under identical conditions. Although the Algae X performance was observed only in a column system and no empirical comparison of Algae X and Chlorella v. has been made under pond growth conditions, there is little evidence to suggest that Algae X could not be cultured in a pond.
A certain degree of care should be taken when drawing conclusions, as only three measurements were performed and so the entire growth curve has not been mapped. The Algae X culture contained larger more filamentous types of algae which could have made collecting a representative sample somewhat more difficult than the homogenous mix of Chlorella v. The results shown for each sample date are however based on the average from two separate tests. The superior growth rate compared to Chlorella v. is discussed further under section below.

4.3 Biomass determination through chlorophyll a analysis

A measurement of algal biomass was sought in order to determine the amount of CO₂ sequestered from the flue gases, bound into organic form, as stipulated in the aims of this project. The chlorophyll a levels monitored during both Phase 1 and Phase 2 of the Growth Trials were correlated to the amount of biomass, as they are indicative of the photosynthetic activity within the algae cultures. The biomass productivity rates of the culture systems were then used to determine the rate of CO₂ sequestration, see section 4.10 Land area estimates for scale-up.

The ratio between chlorophyll a levels and biomass in a culture is however not constant, as the percentage of chlorophyll per algal cell varies. For this reason no entirely correct linear comparison could be made between changes in measured levels of chlorophyll and changes in biomass of the monitored cultures. This was a problem encountered once data compilation had begun, and a means of arriving at a biomass value from chlorophyll calculations was reached by reviewing ratios used in the literature, as well as through consultation with an expert in the field (Benneman, 2010, personal communication). A level of 1.5% (dry weight) chlorophyll to biomass was used in this study. This compares to:

- *Chlorella, the sun-powered supernutrient and its beneficial properties* (Rosenbaum & Lee, 2010) noted 3-5% for Chlorella spp.
- *Chlorophyll Synthesis in Chlorella* (Beale & Appleman, 1971) noted a range of 0-5.5% for Chlorella spp. And also investigated the ratio as a function of average available light.

The amount of chlorophyll per Chlorella v. cell depends on the amount of light available to the cell, light fluctuation, and chemical composition of the growth medium. The correlation between chlorophyll and biomass does however appear to be linear under varying levels of CO₂ (Chinnasamy et al., 2009).

With lower levels of available light, the algae cells synthesize more chlorophyll in order to achieve a higher photosynthetic efficiency per cell. Inversely, the amount of chlorophyll per cell decreases with an increased amount of available light (Beale & Appleman, 1971). Chlorella spp. cultivated in Beale & Appleman’s study were kept in a laboratory environment at light levels far below those recorded during the Growth Trials of this study: 1000-15000 lux compared to an average of 80100 lux. The light to chlorophyll-percentage relationship investigated in their report could therefore not be extrapolated to light and chlorophyll values in this project.

The following calculations were used to determine the biomass concentration, and biomass productivity, with a 1.5% chlorophyll to biomass dry weight ratio used.

\[
c = \frac{Chla}{0.015} \cdot 1000
\]

(6)
Chla in equation (6) is the amount of chlorophyll [µg l⁻¹] in a sample, and the returned value c [g l⁻¹] is the calculated biomass concentration.

\[
P = \frac{c_2 - c_1}{t_2 - t_1} \tag{7}
\]

Equation (7) gives the calculated biomass productivity \( P \) [g l⁻¹ d⁻¹], that is the amount of algal biomass produced per litre, per day, within a culture.

Calculations were performed using the chlorophyll data presented in section 4.2 Chlorophyll a, phaeophytin a and health of the cultures. With a set value for the chlorophyll to biomass ratio, a presentation of biomass would be parallel to the amounts of chlorophyll as seen in graphs in the previous section. Figure 25 and Figure 26 show the calculated changes in biomass in Phase 1 and Phase 2.

**Figure 25.** Biomass concentration during Phase 1.

**Figure 26.** Biomass concentration during Phase 2.
The maximum biomass productivities were calculated at the points in time where the increase in biomass concentration was the greatest, through equation (7). Values used are labelled in Figure 25 and Figure 26. These times were all within the first third of the Growth Trials and occurred between the second and third sets of samples for the columns in Phase 2, and between the first and second sampling times for the pond and the column in Phase 1, as shown in Table 5.

Table 5. Maximum biomass productivities recorded during growth trials at AGL.

<table>
<thead>
<tr>
<th>Productivity</th>
<th>Phase 1</th>
<th>Phase 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>P_CHLOR_POND</td>
<td>0.067</td>
<td>0.264</td>
</tr>
<tr>
<td>P_CHLOR_COLUMN</td>
<td>0.333</td>
<td>0.430</td>
</tr>
<tr>
<td>P_ALGAE_X_COL</td>
<td>n/a*</td>
<td>1.017</td>
</tr>
</tbody>
</table>

*no chlorophyll samples of Algae X were taken during Phase 1, see 3.7 Growth Trial at AGL Power Station

The biomass productivities varied from the above values to negatives as low as -0.19, as can be seen by the negative inclinations of the curves, most notable for the columns at the end of Phase 2.

The trend line in Figure 26 (labelled Poly. Column) is a line of regression automated in MS Excel, and a fourth degree polynomial function. It is of interest since it shows a high regression with the data (R²=0.96) and since the nature of fourth degree polynomial functions fits the characteristics of the five-phase curves often describing algal growth in batch cultures as described in section 2.4 Algae basics. As such, the chlorophyll and biomass analysis point to a common growth pattern.

The biomass calculations for Algae X were also performed with a 1.5 % chlorophyll:biomass ratio, which is reasonable as this is close to the general ratio ascribed to phytoplankton, and also since Chlorella v. was a main constituent (22.4 % by volume) of the species mix within Algae X. This 1.5 % chlorophyll can be assumed as a maximum value for Algae X, as Chlorella v. is a chlorophyll-rich species. The biomass is inversely proportional to the percentage of chlorophyll (equation (6)), therefore the biomass productivity value \( P_{ALGAE_X_COL} \) in Table 5 can be seen as a conservative estimate.

4.4 Biomass determination through Total Solids

Analyses of total solids were performed at the end of Phase 2 on Chlorella v. samples. Values obtained from these samples were used as a further measure of algal biomass, and were compared to the calculated values from the chlorophyll analyses. The difference in total solids weight between the growth medium prior to inoculation, and the totals solids weight of the algal samples are presented in Table 6.

<table>
<thead>
<tr>
<th>Growth medium</th>
<th>Total Solids [mgL⁻¹]</th>
<th>Biomass* [mgL⁻¹]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth medium</td>
<td>980</td>
<td></td>
</tr>
<tr>
<td>Column (10-11-09)</td>
<td>1200</td>
<td>220</td>
</tr>
<tr>
<td>Column (10-11-12)</td>
<td>1200</td>
<td>220</td>
</tr>
</tbody>
</table>

*calculated as difference between growth medium sample and algae sample
A comparison of biomass as total solids, with biomass calculated assuming 1.5 % chlorophyll is presented in Table 7.

**Table 7. Biomass from total solids and from 1.5 % chlorophyll**

<table>
<thead>
<tr>
<th>Biomass [gL⁻¹]</th>
<th>As Total Solids</th>
<th>From 1.5 % chlorophyll a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column (10-11-09)</td>
<td>0.22</td>
<td>0.25</td>
</tr>
<tr>
<td>Column (10-11-12)</td>
<td>0.22</td>
<td>0.15</td>
</tr>
</tbody>
</table>

The measured amounts of total solids were compared with the levels of chlorophyll within the same sample; this gave the mass percentage of chlorophyll as presented in Table 8.

**Table 8. Biomass concentration in grams per litre, expressed as total solids, chlorophyll amounts from the same sample, and the calculated chlorophyll content of the total solids.**

<table>
<thead>
<tr>
<th>Total Solids [gL⁻¹]</th>
<th>Chlorophyll a [gL⁻¹]</th>
<th>Calculated % chlorophyll</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column (10-11-09)</td>
<td>0.22</td>
<td>4550*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.068 %</td>
</tr>
<tr>
<td>Column (10-11-12)</td>
<td>0.22</td>
<td>2300</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.045 %</td>
</tr>
<tr>
<td><strong>Average:</strong></td>
<td></td>
<td><strong>1.557 %</strong></td>
</tr>
</tbody>
</table>

*average of two daily samples 3800, 5300

As can be seen in Table 8, the assumption made in section 4.3 Biomass determination regarding the percent of chlorophyll per cell at 1.5 % was not at all far from the calculated value of 1.557 %. 
4.5 Nitrogen uptake

The initial analysis of the growth medium showed a total nitrogen level of 55 mg nitrogen per litre, which to the most part was dissolved in the water as ammonium, NH$_4^+$. This portion was readily available for algal uptake once the algae systems had been inoculated. The changes in ammonia levels which were observed in Phase 2 in both the column and pond systems corresponded to the remediation of the algae on the eutrophication of the wastewater.

It was observed that the reduction in amounts of available nitrogen (NH$_4^+$) in the algae systems was proportional to the increase of chlorophyll a in the medium. As the algae grew, nitrogen was removed from the waste water and taken up into organic forms within the algal cells. The total calculated level of nitrogen (TCN), remained the same within the systems with the exception of one sampling point, proving that the nitrogen had undergone a change of state.

Figure 27. Chlorophyll a and available nitrogen (NH$_3$) in the column reactors during Phase 2.

Figure 28. Chlorophyll a and available nitrogen (NH$_3$) in the pond reactors during Phase 2.
During the periods of algal growth, observed from the start of the Phase 2 up until 10-11-10 14:00 in the columns and one day later in the pond, the correlation coefficient between the chlorophyll and NH$_3$ variables was calculated in MS Excel as $R^2 = -0.94$ for both systems, confirming a very strong correlation (see Figure 27 and Figure 28). Although this does not imply a causal relation, decreasing amounts of available nitrogen in combination with increasing cell densities are commonly viewed as causes of culture death, which in this study too was observed once levels of available nitrogen reached 8.1 mg nitrogen per litre and 21 mg nitrogen per litre, pond and column. The composition of nitrogen compounds at these points for the column and pond systems are presented in Table 9.

Table 9. Nitrogen levels in the growth medium prior to inoculation, and in the algae systems at the end of their growth periods

<table>
<thead>
<tr>
<th>Nitrogen compound</th>
<th>Growth medium</th>
<th>Column</th>
<th>Pond</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10-11-08, prior to inoc.</td>
<td>10-11-10 14:00</td>
<td>10-11-11 14:00</td>
</tr>
<tr>
<td>TKN/TP (HL) - Total Kjeldahl Nitrogen as N</td>
<td>mgL$^{-1}$</td>
<td>55</td>
<td>55</td>
</tr>
<tr>
<td>TCN - Total Nitrogen as N (Calc)</td>
<td>mgL$^{-1}$</td>
<td>55</td>
<td>55</td>
</tr>
<tr>
<td>NH3* - Ammonia, as N</td>
<td>mgL$^{-1}$</td>
<td>47</td>
<td>8,1</td>
</tr>
<tr>
<td>NOX (DA) - Nitrate + Nitrite, as N</td>
<td>mgL$^{-1}$</td>
<td>0,02</td>
<td>&lt;0,01</td>
</tr>
</tbody>
</table>

*In the analysis process NH$_4^+$ in the samples was measured as NH$_3$

The rate nitrogen uptake by the algae was calculated by measuring the decrease in amounts of available ammonia, NH$_3$ in the culture systems. A maximum uptake rate was obtained by identifying this value at the points in time where the ammonium decrease was the greatest, through equation (8).

$$p_N = \frac{N_2 - N_1}{t_2 - t_1}$$  \hspace{1cm} (8)

$N_2$ and $N_1$ are the amounts of nitrogen as NH$_3$, in milligrams, at the times of sampling $t_2$ and $t_1$. $p_N$ is the calculated uptake rate, expressed as nitrogen mgL$^{-1}$d$^{-1}$. The maximum calculated uptake rates are shown in Table 10.

Table 10. Highest calculated nitrogen uptake rates.

<table>
<thead>
<tr>
<th>Nitrogen uptake maximum rates [mgL$^{-1}$d$^{-1}$]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase 2</td>
</tr>
<tr>
<td>$P_{N,POND}$</td>
</tr>
<tr>
<td>$P_{N,COLUMN}$</td>
</tr>
</tbody>
</table>

4.6 Phosphorous uptake

An initial measurement of total phosphorous in the growth medium was recorded and amounted to 8.5 mgL$^{-1}$. On the second day of trials and onwards reactive phosphate testings were included, so that an appreciation of the organic uptake could be formed, calculated as the difference between total phosphorous and reactive phosphorous.
Comparisons of chlorophyll levels and organic phosphorous levels were inconclusive for both the column and pond systems, as no strong correlation could be seen regarding increase in chlorophyll and possible remediation of the solved phosphorous, as presented in Figure 29 and Figure 30. A correlation coefficient of $R^2=-0.61$ for the columns and $R^2=0.13$ for the pond was calculated.

*Figure 29. Chlorophyll a and reactive phosphorus in the pond reactors during Phase 2.*

It was observed that the levels of organic phosphorus as well as levels of total phosphorus remained quite stable during the entire growth trial. Although this is reasonable for the total levels of phosphorus since no additional P was added to the systems, it is remarkable that the levels of organic P remained so stable during the course of their analysis. The reactive P measurements were however only added to the analysis suites on the second day of trials, and did not permit a long sequence of comparisons. Also, the relative stability of the reactive phosphorus levels was noted for quite low values, and the variance between these values might be ascribed to technical issues in the lab analysis.

*Figure 30. Chlorophyll a and reactive phosphorus in the column during Phase 2*
4.7 Cell composition

The chlorophyll content and overall chemical composition of the algal cell is, apart from light conditions, also affected by the chemical composition of the growth medium. The elemental composition of the *Chlorella* v. cells has been shown to vary depending on the quality of the water in which they grow (Mandalam & Paulsson, 1998). Hence, the Redfield ratio which generalizes the composition of the main nutrients in phytoplankton cells to:

\[ C: N: P = 106: 16: 1 \]  

was not used in this study to determine biomass from data on carbon, nitrogen or phosphorous measurements. Conversely, the formulation of a ratio describing the biomass, based on the compositional changes in carbon, nitrogen, and phosphor observed during the growth trials, was attempted.

However, as values of these changes were only obtained from a few days of growth, and samples of phosphorous and total organic carbon were possibly subject to technical analysis issues and showed inconsistent values, no reliable ratio could be formed.

Mandalaam and Paulsson (1998) and Chisti (2007) mention that around 55% of the microalgal biomass is carbon. This is used in section 4.10 Land area estimates for scale-up, when converting biomass productivity to carbon uptake productivity.

![Figure 31. Changes in levels of algal constituents, carbon, nitrogen and phosphorus, in Phase 2.](image)

As Figure 31 shows, the relationship between the increases in amounts of TOC, P, and N during Phase 2 of the Growth Trial was linear, as shown by the trend lines.

4.8 Heavy metals

Heavy metal tests were performed on three occasions during Phase 2 of the Growth Trial as described in section 3 Methods and Materials. These were performed on the pond and columns containing *Chlorella* v. The results showed very low concentrations of cadmium, mercury and lead in the culture mediums, as shown in Table 11. Cadmium levels were below minimum
detection levels for the given test method at < 0.0002 mg/L on all test occasions, except one. Mercury levels were below minimum detection levels for the given test method at < 0.0001 mg/L on all test occasions. For this reason, cadmium, mercury and lead have not been included in the graphs displayed in Figure 32 to Figure 34, they are instead summarized in Table 11.

In the following sections, the results labelled “Tot” show the total heavy metal content of the un-filtered samples. The results labelled “Sol” shows the total heavy metal content of the sample after having been passed through a Whatman GF/C filter paper. The uptake of heavy metals by the algae was determined by comparing the results from the un-filtered and filtered samples according to equation (4). On the starting day, only the un-filtered test was performed.

Table 11. Summary of heavy metal test results for Cd, Pb and Hg; column and pond.

<table>
<thead>
<tr>
<th>Date:</th>
<th>2010-11-08</th>
<th>2010-11-10</th>
<th>2010-11-12</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Algae Column</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS Total Metals - Cadmium [mgL^{-1}]</td>
<td>&lt;0,0002</td>
<td>&lt;0,0002</td>
<td>&lt;0,0002</td>
</tr>
<tr>
<td>MS Total Metals - Lead [mgL^{-1}]</td>
<td>0,001</td>
<td>0,001</td>
<td>0,002</td>
</tr>
<tr>
<td>MS Total Metals - Mercury [mgL^{-1}]</td>
<td>&lt;0,0001</td>
<td>&lt;0,0001</td>
<td>&lt;0,0001</td>
</tr>
<tr>
<td>MS Sol. Metals - Cadmium [mgL^{-1}]</td>
<td>&lt;0,0002</td>
<td>&lt;0,0002</td>
<td>&lt;0,0002</td>
</tr>
<tr>
<td>MS Sol. Metals - Lead [mgL^{-1}]</td>
<td>&lt;0,001</td>
<td>&lt;0,001</td>
<td>&lt;0,001</td>
</tr>
<tr>
<td>MS Sol. Metals - Mercury [mgL^{-1}]</td>
<td>&lt;0,0001</td>
<td>&lt;0,0001</td>
<td>&lt;0,0001</td>
</tr>
<tr>
<td><strong>Algae Pond</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS Total Metals - Cadmium [mgL^{-1}]</td>
<td>&lt;0,0002</td>
<td>&lt;0,0002</td>
<td>0,0002</td>
</tr>
<tr>
<td>MS Total Metals - Lead [mgL^{-1}]</td>
<td>0,002</td>
<td>0,002</td>
<td>0,002</td>
</tr>
<tr>
<td>MS Total Metals - Mercury [mgL^{-1}]</td>
<td>&lt; 0,0001</td>
<td>&lt; 0,0001</td>
<td>&lt;0,0001</td>
</tr>
<tr>
<td>MS Sol. Metals - Cadmium [mgL^{-1}]</td>
<td>&lt;0,0002</td>
<td>&lt;0,0002</td>
<td>&lt;0,0002</td>
</tr>
<tr>
<td>MS Sol. Metals - Lead [mgL^{-1}]</td>
<td>&lt;0,001</td>
<td>&lt;0,001</td>
<td>&lt;0,001</td>
</tr>
<tr>
<td>MS Sol. Metals - Mercury [mgL^{-1}]</td>
<td>&lt;0,0001</td>
<td>&lt;0,0001</td>
<td>&lt;0,0001</td>
</tr>
</tbody>
</table>

Heavy metal test results for nickel, chromium, zinc and copper in the pond and columns are presented in the sections below.

4.8.1 Heavy metal uptake: Column

The uptake of heavy metals from the growth medium by Chlorella v. growing in the column reactors is shown in Figure 32.

When comparing the un-filtered tests over the course of the week it can be noted that there was an increase in the total heavy metal content. As all components in the column reactor in direct contact with the culture medium were inert, it’s believed that the acidic nature of the exhaust gas caused corrosion within the compressor and hence increased the heavy metal content of the exhaust gas. In particular zinc and copper concentrations increased in the culture medium, but these were also the heavy metals which were most notably taken up by the algae.
The tests performed on the 10th of November 2010 show that after 2 days growth, 93% of the zinc was removed together with the algae when the sample was passed through the filter paper. The same test showed a 73% reduction in copper. The tests on the 12th of November show an 83% reduction in zinc and an 85% reduction in copper when comparing un-filtered and filtered tests. Chromium levels were generally low, but what was there, was removed by the algae to levels below the minimum detection level of the given test method.

Nickel levels in the un-filtered samples over the course of the week remained relatively constant. By the final test day, a 50% reduction in nickel had been achieved when comparing the un-filtered and filtered samples. Cadmium and mercury levels were constantly below detection levels for the given test methods, so no reduction or bioremediation could be observed. Lead was reduced from 0.001 mgL\(^{-1}\) to < 0.001 mgL\(^{-1}\) on the 10th November 2010, whereas on the 12th November the reduction was from 0.002 mgL\(^{-1}\) to <0.001 mgL\(^{-1}\), equivalent to a > 50% reduction.

4.8.2 Heavy metal uptake: Pond

The results from the same tests performed on the pond are displayed in Figure 33 and Figure 34. The zinc concentration in the un-filtered samples over the course of the week increased more in the pond than the column. For this reason the removal of zinc from the growth medium by *Chlorella v.* growing in the pond has been plotted in a separate graph, see Figure 34.
The tests on the 10\textsuperscript{th} of November, after 2 days growth, show a 62\% reduction in copper when comparing un-filtered and filtered tests. The same tests from the 12\textsuperscript{th} of November show a 77\% reduction in copper and a 47\% reduction in nickel. All traces of chromium were taken up by the algae and reduced to levels below detection for the given test method. The increase in zinc in the un-filtered samples from the pond was, as discussed in the previous section, believed to originate from the exhaust gas. In total, more exhaust gas was fed into the pond than the column during the Growth Trial. Also, the paddle wheel was introduced at the start of Phase 2. As the drive shaft and bearing support for the paddle wheel were made of an unknown grade of stainless steel, one of these components may have corroded and added to the heavy metal levels in the pond.

The results from the 10\textsuperscript{th} of November show a 63\% reduction in zinc, whereas the results from the 12\textsuperscript{th} November show a 78\% reduction in zinc when comparing un-filtered to filtered samples from the same day. Mercury levels were constantly below detection levels for the given test method, so no reduction or bioremediation could be observed. On the 12\textsuperscript{th} of November 2010, cadmium was reduced from 0.0002 mgL\textsuperscript{-1} to <0.0002 mgL\textsuperscript{-1}. Lead was reduced from 0.002 mgL\textsuperscript{-1} to <0.001 mgL\textsuperscript{-1}, equivalent to a > 50\% reduction on both the 10\textsuperscript{th} and 12\textsuperscript{th} of November 2010.
4.9 Flue gas analysis

The flue gas analysis equipment was used to determine the composition of the flue gas at the inlet and outlet of the algae column culture system. Sampling was performed on the 11th of November 2010 between 10:00 AM and 2:30 PM. The composition of the flue gas prior to injection was calculated from three separate measurements taken over the course of 15 minutes, see Appendix E: Gas Exhaust Analyser Data, and the average of these readings is shown in Table 12 below.

### Table 12. Flue gas compositions

<table>
<thead>
<tr>
<th>Flue gas constituent</th>
<th>CO₂ [%]</th>
<th>O₂ [%]</th>
<th>SO₂ [ppm]</th>
<th>NOₓ [ppm]</th>
<th>CO [ppm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exhaust measured at stack sampling point</td>
<td>6.5</td>
<td>10</td>
<td>14</td>
<td>300</td>
<td>480</td>
</tr>
<tr>
<td>Exhaust measured at inlet to column (average)</td>
<td>3.5</td>
<td>14.5</td>
<td>5</td>
<td>90</td>
<td>169</td>
</tr>
<tr>
<td>Ambient air at site</td>
<td>0</td>
<td>21.1</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

As can be seen in Table 12, the composition of flue gas being injected into the column and pond reactors is not the same as the composition of the flue gas measured at the stack sampling point. The gas is passed through a number of components prior to injection into the columns (see Figure 16). The condensate trap, textile filter and pressure tank of the compressor could all play a part in changing the flue gas composition. The increased level of O₂ indicates that the most likely explanation is that there was a leak somewhere in the system and the flue gas was being partially diluted with air.

The composition of the flue gas coming from Engines 1 and 2 at the AGL Biogas Power Plant was also stated to vary significantly depending on the quality and quantity of the biogas produced in the anaerobic digesters (Mayhew, 2010). This can be seen when comparing the analysis conducted at the stack sampling point, with the levels reported in Stack emission sampling & analysis (MGT Environmental, 2010).

The flow of gas through the system when the solenoid valves were open was considered constant at a rate of 0.2088 m³ h⁻¹ per column and 0.433 m³ h⁻¹ into the pond (actual cubic metres).

The gas clean-up performance ηᵢ of the culture medium could be calculated as the proportion of CO₂ or NOₓ that remained in the culture divided by the total amount of CO₂ or NOₓ that was introduced through the inlet, see equation (10):

\[
\eta_i = \left( \frac{\int_{t_0}^{t_1} exh_{in}(t) \, dt - exh_{out}(t)}{\int_{t_0}^{t_1} exh_{in}(t) \, dt} \right)
\]  

(10)

Where \( t \) is the elapsed time from the start of gas injection, \( exh_{in}(t) \) was the composition of exhaust gas introduced through the inlet, as shown by the red line in Figure 35 through to Figure 42, \( exh_{out}(t) \) the composition of the exhaust gas measured at the outlet as shown by the blue lines in Figure 35 through to Figure 42. The time \( t_0 \) and \( t_1 \) mark the start and end of the measurement period. The integration was performed numerically using the built in trapezoidal integration function in MATLAB, see Appendix G: MATLAB code numerical integration of gas flows.
The level of SO$_2$ measured at the stack sampling point was 14 ppm. By the time the gas was injected into the column this level was 5 ppm. For all tests performed on Chlorella v. and Algae X, the SO$_2$ level measured at the column outlet was 0 ppm. Therefore SO$_2$ removal, calculated according to equation X, was $\eta_{SO_2} = 100\%$. The removal of CO from the flue gas was observed to follow the CO$_2$ removal quite closely. For this reason, CO$_2$ and NO$_x$ are the flue gas constituents that have been focused upon in the following results.

**4.9.1 Gas clean-up performance for Chlorella vulgaris.**

The first column tested was the second column from the west end growing Chlorella v. *Figure 35* and *Figure 36*, show the results from the first measurements of CO$_2$ and NO$_x$ levels in the exhaust gas at the inlet and outlet of the algae column.

![Flue Gas Analysis: CO$_2$ Chlorella v.](image)

*Figure 35.* CO$_2$ [%] in exhaust gas measured at inlet and outlet.

Gas injection started when the culture pH = 8.92 and was turned off 9 minutes later when the pH had been brought down to pH = 7.55. The red line in *Figure 35* shows the level of CO$_2$ measured at the inlet, and the blue line the level of CO$_2$ measured at the outlet. Results from the NO$_x$ measurement performed at the same time are shown below in *Figure 36.*
Both Figure 35 and Figure 36 show clear signs of reduced levels of CO$_2$ and NO$_x$ in the exhaust gas stream. The gas analysis equipment was turned off shortly after the exhaust gas injection had been turned off. It was then noted that the CO$_2$ and NO$_x$ levels measured at the outlet from this point dropped more slowly than had been anticipated. A second measurement following the same procedure was therefore conducted on the same column. This time, measurements continued to be taken after the injection of flue gas had been turned off, see Figure 37.

Gas injection started when the culture medium had a pH = 9.35 and was turned off when the pH = 7.60, approximately 10 minutes later. Gas measurements continued until CO$_2$ and NO$_x$ levels had reached undetectable levels, approximately 6 minutes later, by which time the pH = 7.70. The NO$_x$ measurements for the same test are shown in Figure 38.
As can be seen in Figure 37 and Figure 38, a significant portion of CO_2 and NO_x continued to leave the culture medium after the injection of exhaust gas had been turned off. The CO_2 reduction in the exhaust gas having passed through the algae column containing *Chlorella v.* was calculated according to equation (10), to \( \eta_{CO_2} = 9\% \). The NO_x reduction for the same column was \( \eta_{NO_x} = 59\% \). These figures confirmed that the sparger system had fulfilled its function, although it was not providing a CO_2 uptake as high as had been wished for.

At the start of Phase 2, one column had been replaced due to a puncture. The opportunity was taken to install a separate fitting with a different type of sparger. The sparger was a micro-porous wooden block fitted by means of a plastic barb directly onto the flexible plastic hose. This column, also growing *Chlorella v.*, was tested in the same manner as previously. This sparger created much smaller bubbles than the bubbles produced in the regular columns. For this column, gas injection started when the culture medium had a pH = 8.64 and was turned off when the pH = 7.51, approximately 6 ½ minutes later. Gas measurements continued until CO_2 and NO_x levels had reached undetectable levels, approximately 5 minutes later, as shown in Figure 39 and Figure 40.

*Figure 38. NO_x removal from exhaust gas by *Chlorella v.*

*Figure 39. CO_2 removal from exhaust gas by *Chlorella v.* using a fine gas sparger.*
A significant portion of CO$_2$ and NO$_x$ continued to leave the culture medium after the injection of exhaust gas had been turned off, although these levels were much lower than those in Figure 37 and Figure 38. The CO$_2$ reduction for this column, calculated according to equation (10), was $\eta_{CO_2} = 44\%$. The NO$_x$ reduction for the same column was $\eta_{NO_x} = 70\%$. This is a significant increase in gas utilization efficiency for such a simple change in design, in comparison to the regular gas spargers.

### 4.9.2 Gas clean-up performance for Algae X

The same method of gas analysis as described above was performed on the column growing *Algae X*. Gas injection started when the culture pH = 9.25 and turned off when the culture pH = 7.46, approximately 8 minutes later. Measurements were continued until CO$_2$ and NO$_x$ levels had reached undetectable levels, which was approximately 6 minutes later, as shown in Figure 41 and Figure 42.

**Flue Gas Analysis: Algae X**

*Figure 41. CO$_2$ removal from exhaust gas by *Algae X*.***
Flue Gas Analysis: Algae X

The CO₂ reduction in the exhaust gas having passed through the algae column containing Algae X was calculated according to equation (10) to \( \eta_{CO_2} = 7\% \). The NOₓ reduction for the same column was \( \eta_{NO_x} = 58\% \).

The simple design for gas injection in this experiment allowed for a reduction in CO₂ up to 44 % and 70 % for NOₓ. Benemann (2008) reports that efficiencies up to 95 % uptake of CO₂ can be achieved by injecting gas through microspargers into 1.5 m deep sections of raceway ponds. This CO₂ utilization is more than two times higher than what was achieved in the column reactors, of similar depth, in this report. A conclusion can be drawn that considerable improvements could be expected with a finer sparger. The sparger used did however serve its purpose for the experiment, as it helped ensure that the algae received sufficient amounts of exhaust gas to grow under their optimal pH conditions. For future reference, it would be worth ensuring that finer spargers are used to lessen CO₂ losses.

Figure 42. NOₓ removal from exhaust gas by Algae X.
4.10 Land area estimates for scale-up

The growth rate of *Chlorella v.* as determined from the Growth Trial at AGL, can be used to give an indication of the required land area for the implementation of such a process on a larger scale.

The growth rate has been translated to biomass productivity $P$ as described in section 4.3 Biomass determination. The biomass productivity can be converted to a volumetric CO$_2$ fixation rate $P_{CO2}$ according to equation (11), which is derived from the typical molecular formula of microalgal biomass as presented in section 2.4 Algae basics (Chisti, 2007), (Khan et al., 2009).

$$P_{CO2} = 1.88 \cdot P$$  \hspace{1cm} (11)

The required cultivation volume $V$ can be calculated according to equation (12):

$$V = \frac{\dot{m}_{CO2}}{P_{CO2}}$$  \hspace{1cm} (12)

Where $\dot{m}_{CO2}$ is the mass flow of CO$_2$ into the cultivation system, assuming that all CO$_2$ is taken up by the algal culture. This was not achieved in this project but is considered possible. Combining equations (11) and (12), gives the required pond area, $A_{pond}$, when taking into account the average pond depth $d_{pond}$.

$$A_{pond} = \frac{\dot{m}_{CO2}}{1.88 \cdot P \cdot d_{pond}}$$  \hspace{1cm} (13)

The total amount of biogas combusted at the AGL Power Plant over the course of 2009 was approximately 19.1 Mm$^3$, equating to roughly 52300 m$^3$/day (Baxter, 2010). The composition of the biogas combusted at AGL varies in accordance with the quality of the raw sewage inflow to the WTP, and hence the exhaust composition varies also. Assuming that the biogas is 75 % pure methane and the remaining 25 % is pure CO$_2$ (Naskeo Environment, 2009), then this would equate to roughly 96.4 tons CO$_2$/day being released from the AGL Biogas Power Plant (U.S. Department of Energy, 2010). Worth noting here is that the biogas produced at the WTP is a on a short carbon cycle and is hence deemed emission free under current legislation.

An algal flue gas sequestration process aiming to sequester all of the CO$_2$ released from the AGL Biogas Power Plant would, according to the assumptions discussed above, need to capture $\dot{m}_{CO2} = 96.4$ tons/day. Assuming furthermore that a continuous system could maintain the maximum biomass productivity achieved in the Growth Trial at AGL of $P = 0.264$ gl$^{-1}$d$^{-1}$ for a pond with a depth of $d_{pond} = 25$ cm, then the required land area, according to equation (13), would be approximately 78 hectares, as is illustrated in Figure 44. This area is directly proportional to the depth of the pond, i. e. a doubled depth would reduce the required land area to half, given that the productivity remained constant. While values for optimum depth varies within the literature from 0.1-0.5m, greater depths are likely to inhibit the biomass productivity, and hence the sequestration rate. Design decisions regarding the trade-off between biomass productivity and pond depth will be affected to a great extent by the prevailing circumstances such as available land areas.

For reference, the required pond area $A_{pond}$ has been plotted as a function of the mass flow of CO$_2$ to be sequestered, $\dot{m}_{CO2}$ in Figure 43.
Figure 43. Required pond area $A_{pond}$ as a function of $\dot{m}_{CO2}$

A portion of the Western Treatment Plant is shown Figure 44, with the predicted 78 ha required for the proposed pond system displayed by the red boxes. The black box shows the location of the AGL Power Plant.

Figure 44. Estimated land area requirements shown in red.
Conclusions that can be drawn from the results of this project include:

- From the literature reviewed, there seems to be a consensus in the scientific community that municipal waste water treatment plants, such as Melbourne Water’s WTP offer among the most favourable conditions for near future large scale cultivation of algae, especially when located in favourable climatic regions such as Australia.
- Gasification has been selected as the most suitable potential end use for the algal biomass.
- *Chlorella v.* has been chosen as a suitable microalgae species to be cultivated in the highly eutrophicated waste water sourced from Melbourne Water’s Pond 2.
- It was observed that a mixed algal colony, denoted *Algae X*, grew much faster in the waste water than the algal cultures individual constituents.
- The partially treated sewage sourced from Melbourne Water’s Pond 2 has proved to be a growth medium rich in nutrients that are readily consumed by the selected algae.
- The exhaust gases from the AGL Biogas Power Plant Engines 1 and 2 have proven to be beneficial to algal growth when introduced to the algal culture medium in response to the culture mediums pH.
- Condensate removed from the exhaust gas was measured to have a pH = 3.0 and is likely sulphuric and nitric acid.
- For the selected column bioreactor design growing *Chlorella v.*, a CO\textsubscript{2} utilization efficiency up to 44 % was achieved.
- The column bioreactor growing *Chlorella v.* was proven to remove 70 % of the NO\textsubscript{x} content from the exhaust gas.
- The column bioreactor growing *Algae X* had a CO\textsubscript{2} utilization efficiency of around 7 % and removed 58 % of the NO\textsubscript{x} content from the exhaust gas.
- All tests showed a 100 % reduction in SO\textsubscript{2} but these levels were low to start with.
- Better growth performance was achieved in the column reactors, compared with the pond. Several possible reasons for this are discussed but generally it was believed that better light access combined with better mixing was achieved in the column reactors.
- The maximum biomass productivity for *Chlorella v.* growing in the column reactor was achieved in Phase 2 of the Growth Trial, at a rate of \( P = 0.43 \, \text{gL}^{-1}\text{d}^{-1} \).
- The maximum biomass productivity for *Chlorella v.* growing in the pond reactor was achieved in Phase 2 of the Growth Trial, at a rate of \( P = 0.26 \, \text{gL}^{-1}\text{d}^{-1} \).
- The maximum rate of nitrogen removal for *Chlorella v.* growing in the column reactor during Phase 2 of the Growth Trial, was a rate of \( P_N = 54.62 \, \text{mgL}^{-1}\text{d}^{-1} \).
- The maximum rate of nitrogen removal for *Chlorella v.* growing in the pond reactor in Phase 2 of the Growth Trial, was a rate of \( P_N = 19.86 \, \text{mgL}^{-1}\text{d}^{-1} \).
- Heavy metal concentrations in the growth medium were generally low. Over the course of 2 days growth, when *Chlorella v.* was filtered out from the growth medium, heavy metal levels were significantly reduced. More specifically in the column reactor:
  - Copper levels were reduced by up to 85 %.
  - Zinc levels were reduced by up to 93 %.
  - Nickel levels were reduced by up to 50% after 4 days of growth.
o All traces of chromium were taken up by the algae and reduced to levels below detection for the given test method.

- In the pond reactor:
  o Copper levels were reduced by up to 77%.
  o Zinc levels were reduced by up to 78%.
  o Nickel levels were reduced by up to 47%.
  o All traces of chromium were taken up by the algae and reduced to levels below detection for the given test method.

- If certain improvements are made to the experimental design in this project, it is believed that a raceway pond system occupying approximately 78 hectares would be sufficient to sequester 100% of the CO₂ emitted from the AGL Biogas Power Plant.

6 FUTURE WORK

A future large scale system can be designed around the growth conditions identified in this project. A larger continuous system would require further regulation and more automated control systems, compared with those used in this project. A key parameter to keep at a stable and optimum level will be the nutrient levels. Nutrients should be provided in excess, and the inlet of nutrient, i.e. wastewater, should be regulated so to keep the algae in constant supply of nitrogen. This could be achieved in a fashion similar to the pH-regulated exhaust gas inlet in this project. Monitoring and regulating an inlet of wastewater could be done through a probe measuring the amounts of ammonia, NH₃ in the culture systems.

A future system would need to have automated systems in place also to cope with the shifting quality and quantity of biogas, and hence exhaust, as this varies significantly depending on weather conditions, weekday, and inflows to the WTP. Future work should therefore investigate how to apply a buffer system where exhaust could be stored so as to be able to sustain algal cultures during periods of no biogas production.

Future work should also investigate ways to improve the CO₂ utilization. To do so, further work on improving the sparger system should be performed. As discussed previously, the experimental system succeeded in supplying the algae with CO₂ upon demand, however it’s believed that less CO₂ would go to waste if a finer sparger with even smaller bubbles were to be used.

Focus should also be laid upon investigating different algal strains under the conditions at the WTP. In particular it may be of interest to study the growth and performance of diverse algal colonies rather than monocultures as these can be more robust and fast growing, as indicated by the limited data on the performance of *Algae X*.

Also how to harvest and further process the biomass should be focused upon, as this has been outside this project’s scope. Of particular interest may be investigating how the algae can be treated so as to remove any uptake of heavy metals before being further processed, so as to remove the heavy metals from the material flow loop. The pyrolysis process mentioned in 1.1 Background may be such a process.

A continuous culture system should be operated over a longer period, such as 1 year, to measure performance over the different seasons. Particularly for the winter months, it would be of interest
to make use of excess heat from the biogas combustion process, both to improve the growth conditions for the algae and to improve the overall efficiency of the system.

It may also be of interest to investigate the algae’s performance regarding uptake of the heavy metals cadmium and mercury, that were of levels too low to detect in this experiment.
REFERENCES


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<th>Figure</th>
<th>Source</th>
</tr>
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<tr>
<td>Figure 1</td>
<td>Larsson &amp; Lindblom, 2010</td>
</tr>
<tr>
<td>Figure 2</td>
<td>Reproduced with permission, from (Mata et al., 2010)</td>
</tr>
<tr>
<td>Figure 3</td>
<td>Google Earth Image, 2010, internet: maps.google.com.au</td>
</tr>
<tr>
<td>Figure 4</td>
<td>With permission from Keren Gabay, Seambiotic homepage. Internet: <a href="http://www.seambiotic.com">www.seambiotic.com</a></td>
</tr>
<tr>
<td>Figure 5</td>
<td>(left) With permission from Dr. Eric Capelle, South Australian Research and Development Institute. Internet: <a href="http://www.sardi.sa.gov.au/__data/assets/image/0019/123490/Photobioreactor.jpg">http://www.sardi.sa.gov.au/__data/assets/image/0019/123490/Photobioreactor.jpg</a> (right) Modified from picture available at Flickr.com 2010, user lowcarboneconomy. Internet: <a href="http://www.flickr.com/photos/lowcarboneconomy/2065631607">http://www.flickr.com/photos/lowcarboneconomy/2065631607</a></td>
</tr>
<tr>
<td>Figure 6</td>
<td>(left) Larsson &amp; Lindblom (2010). (right) Available at Flickr.com 2005, user juventson. Modified from: <a href="http://www.flickr.com/photos/jurvetson/58591531/">http://www.flickr.com/photos/jurvetson/58591531/</a></td>
</tr>
<tr>
<td>Figure 7</td>
<td>(left) Modified from (Richmond &amp; Cheng Wu, 2001) (right) Modified from: <a href="http://www.nerdmodo.com/wp-content/uploads/2009/08/algaeecultivationforenergy_thumb.jpg">http://www.nerdmodo.com/wp-content/uploads/2009/08/algaeecultivationforenergy_thumb.jpg</a></td>
</tr>
<tr>
<td>Figure 9</td>
<td>(left) With permission from (Richmond &amp; Cheng Wu, 2001). (right) With permissions, Karlsruhe Inst. Of Technology. Photo by Florian Lehr.</td>
</tr>
<tr>
<td>Figure 10</td>
<td>Larsson &amp; Lindblom, 2010</td>
</tr>
<tr>
<td>...</td>
<td>ibid</td>
</tr>
<tr>
<td>Figure 32</td>
<td>Larsson &amp; Lindblom, 2010</td>
</tr>
</tbody>
</table>
APPENDIX A: ALGAE INOCULATION
DOCUMENTATION

Documentation was kept of all algae inoculations preceding and throughout the growth trials. Data was collected regarding:

- Container number
- Algae content
- Container type, location
- Water type
- Gas flows
- Added nutrients
- Inoculum volume
- Growth medium volume
- Date of inoculation

The document containing this data was too large to fit into the printed format. For a copy of this documentation, please contact the authors.
APPENDIX B: SAMPLING PLAN DURING PHASE 1

All samples were collected by a sampler from ALS at 09:00 am and delivered to the laboratory in Scoresby for analysis the same day.

<table>
<thead>
<tr>
<th>Date:</th>
<th>Description:</th>
<th>Sample Point:</th>
<th>Sample Group:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tue</td>
<td>Growth medium – waste water</td>
<td>Point 1: Upstream of AGL Biogas Scrubber</td>
<td>Full Suite</td>
</tr>
<tr>
<td>26/10</td>
<td>AGL-Column</td>
<td>Point 2: Algae Column</td>
<td>Chlorophyll</td>
</tr>
<tr>
<td></td>
<td>AGL-Pond</td>
<td>Point 3: Algae Pond</td>
<td>Chlorophyll</td>
</tr>
<tr>
<td>Thu 28/10</td>
<td>AGL-Column</td>
<td>Point 2: Algae Column</td>
<td>Chlorophyll</td>
</tr>
<tr>
<td></td>
<td>AGL-Pond</td>
<td>Point 3: Algae Pond</td>
<td>Chlorophyll</td>
</tr>
<tr>
<td>Fri 29/10</td>
<td>AGL-Column</td>
<td>Point 2: Algae Column</td>
<td>Chlorophyll</td>
</tr>
<tr>
<td></td>
<td>AGL-Pond</td>
<td>Point 3: Algae Pond</td>
<td>Chlorophyll</td>
</tr>
<tr>
<td>Mon 1/11</td>
<td>AGL-Column</td>
<td>Point 2: Algae Column</td>
<td>Chlorophyll</td>
</tr>
<tr>
<td></td>
<td>AGL-Pond</td>
<td>Point 3: Algae Pond</td>
<td>Chlorophyll</td>
</tr>
<tr>
<td>Wed 3/11</td>
<td>AGL-Column</td>
<td>Point 2: Algae Column</td>
<td>Chlorophyll</td>
</tr>
<tr>
<td></td>
<td>AGL-Pond</td>
<td>Point 3: Algae Pond</td>
<td>Chlorophyll</td>
</tr>
</tbody>
</table>
## APPENDIX C: FLUE GAS ANALYSER TECHNICAL DATA

<table>
<thead>
<tr>
<th>Storage temperature</th>
<th>-4 to +122 °F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Operating temperature</td>
<td>+23 to +113 °F</td>
</tr>
<tr>
<td>Dimensions</td>
<td>15.55 x 10.83 x 3.74 in</td>
</tr>
<tr>
<td>Material/Housing</td>
<td>ABS</td>
</tr>
</tbody>
</table>

### Probe type Type K

<table>
<thead>
<tr>
<th>Meas. Range</th>
<th>-40 to +2192 °F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy</td>
<td>±0.5% of rdg. (+212 to +2192 °F) ±0.9 °F (-40 to +211.82 °F)</td>
</tr>
<tr>
<td>Resolution</td>
<td>0.1 °F (-40 to +2192 °F)</td>
</tr>
</tbody>
</table>

#### Probe type Exhaust gas loss measurement

<table>
<thead>
<tr>
<th>Meas. Range</th>
<th>-20 to +99.9 % qA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resolution</td>
<td>0.1 % qA (-20 to +99.9 % qA)</td>
</tr>
</tbody>
</table>

#### Probe type Electrochemical measurement CO

<table>
<thead>
<tr>
<th>Meas. Range</th>
<th>0 to +10000 ppm CO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy</td>
<td>±5% of rdg. (+200 to +2000 ppm CO) ±10% of rdg. (+2001 to +10000 ppm CO) ±10 ppm CO (0 to +199 ppm CO)</td>
</tr>
<tr>
<td>Resolution</td>
<td>1 ppm CO (0 to +10000 ppm CO)</td>
</tr>
</tbody>
</table>

#### Probe type Electrochemical measurement CO₂

<table>
<thead>
<tr>
<th>Meas. Range</th>
<th>0 to +50 Vol. % CO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy</td>
<td>±0.3 Vol. % CO₂ + 1% of rdg. (0 to 25 Vol. % CO₂) ±0.5 Vol. % CO₂ + 1.5% of rdg. (&gt;25 to 50 Vol. % CO₂)</td>
</tr>
<tr>
<td>Resolution</td>
<td>0.01 Vol. % CO₂ (0 to 25 Vol. % CO₂) 0.1 Vol. % CO₂ (&gt;25 Vol. % CO₂)</td>
</tr>
</tbody>
</table>

#### Probe type Electrochemical measurement H₂S

<table>
<thead>
<tr>
<th>Meas. Range</th>
<th>0 to +300 ppm H₂S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy</td>
<td>±5% of rdg. (+40 to +300 ppm) ±2 ppm (0 to +39.9 ppm)</td>
</tr>
<tr>
<td>Resolution</td>
<td>0.1 ppm (0 to +300 ppm)</td>
</tr>
</tbody>
</table>

#### Probe type Efficiency Measurement (Eta)

<table>
<thead>
<tr>
<th>Meas. Range</th>
<th>0 to +120 %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resolution</td>
<td>0.1 % (0 to +120 %)</td>
</tr>
</tbody>
</table>

#### Probe type Pressure probe

<table>
<thead>
<tr>
<th>Meas. Range</th>
<th>-80.29 to +80.29 inH₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy (absolute)</td>
<td>±0.5 inH₂O (-20.03 to +20.03 inH₂O) ±0 inH₂O (-20.07 to -80.29 inH₂O)</td>
</tr>
<tr>
<td>Accuracy (relative)</td>
<td>0.1 inH₂O (-80.29 to +80.29 inH₂O)</td>
</tr>
</tbody>
</table>

#### Probe type Pressure Probe

<table>
<thead>
<tr>
<th>Meas. Range</th>
<th>-16.06 to +16.06 inH₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy (absolute)</td>
<td>±1.5% of rdg. (-16.06 to -1.2 inH₂O) ±1.5% of rdg. (+1.2 to +16.06 inH₂O) ±0.03 inH₂O (-1.2 to +1.2 inH₂O)</td>
</tr>
</tbody>
</table>
APPENDIX D: COD, BOC, SS & TOC FACTORS AT STOCKHOLM WATER

Calculations made on 24h samples data analysed in 2003.

<table>
<thead>
<tr>
<th>2003-12-12/ABH</th>
<th>COD</th>
<th>BOD</th>
<th>Susp</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Henriksdal</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hin</td>
<td>TOC*(3,82 ± 1,0)</td>
<td>TOC*(1,97 ± 0,6)</td>
<td>TOC*(2,21 ± 1,2)</td>
</tr>
<tr>
<td>Sin</td>
<td>TOC*(3,73 ± 0,8)</td>
<td>TOC*(1,65 ± 0,4)</td>
<td>TOC*(2,39 ± 1,0)</td>
</tr>
<tr>
<td>Hfv</td>
<td>TOC*(3,33 ± 1,4)</td>
<td>TOC*(1,65 ± 0,4)</td>
<td>TOC*(1,86 ± 0,6)</td>
</tr>
<tr>
<td>Hut</td>
<td>TOC*(3,52 ± 0,8)</td>
<td>TOC*(0,22 ± 0,3)</td>
<td>TOC*(0,19 ± 0,3)</td>
</tr>
<tr>
<td><strong>Bromma</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bfv</td>
<td>TOC*(3,60 ± 0,8)</td>
<td>TOC*(1,70 ± 0,4)</td>
<td>TOC*(1,98 ± 0,6)</td>
</tr>
<tr>
<td>But</td>
<td>TOC*(3,29 ± 1,2)</td>
<td>TOC*(0,24 ± 0,2)</td>
<td>TOC*(0,13 ± 0,1)</td>
</tr>
<tr>
<td><strong>Hammarby Sjöstad</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In</td>
<td>TOC*(3,39 ± 0,4)</td>
<td>TOC*(1,67 ± 0,8)</td>
<td>TOC*(1,33 ± 0,8)</td>
</tr>
<tr>
<td>Ut</td>
<td>TOC*(3,54 ± 1,0)</td>
<td>TOC*(0,23 ± 0,2)</td>
<td>TOC*(0,18 ± 0,2)</td>
</tr>
<tr>
<td><strong>Optiroc</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TOC*(3,48 ± 1,8)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Divide by</th>
<th>COD to TOC</th>
<th>BOD to TOC</th>
<th>Suspended solids to TOC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3,522</td>
<td>1.16625</td>
<td>1.28375</td>
</tr>
</tbody>
</table>
APPENDIX E: GAS EXHAUST ANALYSIS DATA

Data was collected and digitally compiled into a spreadsheet, covering measurements of:

- % CO
- % CO2
- ppm SO2
- ppm CO
- ppm NO2
- ppm NOx
- ppm NO
- ppm H2
- gas temperature

The document containing this data was too large to fit into the printed format. For a copy of this documentation, please contact the authors.
Data was collected and digitally compiled into a spreadsheet, covering measurements of:

- Date and time
- Lux values
- Gas volumetric flows
- Culture pH
- Culture temperatures

The document containing this data was too large to fit into the printed format. For a copy of this documentation, please contact the authors.
APPENDIX G: MATLAB CODE NUMERICAL INTEGRATION OF GAS FLOWS

%--- Flue gas analysis: Thursday 11th November 2010-----------------------
clf; clc; clear all; close all;

%--- Readings from measurements -----------------------------------------
%--- Analysis: Column 2 chlorella PM ca 14:30-----------------------------

time_1 = [0, 26, 42, 64, 86, 109, 130, 192, 234, 289, 351, 393, 428, ...
          482, 561, 628, 660, 689, 747, 777, 833, 893, 990];
  % Time of gas analysis [s]
exh_CO2_in_1 = [3.47, 3.47, 3.47, 3.47, 3.47, 3.47, 3.47, ...
                3.47, 3.47, 3.47, 3.47, 3.47, 3.47, 3.47, ...
                3.47, 3.47, 3.47, 3.47, 3.47, 0, 0, 0, 0, 0, 0, 0];
  % CO2 in exhaust measured at inlet (manifold) [%]
exh_CO2_out_1 = [0, 0, 0, 1.16, 1.60, 2.01, 2.25, 2.75, 2.93, 3.12, ...
                 3.25, 3.32, 3.36, 3.40, 3.44, 1.59, 1.07, 1.00, 0.86, ...
                 0.75, 0.64, 0.37, 0.05];
  % CO2 measured outlet [%]
%

% % -- Analysis: Column 2 Chlorella AM ------------------------------------
% time_2 = [0, 25, 43, 63, 134, 193, 221, 278, 310, 374, 442, 489, ...
%          517, 537];
%  % trial time [s]
% exh_CO2_in_2 = [3.47, 3.47, 3.47, 3.47, 3.47, 3.47, 3.47, ...
%                3.47, 3.47, 3.47, 3.47, 3.47, 3.47, 3.47];
%  % CO2 in exhaust measured at inlet (manifold) [%]
% exh_CO2_out_2 = [0, 0, 0, 0.78, 1.42, 1.61, 1.68, 1.68, 1.76, ...
%                 1.76, 1.81, 2.02, 1.89, 1.85, 2.17];
%  % CO2 measured outlet [%]
% exh_NOx_in_2 = [90, 90, 90, 90, 90, 90, 90, 90, 90, 90, 90, 90, ...
%                90, 90, 90, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0];
%  % NOx content measured exhaust in [ppm]
% exh_NOx_out_2 = [1, 1, 2, 4, 6, 7, 10, 27, 30, 32, 42, 46, 46, 47, ...
%                  55, 21, 13, 11, 8, 7, 5, 2, 0];
%  % NOx content measured exhaust out [ppm]
%
%
% % -- Analysis: Column 6 Chlorella with wooden sparger---------------------
% time_6 = [0, 152, 177, 184, 214, 249, 271, 283, 312, 327, 338, 346, ...
%          368, 381, 407, 448, 487, 563, 633, 670, 781, 846, 924, 1247, ...
%          1396, 1468, 2012, 2245];
%  % trial time [s]
% exh_CO2_in_6 = [3.47, 3.47, 3.47, 3.47, 3.47, 3.47, 3.47, ...
%                3.47, 3.47, 3.47, 3.47, 3.47, 3.47, 3.47];
%  % CO2 in exhaust measured at inlet (manifold) [%]
% exh_CO2_out_6 = [0, 0, 0, 0.67, 0.76, 1.13, 1.19, 1.22, 1.53, 2.17, ...
%                 2.46, 2.47, 2.55, 2.66, 2.65, 1.79, 1.76, 0.99, ...
%                 0.87, 0.78, 0, 0, 0, 0, 0, 0, 0, 0];
%  % CO2 measured outlet [%]
% exh_NOx_in_6 = [90, 90, 90, 90, 90, 90, 90, 90, 90, 90, 90, 90, ...
%                90, 90, 90, 90, 90, 90, 90, 90, 90, 90, 90, 90, 90, ...
% NOx content measured exhaust in [ppm] measured from avg % of 3 latest manifold readings, 15min prior to test
% exh_NOx_out_6 = [2, 2, 9, 9, 10, 13, 15, 19, 21, 20, 20, 19, 19, ...
% 27, 17, 18, 11, 10, 9, 6, 5, 4, 1, 1, 1, 0, 0];
% NOx content measured exhaust out [ppm]

% -- Analysis: Column X -----------------------------------------------
% time_x = [0, 60, 67, 74, 81, 112, 126, 156, 182, 200, 225, 249, ...
% 277, 294, 315, 346, 389, 472, 494, 513, 584, 616, 677, 746, ...
% 827, 848, 874, 961, 1002];
% % trial time [s] 29
% exh_CO2_in_x = [3.82, 3.82, 3.82, 3.82, 3.82, 3.82, 3.82, 3.82, ...
% 3.82, 3.82, 3.82, 3.82, 3.82, 3.82, 3.82, 3.82, ...
% 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0];
% % CO2 in exhaust measured at inlet (manifold) [%]
% exh_CO2_out_x = [0, 0, 0, 0, 1.149, 1.82, 2.28, 2.64, 2.77, ...
% 2.91, 3.03, 3.11, 3.82, 3.28, 3.24, 3.25, 3.28, ...
% 2.13, 1.94, 1.42, 1.28, 1.01, 0.81, 0.92, 0.59, 0, 0, ...
% 0];
% % CO2 measured outlet [%]
% exh_NOx_in_x = [90, 90, 90, 90, 90, 90, 90, 90, 90, 90, 90, 90, ...
% 90, 90, 90, 90, 90, 90, 90, 90, 90, 90, 90, 90, 90, 90, 90, 90, ...
% 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0];
% % NOx content measured exhaust in [ppm]
% exh_NOx_out_x = [0, 0, 2, 2, 7, 8, 10, 15, 22, 31, 36, 37, ...
% 37, 37, 38, 44, 29, 26, 23, 15, 14, 10, 8, 4, 5, 4, 3, 2];
% % NOx content measured exhaust out [ppm]

% --- Calculate integrals ---------------------------------------------
tot_CO2_in_1 = trapz(time_1, exh_CO2_in_1);
tot_CO2_out_1 = trapz(time_1, exh_CO2_out_1);
tot_CO2_absorbed = tot_CO2_in_1 - tot_CO2_out_1;
perc_CO2_absorbed = (tot_CO2_absorbed / tot_CO2_in_1)*100

tot_NOx_in_1 = trapz(time_1, exh_NOx_in_1);
tot_NOx_out_1 = trapz(time_1, exh_NOx_out_1);
tot_NOx_absorbed = tot_NOx_in_1 - tot_NOx_out_1;
perc_NOx_absorbed = (tot_NOx_absorbed / tot_NOx_in_1)*100

%--- Plots -------------------------------------------------------------
figure(1)
title('Flue Gas Analysis: Chlorella Column 2')
xlabel('Time [s]'); ylabel('CO2 [%] in exhaust')
plot(time_1, exh_CO2_in_1, 'b', time_1, exh_CO2_out_1, 'r')
legend('Exhaust Inlet', 'Exhaust outlet'); axis([0 990 -0.25 3.7]); grid

figure(2)
title('Flue Gas Analysis: Chlorella Column 2'); xlabel('Time [s]');
ylabel('NOx [ppm] in exhaust')
plot(time_1, exh_NOx_in_1, 'b', time_1, exh_NOx_out_1, 'r')
legend('Exhaust Inlet', 'Exhaust outlet'); axis([0 990 -5 100]); grid