UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

The development of an approach for the production and use of algae to treat urban wastewater

by

Robson Masaraure

Submitted in partial fulfillment of the requirements for the degree

MSc Water Resources Management

in the

Faculty of Natural and Agricultural Sciences

University of Pretoria

28 April 2016

DECLARATION

I, Robson Masaraure, declare that the thesis, which I hereby submit for the degree MSc Water Resources Management at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

_____ _____

SIGNATURE DATE

TABLE OF CONTENTS

Page

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

I would like to thank my beautiful wife, Fadzai Rufaro Lorraine Kwambana and my beautiful daughter Kayla Maita Masaraure. If it were not for their love, support, patience and encouragement, I would not be where I am today.

In addition, I would want to thank my supervisor, Dr. Marius Claassen, for his guidance and support throughout the course of this research. Thank you for this opportunity, it taught me a lot about microalgae. Thank you for having confidence in me, you have been a great mentor.

I would wish to thank the Council for Scientific and Industrial Research (CSIR) for allowing the main research work to be conducted at their premises. I would also want to offer my appreciation to Ms Leanie de Klerk from CSIR for her inputs, my friends, colleagues, the Faculty of Natural and Agricultural Sciences and staff for making my time at the University of Pretoria a wonderful experience.

Finally, I would like to thank God the almighty for giving me the strength and guidance during the course of this research.

ABSTRACT

The presence of huge quantities of nutrients in water bodies heavily promotes the growth of algae. Excess nitrogen and phosphorus in discharged wastewaters can lead to downstream eutrophication thereby damaging the ecosystem. Algae bioremediation involves the use of live algae in the removal of excess dissolved nutrients from wastewater and subsequently diminish the pollution load. It is an alternative economical and sustainable way of treating sewage wastewater compared to conventional treatment processes. With dwindling water resources, it is imperative to find ways to minimise pollution of many streams. The main objectives of this investigation were to determine whether there is a difference in the optimum conditions in pools and raceways that are required to cultivate *Chlorella vulgaris* and *Chlorella prototheco*ides, and for using microalgae to reduce nutrient loads in urban wastewater treatment works. Several physicochemical variables that includes pH, dissolved oxygen (DO), temperature, conductivity, total dissolved solids (TDS), chlorophyll *a* and ammonium (NH_4^+) were studied. These variables were measured to ascertain how they affect the production of these two selected *Chlorella* spp. *Chlorella vulgaris* was cultured in round pool 1 and *C. prototheco*ides in round pool 2. These two *Chlorella* spp. were then mixed together and cultured in the raceway pond. *Chlorella vulgaris* showed that they grow faster than *C. prototheco*ides. This was indicated by the high concentration levels of chlorophyll *a* averaging 179.03 µg/ℓ in round pool 1. Round pool 2 showed different results as compared to round pool 1 with low levels of chlorophyll *a* averaging 41.32 µg/ℓ and high levels of ammonium averaging 104.91 mg/ℓ. The algae cultures were introduced to stabilization ponds at Motetema wastewater treatment works with a depth of 2 m and a capacity of 20 Mℓ but without mechanical aeration, and the effluent quality was monitored. The concentration of nitrates decreased from 0.88 mg/ℓ to 0.81 mg/ℓ and chlorophyll *a* showed a 25.94 % decrease from 45.38 mg/ℓ to 33.61 mg/ℓ after the introduction of microalgae. The study showed that these two *Chlorella* spp. responded differently to the prevailing ranges of physicochemical variables during culturing, and they have the potential to be utilised for wastewater treatment on a large scale.

KEYWORDS: microalgae, raceway pond, *C. vulgaris*, pH, temperature, *C. prototheco*ides, dissolved oxygen, conductivity, round pools, nitrates

CHAPTER 1

1.1 Introduction

Rapid population growth that subsequently leads to vast increase in the number of industries and urban settlements often leads to environmental pollution. Anthropogenic activities particularly agriculture, industrialisation and urbanisation, have led to the increase in the quantity of nitrogen and phosphorus discharged to inland water systems. This is due to the amount of untreated wastewater discharged into water bodies. Untreated or partially treated wastewater finds its way to water bodies resulting in the enrichment of nutrients or eutrophication (Mahapatra et al., 2013). Due to continuous depletion of natural resources, it is imperative that pollution level is reduced. An increase in the amount of nitrogen and phosphorus entering water bodies have severe effects on the whole ecosystem. In order to prevent these problems we should handle nitrogen and phosphates as nutrients resource rather than pollutants that only has to be disposed of (Choi and Lee, 2013). Both nitrogen and phosphorus in wastewater should be treated properly before being discharged to minimise their contaminant effect in aquatic ecosystems (An et al., 2003). One of the possible ways of wastewater treatment is the use of microalgae species such as *C. protothecoides* (formerly known as *Chlorella pyrenoidosa*) (Zeng et al., 2009) and *C. vulgaris.*

1.2 Research problem

Many studies have been carried out to investigate the wastewater phycoremediation using microalgae to remove different wastewater pollutants. Sustainability and environmental concerns are major issues in the world today (Singh et al., 2013). The idea of employing microalgae for the removal of high concentrations of nutrients in wastewater has been developed for years (Oswald et al., 1957; De la Noue et al., 1992). Phycoremediation is an effective and low technology process with huge cost savings. It provides a more appropriate method of water treatment for developing countries (Pittman et al., 2011).

The realisation that the quality of water resources are depleting and the costly nature of chemicals to treat the wastewater has brought about the necessity to find economical ways of treating wastewater. The specific advantage of using microalgae is that they contribute to

carbon sequestration during their production. They also provide food and oxygen for many species in the aquatic environment (Anesio et al., 2009) and they are involved in symbiosis with bacteria in various ecosystems (Gast et al., 2009). Recent studies have demonstrated that microalgae can be utilised for the removal of nitrogen and phosphorus from wastewater (An et al., 2003; Órpez et al., 2009). Globally *Chlorella* spp. are widely used microalgae for nutrient removal (Wang et al., 2013). According to Lee and Lee (2001), most of the studies have been focusing on the economic viability of implementing microalgae for wastewater treatment.

This topic has gained momentum due to its cost effectiveness and environmental benefits as mentioned by Vacca et al. (2005). Mallick (2002) and, Ahluwalia and Goyal (2007) also support the fact that microalgae are viable and effective in the removal of phosphorus and nitrogen from wastewater hence very essential for bioremediation. The aim of this study was to establish an approach (see Appendix A) on how best to culture algae in the round pools and raceway pond, and their efficiency in removing nutrients in urban wastewater.

1.3 Research aim and objectives

The principal aim of this project was to determine the conditions of culturing algae (*C. vulgaris* and *C. protothecoides)* in the round pools and raceway pond in order to facilitate the effective and efficient removal of nutrients in urban wastewater. The project objectives were:

- To determine whether there is a difference in the optimum conditions in pools and raceways that are required to cultivate *Chlorella vulgaris* and *Chlorella protothecoides*.
- To monitor the effluent quality from wastewater stabilization ponds. This is done after introducing microalgae cultures to stabilization ponds.

CHAPTER 2

2.1 Overview

The emphasis of implementing the most economical and natural methods of removing nutrients in wastewater has been considered in many research works. According to Yaakob et al. (2014) and Singh et al. (2013), this is mainly due to rapid industrialization resulting in many industries disposing of wastewater into water bodies without proper treatment. Nijboer and Verdonschot (2004) identified ammonium (NH_4^+), nitrates (NO_3^-) and phosphates (PO_4^{3-}) as major sources of eutrophication. These nutrients may occur artificially and naturally. Artificial occurrence of these nutrients may results from industrial discharges, domestic effluent and anthropogenic activities such as fertilizer application whilst natural occurrence might be due to the decomposition of cellular material or gaseous exchange from the atmosphere. Bhatt et al. (2014) noted that nitrogen and phosphorus are the two important nutrient compounds to analyse a water source for potential algae growth. The principal forms in which they arise in wastewater are ammonia (NH_3), nitrite (NO_2^-), nitrate, and orthophosphate. Correll (1998) reiterates that if no proper treatment is applied to excess nutrients, eutrophication will take place hence affecting the level of dissolved oxygen (DO) and subsequently aquatic life. On the other hand, microbial water pollution has become a major concern for environmental safety and public health (Bouali et al., 2012).

2.1.1 Algae culturing in open raceway ponds

Algae are photosynthetic, pigment-producing, protein-rich microorganisms that play a vital role in wastewater treatment through their ability to generate their own carbon source and oxygen (Job et al., 2014). Microalgae are prokaryotic or eukaryotic photosynthetic microorganisms, some of which can also form a chain or colony ranging from a few micrometres to a few hundred micrometres and are generally ubiquitous in nature. According to Barsanti and Gualtieri (2006), algae are a broad category that has no proper taxonomic classification. Lee (1980) added that they are thallophytes, meaning they lack roots, stems and leaves. They do not have a sterile covering of cells around the reproductive cells and have chlorophyll *a* as their primary photosynthetic pigment. Sheehan et al. (1998) classified algae into two types based on their sizes, microalgae and macroalgae. Microalgae being the

microscopic photosynthetic organisms that are found in both marine environment and freshwater environment.

High rate algal (raceway) ponds are open shallow ponds with paddlewheel to circulate algae and the nutrients (Brennan and Owende, 2010) as shown in Fig. 1. The water level is usually kept at not less than 15 cm. Pedroni et al. (2001) indicated that the typical size of open pond for microalgae production systems ranges from 0.2 to 0.4 hectares, however, there is evidence of very large-scale open pond systems. Achara (2012) added that the ponds are kept shallow because algae need to be exposed to sunlight, and sunlight can only penetrate the pond water to a limited depth. Raceway ponds are the most often used for large-scale production systems for wastewater treatment and biofuel production, and are relatively cheap to construct and operate.

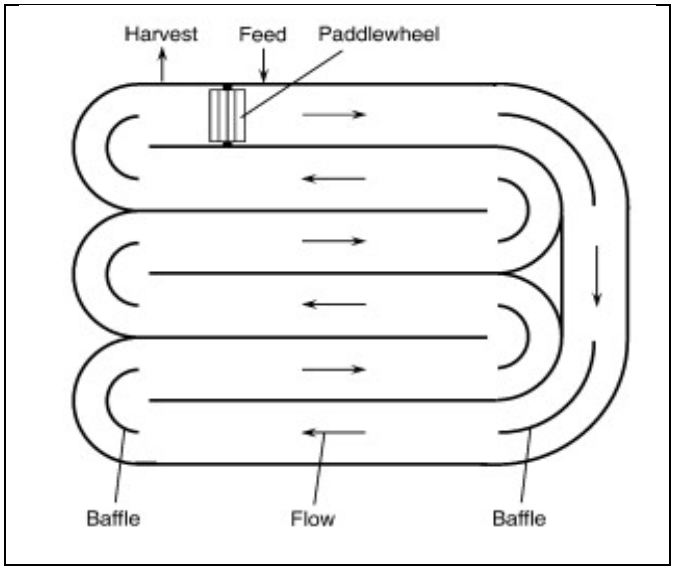


Figure 1: Schematic layout of an open raceway pond (Chisti, 2007)

There are basic principles of operation in algal wastewater treatment ponds as illustrated in Fig.2. Algae produce oxygen from water as a by-product of photosynthesis. Bacteria will then use this oxygen (O_2) as they aerobically bio-oxidise the organic compounds in wastewater to produce carbon dioxide (CO_2) which is the end-product of this bio-oxidation process (Sharma and Khan, 2013). Achara (2012) indicated that in algae culture, different algae have different requirements but the basic reaction during photosynthesis of the single celled phytoplankton in

water has been given as: CO_2 + light energy + H_2O = glucose + O_2 + H_2O. Algae can also act as a sink for carbon dioxide because they absorb it for photosynthesis (Singh et al., 2013). Based on a theoretical ratio, algae are able to fix approximately 1.8 kg of carbon dioxide fixed for every 1 kg algal biomass produced (Chisti, 2007).

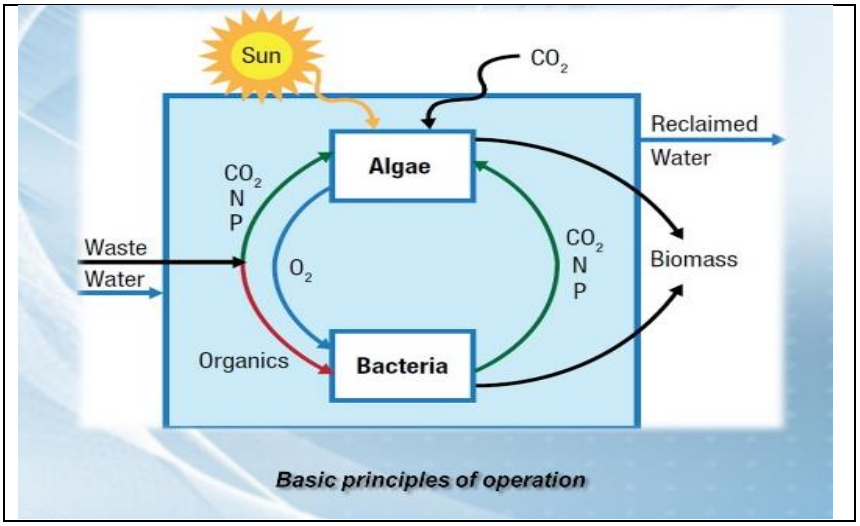


Figure 2: Basic principles of operation in the open raceway ponds (Paliwal, 2012)

According to Chisti (2007) and Shen et al. (2009), raceway ponds have advantages and disadvantages for algae production as indicated in Table 1. They have culture density of 0.25 – 1 g.ℓ^{-1}, low gas exchange and high scalability. Carvalho et al. (2006) and Chisti (2007) added that raceway ponds are mainly used to culture suspended algae but there are advantages and disadvantages of using this approach.

Table 1: Advantages and Disadvantages of raceway ponds (Chisti, 2007; Shen et al., 2009)

Cultivation System	**Advantages**	**Disadvantages**
Open raceway pond	• Inexpensive to build and operate • Easy and cheap to maintain • Low energy inputs	• Biomass productivity is minimal • Inefficient mixing and poor light penetration • Needs large areas for considerable biomass yield • High risk of pollution and contamination • Limitation of species • Poor dispersion of carbon dioxide to the environment

2.1.2 Physicochemical parameters essential for microalgae growth

2.1.2.1 Overview

Microalgae growth is affected by certain physicochemical factors that include pH, right nutrients, light and temperature range of the medium (Farhadian et al., 2008; Achara, 2012). This was supported by Blair et al. (2014), who emphasized the importance of light (energy), carbon source autotrophic metabolism, growth medium (water) and nutrients (N and P) for reproduction of microalgae. Sudhakar and Premalatha (2012) also added that algae require only a few basic resources to grow successfully, these includes carbon dioxide, water, sunlight and nutrients. The ideal range of values for these factors is indicated in Table 2.

Table 2: A generalised set of conditions for microalgae culturing (Lavens and Sorgeloos, 1996)

Parameters	**Range**	**Optima**
Temperature (°C)	16 – 27	18 - 24
Light intensity (lux)	1 000 - 10 000 (depends on volume and density)	2 500 - 5 000
pH (units)	7 – 9	8.2 - 8.7

2.1.2.2 Nutrients

Nutrients and physical factors are essential for culturing of algae in wastewater. Alsull and Omar (2012) indicated the importance of salinity and nutritional factors that have an influence on algae growth, physiological activities and biochemical composition. Nitrogen is supplied in the nitrate and ammonium form but ammonia nitrogen is preferred for algal growth (Kaplan et al., 1986; Berman and Chava, 1999). Phosphorus is used by algae in orthophosphate form and should be supplied to significant excess as phosphates because not all phosphorus compounds are bioavailable (Kumar et al., 2010).

Hoffmann (1998), Shen et al. (2009) and Lundquist et al. (2010) claims that raceway ponds have phosphorus loading of 1.2 - 7.5 mg.ℓ^{-1} day^{-1} and 96 % phosphorus removal. There is still more research on the ideal levels of these nutrients. Brzezinski (1985) found that in most cultures the macronutrients required are nitrogen (N) and phosphorus (P) in the ratio of

16N : 1P, however, in most cultures nutrients are often added in excess in order to maximize nutrient limitation. Ramaraj et al. (2010) reported that 1 g algae production needs 0.035 g of nitrogen and 0.004 g of phosphorus. Chaumont (1993) indicated that algal growth rates of 10 to 50 g m^2/day (grams of algal mass per square metre per day) have been reported.

2.1.2.3 Temperature requirements

Konopka and Brock (1978) emphasized the role of temperature in algal growth. Temperature variations affect biochemical reactions and subsequently biochemical composition of algae. Favourable high temperatures stimulate algal growth but once optimal temperature is reached, any further increase will deter algal growth due to stress. Muñoz and Guieysse (2006) added that temperature regulate cellular, morphological and physiological responses of microalgae: higher temperatures generally accelerate the metabolic rates of microalgae, whereas low temperatures lead to inhibition of growth. According to Ono and Cuello (2003), the optimal temperatures varies among microalgal species and optimal growth temperatures of 15 – 26 ºC have been reported for some species.

Cho et al. (1994) indicated that generally temperature up to 15 °C is the most favoured for algal cultivation. However, microalgae species react differently to a range of temperatures some thrive effectively at high temperatures whilst others prefer low temperatures. According to Li (1980), microalgae species are capable of photosynthesising over a wide range of temperatures generally stated between 15 and 30 ºC but with optimal conditions between 20 and 25 ºC. A bell shaped growth curve is generally observed for describing the relationship between temperature and microalgae growth rate (Fig. 3). However according to Suzuki and Takahashi (1995), it appears that the shapes widely differs between species. The evidence from the study of growth rates versus optimal temperature for 17 different *Chlorella* strains showed that *Chlorella* spp. grew effectively between 26 ºC (with *C. vulgaris, C. protothecoides)* and 36 ºC (with *Chlorella fusca, Chlorella kessleri)* (Kessler, 1985).

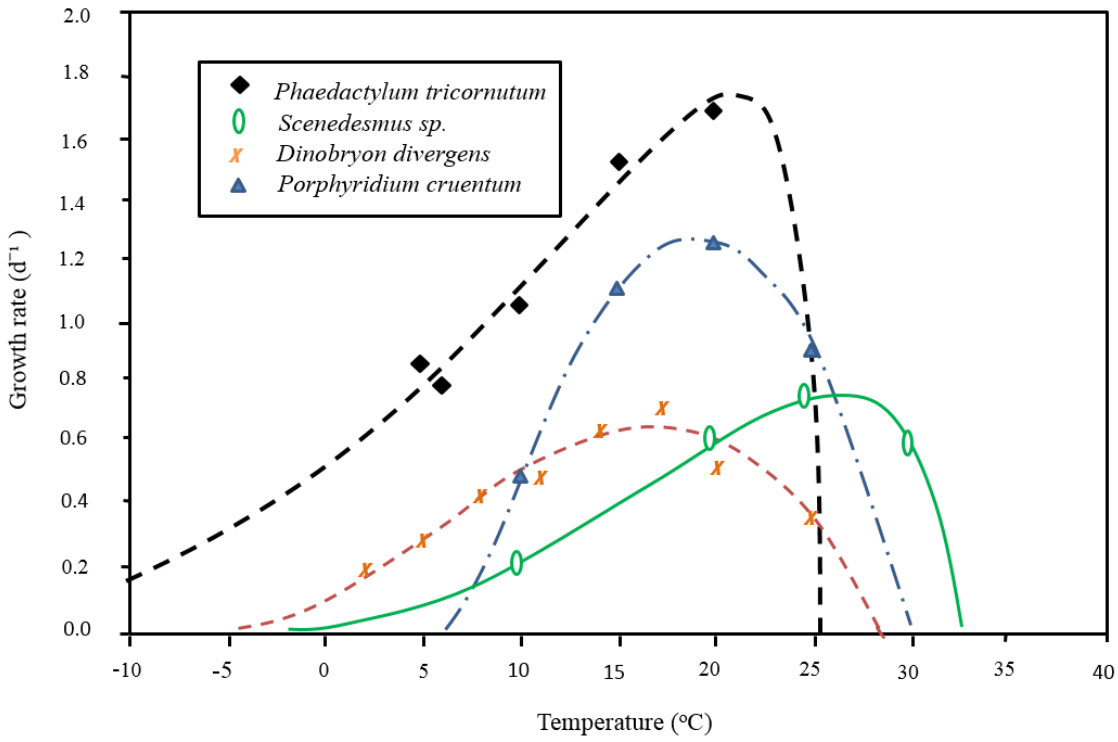


Figure 3: The effect of temperature on microalgae growth rate (Redrawn from Butterwick et al., 2005; Kudo et al., 2000)

2.1.2.4 Light requirements

Light is also essential for microalgae growth. Depriving microalgae of optimum light will hinder their growth. Turbulence can be created in the culture in order to facilitate enough supply of light especially in wastewater due to high particulate content. This is done to enable cells getting exposed to light for energy build-up to enhance productivity. Light deprivation can also be limited by designing raceway ponds with shallow depth in order to facilitate light penetration to the bottom of the ponds. De la Noue et al. (1992) claims that depth of 15 to 50 cm is generally recommended. On the other hand Muñoz and Guieysse (2006) and Li et al. (2008) state that the depth of water in the open raceway pond should not be more than 30 cm.

Friedman et al. (1991) however indicated that microalgae exposure to light must be monitored since high intensities of light tend to increase polysaccharide production in the algal cell. For photosynthesis, generally microalgae utilise light with a wavelength from 400 nm to 700 nm.

However, the wavelength absorbed differs with the algae species. Considering the green microalgae, they absorb light energy for photosynthesis through chlorophyll *a* as a major pigment, absorbing light energy in the range of 450 - 475 nm and 630 - 675 nm and, carotenoids as an accessory pigment absorbing light energy of 400 - 550 nm (Blair et al., 2014).

According to Andersen (2005), some algae prefer low light intensities (< 60 µmol m^{-2} s^{-1} Photosynthetic Photon Flux) while others need higher light intensities (> 100 µmol m^{-2} s^{-1} Photosynthetic Photon Flux). Hanagata et al. (1992) reported that saturation light of *Chlorella* spp. is approximately 200 µmol/sec/m^2 Photosynthetic Photon Flux. Excess light conditions results in photoinhibition and this reduce algal productivity. Fig. 4 illustrates the photosynthetic irradiance (P/I) response of microalgae, The open arrows indicate the high light acclimated response direction and the filled arrows indicate the low light acclimated direction (Grobbelaar, 2006).

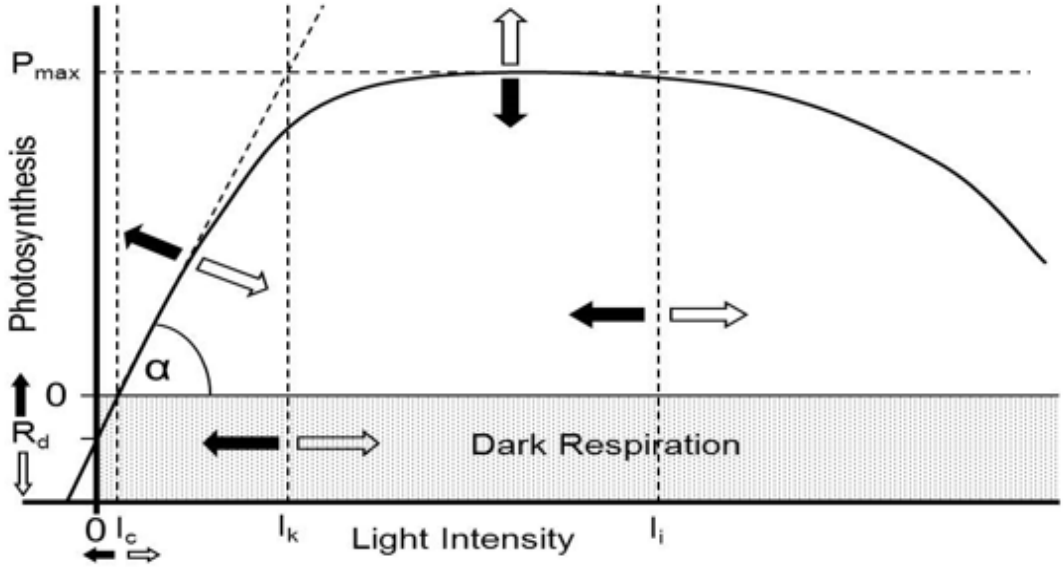


Figure 4: The photosynthetic irradiance response of microalgae (Grobbelaar, 2006)

2.1.2.5 pH requirements

Azov (1982) emphasised the importance of pH in microalgae growth as it affects the availability of inorganic carbon. According to Knud-Hansen et al. (1998) and Round (1984), the three principle forms of dissolved organic carbon needed for algae growth exists in equilibrium as carbon dioxide, bicarbonate and carbonate. Carbon dioxide and sometimes

bicarbonate are essential to algae but rarely carbonate. The pH increases due to photosynthetic carbon dioxide assimilation. According to Oswald (1988), microalgae have been shown to cause a rise in pH to between 10 and 11 in open ponds because of CO_2 uptake.

Intake of nitrogen by microalgae affects the pH of the medium this is because nitrate ions assimilation may increase the pH. In addition, as pH becomes high it may cause precipitation of phosphate medium. The pH affects the abundance of algae, this was indicated in one study where pH was lowered from 6.6 to 5.0, algal abundance increased (Leavitt et al., 1999) and reduced growth was observed in a group of pH tolerant algae when pH exceeded 9.5 (Pedersen and Hansen, 2003). This shows how different algal species reacts differently in numerous pH ranges.

2.2 Challenges of algal culturing in open raceway ponds

Although the open raceway ponds proved to be the simplest of all the algae growing techniques, it also has some challenges owing to the fact that the environment in and around the pond is not completely under control. Often problems of low productivity associated with poor mixing, contamination, dark zones and inefficient use of carbon dioxide are experienced in these raceway ponds (Chisti, 2007; Mata et al., 2010). According to Achara (2012), open pond cultivation is limited to strains that are resistant to contamination by other microorganisms, such as other algal species or bacteria. Issues like bad weather and contamination from strains of bacteria or other outside organisms often results in undesirable species taking over the desired algae growth in the ponds. According to Lee (2001), culturing *Chlorella* spp. in open ponds is often not possible due to the harsh culture conditions. Due to temperature fluctuations up to 20 °C between day and night, it makes it very difficult to maintain the optimum growth temperature (Borowitzka, 2005).

Grima et al. (2003) and Uduman et al. (2010) pointed out the cost implications of harvesting suspended algae. Harvesting of the algae from water remains another challenge due to the small size algal cells, with unicellular eukaryotic algae typically, 3 - 30 µm. Chorus and Bartram (1999) also added that cyanobacteria as small as 0.2 - 2 µm poses harvesting challenges. Reducing the costs of algae harvesting still remains a major challenge. To overcome the challenges of harvesting suspended algae, Hoffmann (1998) indicated that it is better to use

surface-attached algae biofilm systems that are naturally concentrated and more readily harvestable. According to Azov et al. (1980), mass algal cultivation in open basins encounters difficulties including among others, the maintenance of the introduced algal species as unialgal cultures.

The uncontrolled replacement of algae with one that has inferior properties with respect to nutrient uptake efficiency, growth rate, chemical composition and so forth can lead to a process that is not economically acceptable (Gantar et al., 1991). According to De la Noue et al. (1992), phytoremediation yields favourable benefits but there are also major problems of using microalgae, which includes their recovery from the treated effluent. Among the challenges experienced in algae growth, Carvalho et al. (2006) indicated that in order to prevent photooxidative damage, excess oxygen should be removed. This is because oxygen concentrations above air saturation will begin to hinder photosynthesis.

Light deprivation is also another problem in algae culturing. Algae at the bottom of the culture are susceptible to lack of enough light due to the fact that most of the light has already been absorbed by the outermost layer of cells (Borowitzka, 1999). Achara (2012) supported this by emphasizing that in open ponds there is evaporation losses, poor light utilization by the cells and diffusion of CO_2 to the atmosphere. According to Qiang and Richmond (1996), to overcome the light problem a reactor with a high surface area to the volume ratio should be designed. A vigorous mixture is also essential to ensure that all cells reside in the illuminated area for considerable time.

Aksu (2005) reported that one limitation of using an algal system, as secondary treatment process is the presence of high concentrations of ammonia and urea in raw wastes that hinder algal growth and physiological activity. Nutrient removal can be effectively achieved through the controlling of the essential growth parameters including temperature and pH but all these controls require additional costs (Abu-Rezq et al., 1999). In addition, the availability of land and water for large-scale microalgae production often is a challenge to algal-based wastewater treatment (Achara, 2012).

2.3 Wastewater treatment by microalgae in stabilization ponds

The microalgae-based technology for wastewater treatment is cost-effective and environmentally friendly. Wastewater treatment with algae can be done using stabilization ponds. According to Sekhukhune District Municipality (SDM, 2013), primary ponds are the first to receive untreated but screened wastewater followed by the secondary ponds and lastly the tertiary pond. Tertiary ponds are used to polish the effluent and remove algae before treated wastewater is discharged into water bodies. Stabilization ponds can be operated either in series or in parallel connection (Fig. 5). Ponds connected in series reduce the amount of algae present in the last pond and generally achieves better quality effluent. Parallel-connected ponds on the other hand are aimed at reducing organic loading in the primary ponds. Hamzeh and Ponce (2016) emphasized the classification of ponds in relation to their biological activities, namely anaerobic, facultative and maturation.

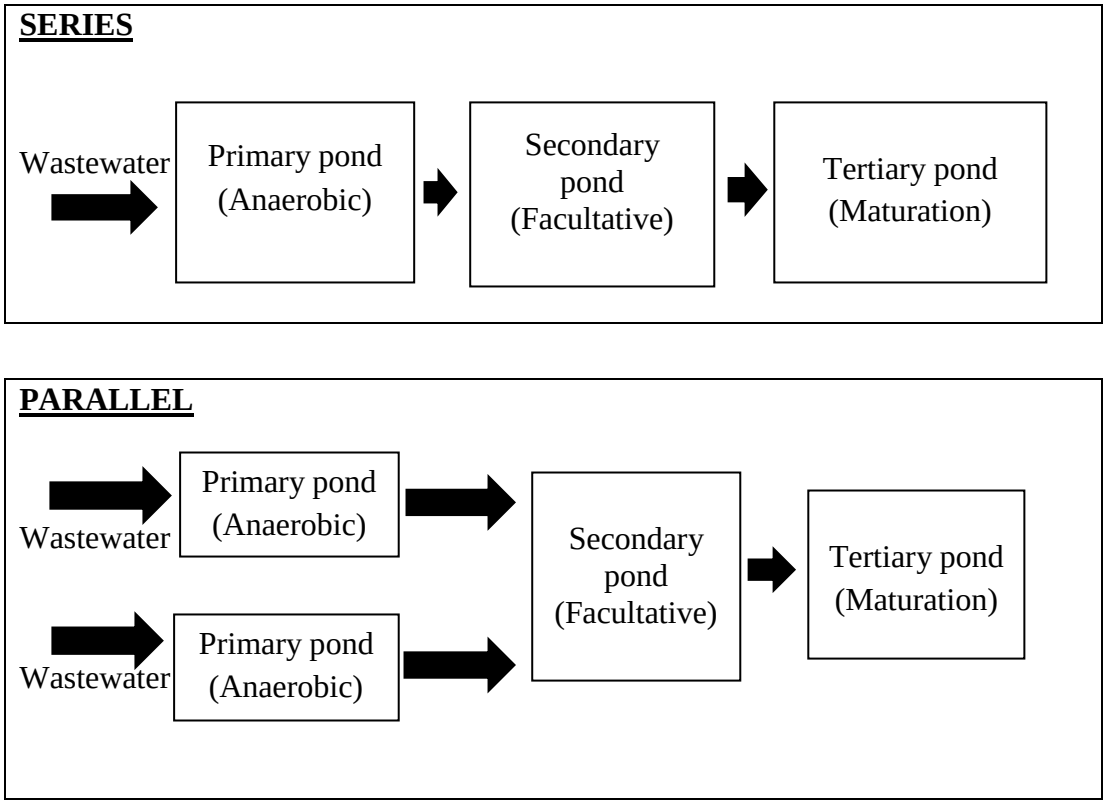


Figure 5: Series and parallel connection of stabilization ponds (Adapted from SDM, 2013)

The operation of stabilization ponds involves the stabilization of organic wastewater through complex natural processes involving sunlight, oxygen, water currents, algae and bacteria. Wastewater stabilization ponds are simple in design and construction, and they require low capital in operation and maintenance. Besides their merits, they also have demerits, which include large land requirements and odour problems if not properly designed (DFID, 1998). The *Chlorella* spp. proved to be the most common and effective unicellular species used for the bioremediation purposes (Lim et al., 2010; Tam et al., 1994; Lau et al., 1997). Kim et al. (2010) and Valderrama et al. (2002) suggested and proved the use of *C. vulgaris* in the removal of nitrogen (ammonia and ammonium ion) from wastewater at a reasonable uptake rate.

Different cultured microalgae species have been used in wastewater treatment practically due to low cost and high efficiency (Oswald., 1988; De la Noue et al., 1992) and this is mainly based on natural processes of eliminating several wastewater pollutants (Ran et al., 2004). Graham et al. (2009) and Oswald (2003) supported this by claiming that the use of algae-based treatment will have more benefits including monetary and ecological. Hii et al. (2011) also added that the use of microalgae for nutrient removal in wastewater is considered the most efficient, environmental friendly, relatively low cost and simple alternative wastewater treatment as compared to conventional wastewater treatment techniques.

Fallowfield and Garrett (1985) reported that algae use for treatment of both domestic and agricultural wastewaters is attractive since the technology is relatively simple and it requires less energy. By utilising harmful nutrients (N and P) as food source, algae hold promise of being able to accomplish nutrient removal with a net energy savings to water treatment system (Singh et al., 2013). Lundquist et al. (2010) and Pittman et al. (2011) also claims that there are several benefits resulting from using algae for wastewater treatment. They emphasized that so much research has been done to consider the integration of wastewater treatment and algae production but Carvalho et al. (2006) argues that the growth of algae will need primary and micronutrients, which can be very expensive when a huge quantity is required. These primary nutrients include carbon, nitrogen and phosphorus. Micronutrients are also essential even though they rarely affect the growth of algae in wastewater and they include silica, calcium, magnesium, potassium, iron, manganese, sulphur, zinc, copper, and cobalt (Knud-Hansen et al., 1998).

The introduction of algal-based treatment approach was a way to avoid costs and demerits of using chemical and physical processes. The use of chemical or physical processes in the removal of nutrients in wastewater is very expensive, less efficient and ecologically not safe as compared to algae assimilation although it must be noted that the acceptable levels of nutrients in wastewater cannot be achieved by algae assimilation alone (Oswald, 2003). In addition Tchobanoglous and Burton (1991) also claims that the use of available chemical and physical based technologies in the removal of nutrients are proving to be energy consuming and chemical demanding hence making them very expensive and uneconomical. Chemical usage in wastewater treatment often leads to secondary contamination of sludge affecting its usage and disposal (Hoffman, 1998).

Amengual-Moro et al. (2012) indicated that the combination of algae and bacteria in the ponds aids in the reduction of Biochemical Oxygen Demand (BOD). This is achieved through the oxidation of organic matter by chemoorganotrophic bacteria using oxygen (O_2) released by algae during photosynthesis. Muñoz and Guieysse (2006) illustrate the removal of BOD through the exchange of oxygen and carbon dioxide between the algae and bacteria (Fig. 6).

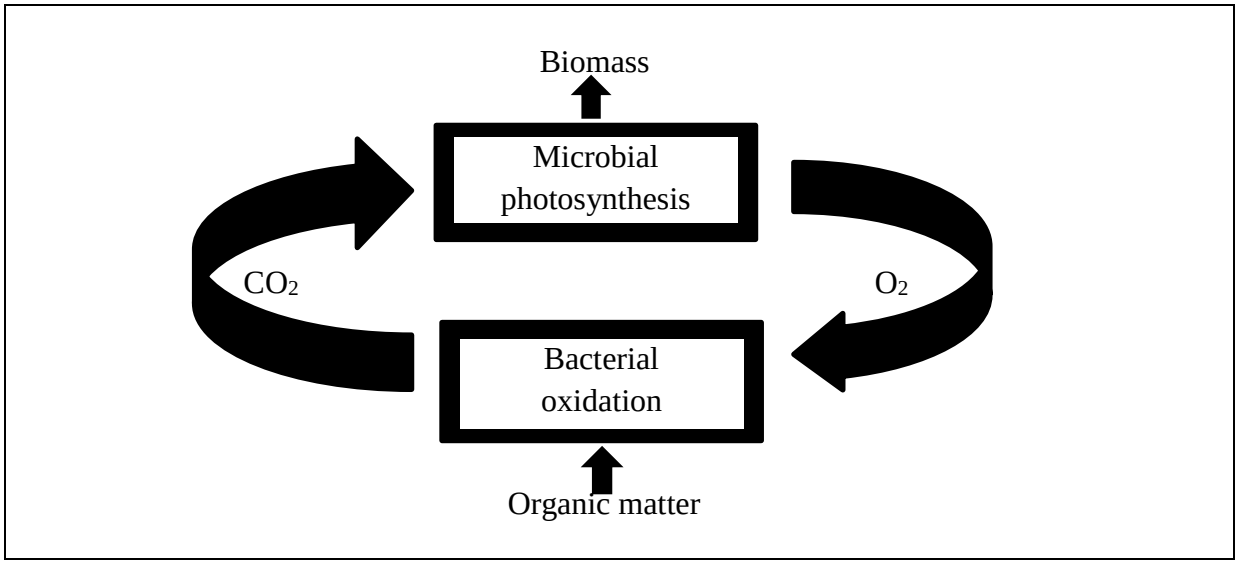


Figure 6: Illustration of Biochemical Oxygen Demand (BOD) removal (Adapted from Muñoz and Guieysse, 2006)

CHAPTER 3

3.1 Materials and Methods

3.1.1 Study area and sampling

Motetema wastewater treatment works was selected as the study area for algal-based wastewater treatment. Motetema lies in Groblersdal, Limpopo Province in South Africa and its geographical coordinates are 25° 6' 3.87" South and 29° 28' 6.78" East, about 141 km North East of Pretoria (Fig. 7). According to Urban Main Place (UMP, 2011), Motetema covers an area of approximately 2.5 km^2 with a population of more than 8500 and approximately 2400 households. Motetema uses stabilization ponds for treatment of the wastewater derived from the surrounding community. According to Wiley et al. (2009), stabilization ponds are used as an appropriate alternative for wastewater treatment and they effectively stabilize wastewater by reducing Biochemical Oxygen Demand (BOD).

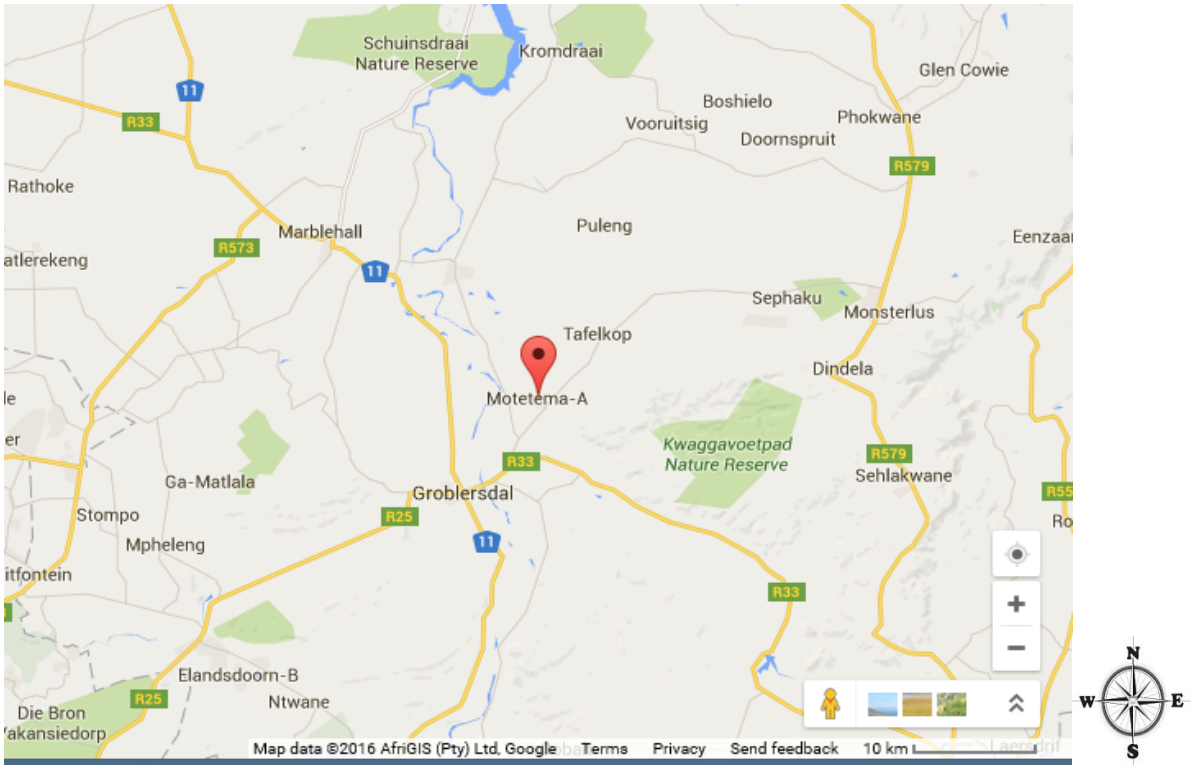


Figure 7: Map showing the geographical location of Motetema wastewater treatment works in Limpopo Province, South Africa (Google Map, 2016)

3.1.2 Algae culture and media preparation

Chlorella vulgaris and *Chlorella protothecoides* were cultured from pure stock cultures in Erlenmeyer flasks, using sterile methods (i.e., work in laminar flow hood, use filter-sterilised algal medium, autoclaved glassware, etc.). The flasks were allowed to shake at 100 rpm at room temperature with a 12:12 circadian cycle. A scale-up approach was followed for the mass culturing of these two species: 250 mℓ flask → 15 ℓ fish tank → 50 ℓ fish tank → 10 000 ℓ round pools → raceway pond. When cultures reached a maximum or near-maximum density, they were transferred to the next container and topped up with concentrated media and water. The fish tanks, round pools and raceway pond were fitted with a pump to allow a constant flow of the water. When the cultures were transferred to a raceway pond, they were combined together. *Chlorella vulgaris* was selected for this experiment because it has great potential for capturing CO_2. It will grow at a faster rate of 0.6 g/ℓ per day and tolerate 10 – 15 % CO_2 (Lee et al., 2000). According to Converti et al. (2009), *C. vulgaris* can also grow in extreme environments with high temperatures of 30 - 35 °C, and acidic environments such as pH of 3 (Mayo, 1997).

The algal cultures in the fish tanks, round pools and raceway pond were monitored on a weekly basis by measuring the cell concentration and chlorophyll *a* levels, as well as using microscopic examination to make sure that the cultures remained free from contamination by other species. Algal culture medium was prepared according to the green algae, *Selenastrum capricornutum*, growth test method 1003.0 (USEPA, 2002). Normal concentration (for use in the Erlenmeyer flasks) as well as ten times concentrated stock solutions (for use in the fish tanks, round pools and raceway pond) were prepared according to the Table 3 below. Milli-Q water was used during all dilutions. For the final algal media the following was done: 1 mℓ of A, B, C, D and E (in this order) was added to 900 mℓ of water. Each addition was mixed well. 1 000 mℓ mix was diluted and the pH was adjusted to 7.5 ± 0.1, using sodium hydroxide (NaOH) or hydrogen chloride (HCl). The normal concentration medium was filter-sterilized using 0.22 µm pore size sterile filtration units.

Table 3: Preparation of stock solution concentration

Stock solution	Compound	Amount dissolved in water	
Macronutrients			
		Normal concentration	Ten times concentration
A	$MgCl_2.6H_2O$ $CaCl_2.2H_2O$ $NaNO_3$	6.08 g 2.2 g 12.75 g Dissolve in 500 mℓ water	60.8 g 22.0 g 127.5 g Dissolve in 500 mℓ water
B	$SO_4.7H_2O$	7.35 g Dissolve in 500 mℓ water	73.5 g Dissolve in 500 mℓ water
C	K_2HPO_4	0.522 g Dissolve in 500 mℓ water	5.22 g Dissolve in 500 mℓ water
D	$NaHCO_3$	7.5 g Dissolve in 500 mℓ water	75.0 g Dissolve in 500 mℓ water
Micronutrients			
E1	H_3BO_3 $MnCl_2.4H_2O$ $FeCl_3.6H_2O$ $Na_2EDTA.2H_2O$	92.8 mg 208 mg 79.9 mg 150 mg Dissolve in 400 mℓ water	928 mg 2.08 g 799 mg 1.5 g Dissolve in 400 mℓ water
E2	$ZnCl_2$	164 mg Dilute to 100 mℓ	1.64 g Dilute to 100 mℓ
E3	$CoCl_2.6H_2O$	71.4 mg Dilute to 100 mℓ	714 mg Dilute to 100 mℓ
E4	$Na_2MoO_4.2H_2O$	36.6 mg Dilute to 10 mℓ	363 mg Dilute to 10 mℓ
E5	$CuCl_2.2H_2O$	60 mg Dilute to 1 000 mℓ Take 1 mℓ of this solution and dilute to 10 mℓ	600 mg Dilute to 1 000 mℓ Take 1 mℓ of this solution and dilute to 10 mℓ
E6	Na_2SeO_4	119.6 mg Dilute to 100 mℓ	1.196 g Dilute to 100 mℓ
Total E	1 mℓ of E2 + E3 + E4 + E5 + E6 to E1 was added and brought to a volume of 500 mℓ		

3.2 Experimental setup

Algal samples for the study were cultured at the Council for Scientific and Industrial Research (CSIR) premises in Pretoria. In order to test the growth rate of microalgae, three ponds were constructed. These ponds consisted of two round shaped and one rectangular shaped (see Appendix B). In the round shaped pools *C. vulgaris* and *C. protothecoides* were cultured separately whilst in the rectangular shaped raceway pond, the two *Chlorella* spp. were mixed together.

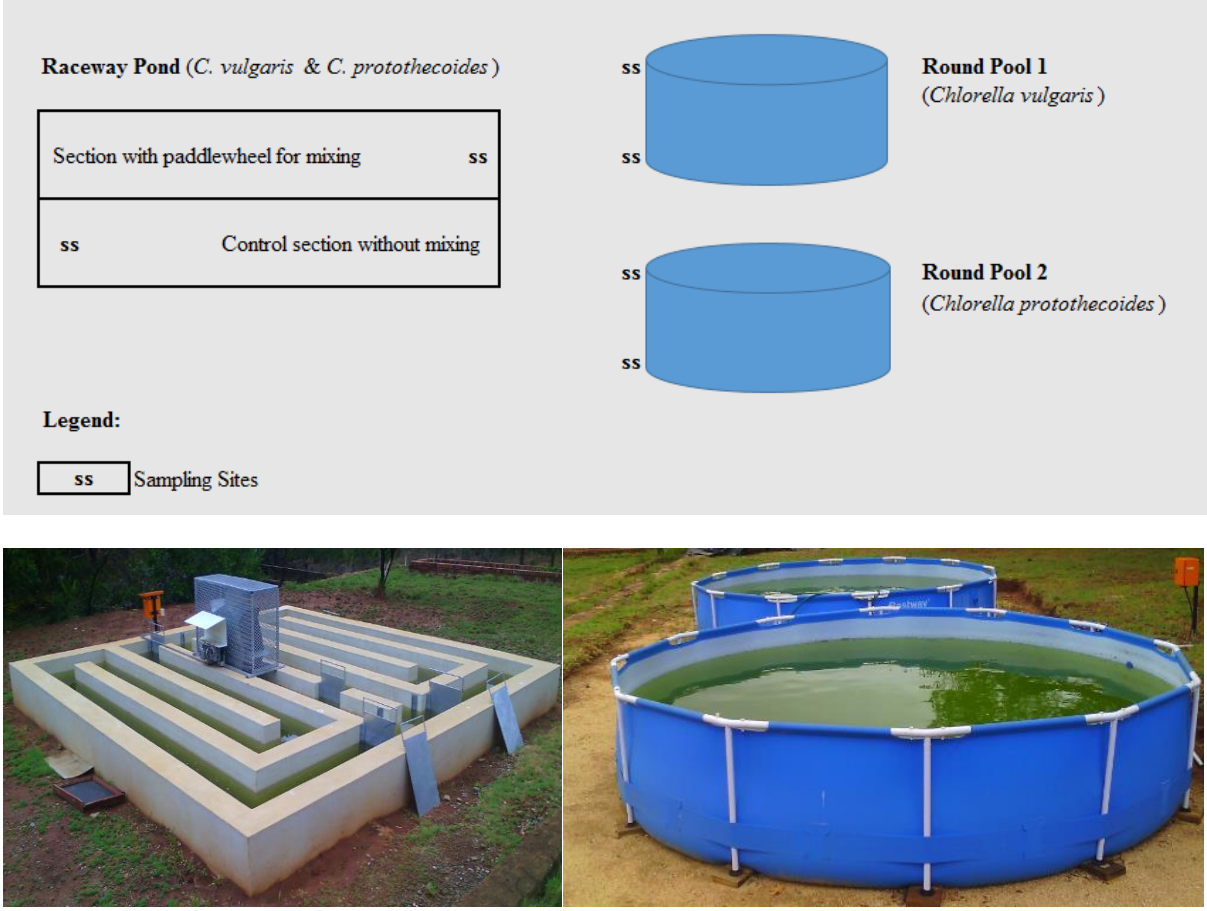


Figure 8: Schematic layout of the experimental setup

The recordings were taken after every 1 hour for 24 hours, from the top and at the bottom of the round pools. The readings were also taken in the raceway pond from the control section without mixing and from another section with paddlewheel for mixing as shown in Fig. 8 above. The physicochemical parameters monitored included pH (units), temperature (°C), conductivity (µS/cm), total dissolved solids (mg/ℓ) and DO (mg/ℓ) and this was done using Thermo Scientific Orion Star pH/ORP/ISE/Conductivity/RDO/DO meter on the 7th and 8th of August 2015 (Fig. 9). The same instrument was also used to measure DO from the stabilization ponds at Motetema wastewater treatment works on the 16th of January 2016.

Figure 9: Thermo Scientific Orion Star pH/ORP/ISE/Conductivity/RDO/DO meter

On the 25th, 26th and 27th of November 2015, the Hydrolab DS 5 Multipleparameter Data Sonde (Fig. 10) was used for data collection in the round pools. The same instrument was also used for data collection from the stabilization ponds at Motetema wastewater treatment works on the 16th of January 2016. In order to take recordings from the stabilization ponds a bucket attached to a rope was used and the samples were collected from a turbulent well-mixed point, which was the manhole at the outlet diversion control structure (Fig. 10). The data collected using the Hydrolab DS 5 included: temperature (°C), pH (units), conductivity (µS/cm), total dissolved solids (g/ℓ), nitrates (mg/ℓ) and chlorophyll *a* (µg/ℓ).

Figure 10: Experimental setup using Hydrolab DS 5 Multipleparameter Data Sonde

The following activities were carried out for the preparation and maintenance of the raceway pond and round pools: this involved nutrient dosing to feed growing algal community and monitoring of water chemistry. Algal densities at different depths were recorded and monitored. The algal culture was released through three phases to coincide with the increase in algal density (Fig. 11).

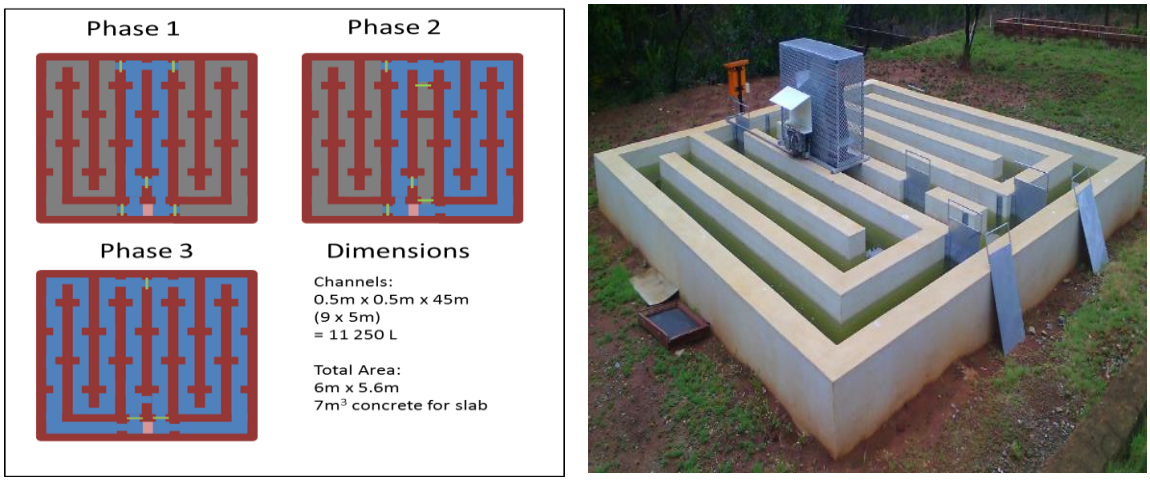


Figure 11: Three phases of algae culturing in the raceway pond (Oberholster, 2014)

3.3 The introduction of microalgae to the stabilization ponds

The algal cultures from the raceway pond and round pools at CSIR premises were then transported using a truck and introduced to the stabilization ponds at Motetema wastewater treatment works. The construction of these stabilization ponds was done in such a way that the wastewater will undergo different treatment processes in different kinds of stabilization ponds such as anaerobic ponds, facultative ponds and saturated ponds. The black arrows in Fig. 12 show the flow direction of influent and effluent. During the sampling period, the white stabilization ponds were being dried to remove sludge.

The algae cultures were introduced to facultative ponds with a depth of 2 m and a capacity of 20 Mℓ but without mechanical aeration. The introduced algae cultures were allowed to mature in the green stabilization ponds with a capacity of 7.5 Mℓ each (Fig. 12). The maintenance process involved the monthly monitoring of total cell counts of algae in collected water samples, monitoring of outflow water quality, inoculation of algae as established through water monitoring and recording, and monitoring of algal profiles.

3.4 Statistical analysis

Data was analysed by Pearson's correlation analysis. Different variables were evaluated to determine if they have a relationship. This was done by comparing the behaviour of one variable in relation to the other. For positive correlation, as one variable increases so does the other whilst for negative correlation, as one variable increases the other decreases. This relationship was shown using scatter plots (see Appendix L). Pearson's correlation coefficient r will measure the strength of the linear relationship between the paired variables. The closer the r value to 1 or –1, the stronger the positive or negative linear correlation respectively.

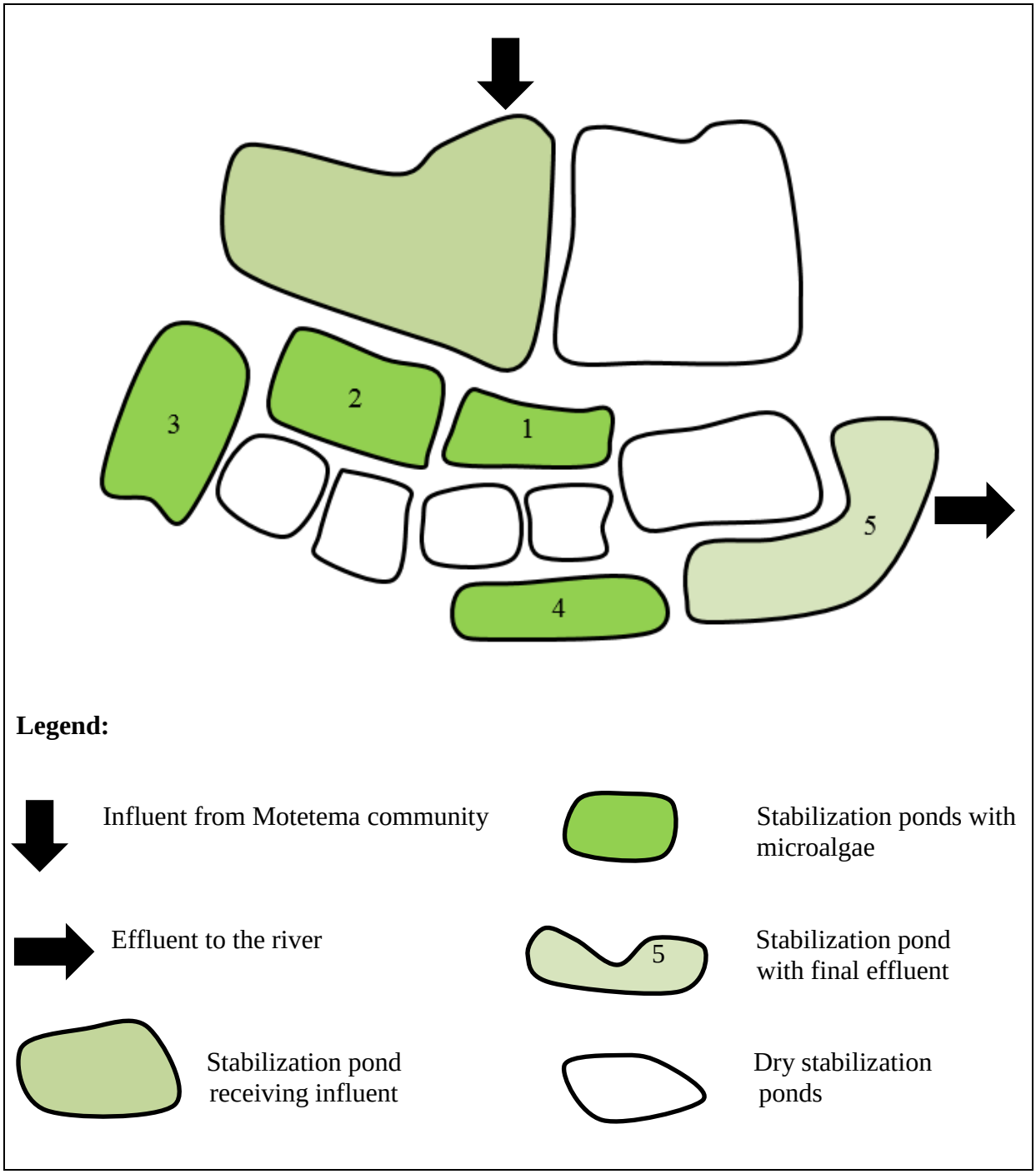


Figure 12: Schematic layout of the stabilization ponds at Motetema wastewater treatment works (Redrawn from Google Map, 2016)

CHAPTER 4

4.1 Results and Discussion

The abundance of *C. vulgaris* and *C. protothecoides* in the round pools and raceway pond, and its daily yield was highly influenced by the values of the physicochemical variables. The results of the water quality for August and November 2015 reflects changes in the curves for almost all the physicochemical parameters including temperature, pH, conductivity, total dissolved solids, DO, nitrates, ammonium and chlorophyll *a*.

4.1.1 Temperature

The experimental results for temperature recorded on the 7th and 8th of August 2015, and 25th, 26th and 27th of November 2015 are presented in Fig. 13 and Fig. 14 respectively. Fig. 13 depicts the temperature results for both round pools and raceway pond ranging from 12.6 °C and 16.6 °C, and 10.6 °C and 15.1 °C respectively. These results were obtained in August and they differ with those obtained in November (Fig. 14) with temperature range of 22.14 °C and 27.27 °C for round pools only. This indicates that November recorded high temperatures during algae growth in round pools 1 and 2. The average temperatures for the round pools were 14.5 °C whilst for the raceway pond were 12.9 °C.

This indicates that these two *Chlorella* spp. are capable of photosynthesis over a wide range of temperatures, in this case 10.6 °C and 27.27 °C. The raceway pond recorded the lowest temperature of 10.6 °C around 8 am on the 8th of August 2015, this was possibly due to their location, being affected by the shade of the nearest trees. The round pools 1 and 2 had the same geometry and they were exposed to the same environmental condition, their results indicate uniform temperature values throughout the whole sampling process. Ono and Cuello (2003) indicated that the optimal growth temperatures of 15 – 26 °C have been reported for some species, whilst others grow effectively in optimal conditions between 20 – 25 °C (Li, 1980). Muñoz and Guieysse (2006) added that temperature regulates cellular, morphological and physiological responses of microalgae. Higher temperatures generally accelerate the metabolic rates of microalgae, whereas low temperatures lead to inhibition of growth. The previous study done by Oswald et al. (1957) showed that *Chlorella* spp. showed a good adaptation for low-

temperature conditions and these species could even sustain photosynthetic oxygen production at temperature nearly as low as freezing. According to this research, these two *Chlorella* spp. showed better tolerance to temperatures as low as 10.6 °C and as high as 27.27 °C.

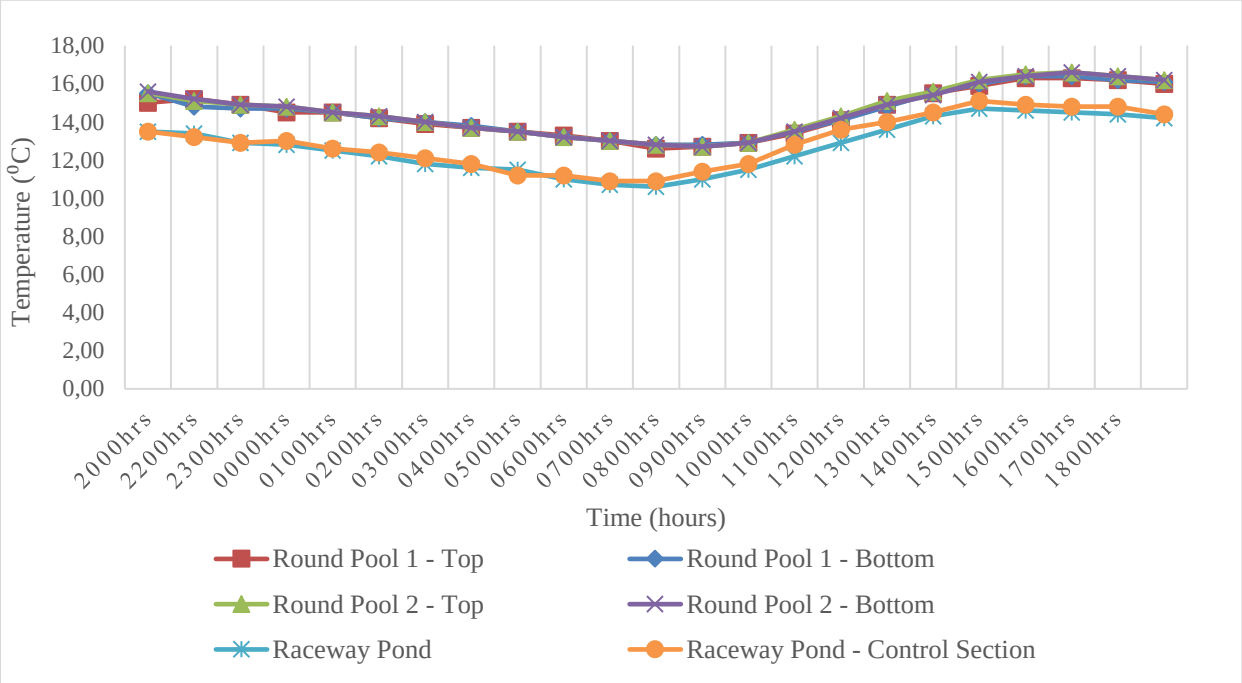


Figure 13: Temperatures recorded in the round pools and raceway pond on the 7th and 8th of August 2015

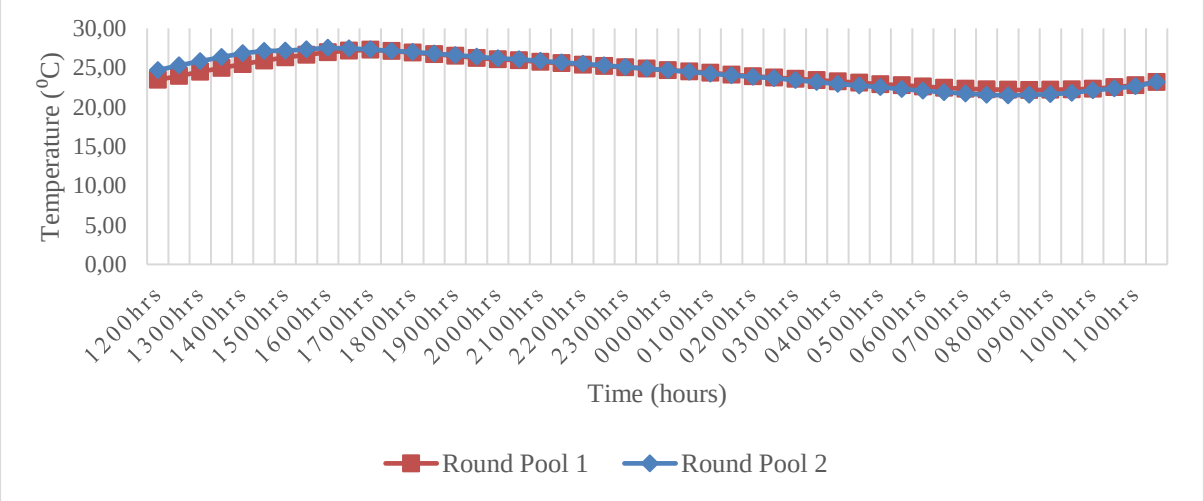


Figure 14: Temperatures recorded in round pool 1 on the 25th and 26th of November 2015 and round pool 2 on the 26th and 27th of November 2015

4.1.2 Conductivity

Conductivity is the numerical expression of the water's ability to conduct electric current (Moeeni and Kumar, 2014). The conductivity of the algae culture (Fig. 15) showed little variation of 22 µS/cm during the 24-hour sampling period on the 7th and 8th of August 2015 for round pools, ranging between 325 µS/cm and 347 µS/cm. In the raceway pond the variation was even much lower as compared to the round pools especially the control section. The control section recorded the lowest conductivity value of 337 µS/cm whilst the other side of the raceway pond where mixing took place recorded conductivity value of 343 µS/cm.

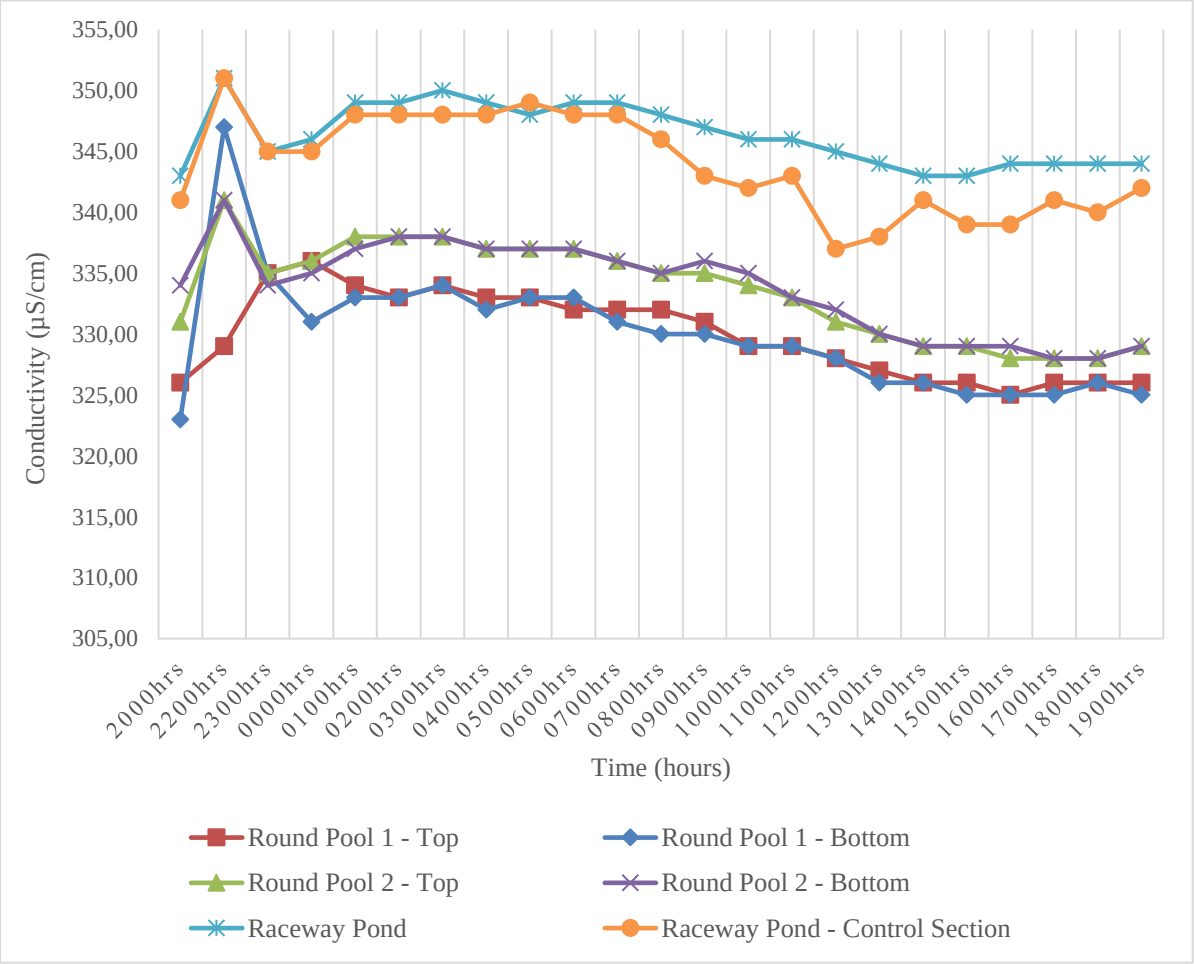


Figure 15: Conductivity recorded in the round pools and raceway pond on the 7th and 8th of August 2015

On the 25th, 26th and 27th of November 2015, conductivity measurements were taken from round pools only, with the conductivity values for round pool 2 for the 24-hour sampling period showing a difference of 16 µS/cm between minimum and maximum values. This was less variation as compared to round pool 1, which recorded a difference of 38 µS/cm (Fig. 16). Round pool 1 showed fluctuations probably due to the disturbances of the culture during the sampling process. The minimum conductivity values recorded in round pool 1 and 2 in November were 324 µS/cm and 292 µS/cm respectively. Round pool 1 recorded the highest conductivity value of 362 µS/cm, a difference of 17.5 % as compared to round pool 2 that recorded 308 µS/cm as the maximum conductivity value.

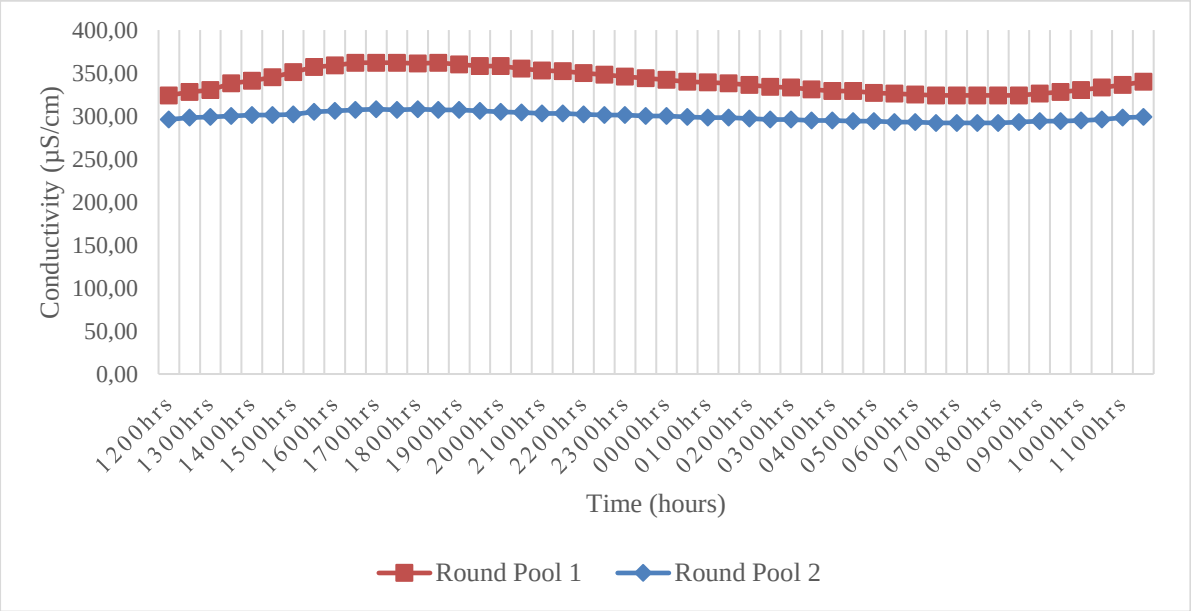


Figure 16: Conductivity recorded in round pool 1 on the 25th and 26th of November 2015 and round pool 2 on the 26th and 27th of November 2015

4.1.3 Dissolved oxygen and Total dissolved solids

The DO concentration from round pools and the raceway pond (Fig. 17) showed a marked variability over the 24-hour sampling period with minimum values of 8.09, 9.99 and 7.82 mg/ℓ, and maximum values of 10.57, 13.21 and 8.91 mg/ℓ obtained in round pool 1, round pool 2 and raceway pond respectively. The DO levels change and vary according to the time of the day and temperature.

The results showed that there is moderate negative correlation (r = - 0.6218) and strongly negative correlation (r = - 0.9104) in round pool 1 and round pool 2 respectively, between DO and total dissolved solids. The round pool 1 and round pool 2 had p-values of 0.001531 and 0.00001 respectively (see Appendix L). The p-values for both pools were less than the significant level of 0.05, which indicates that the correlation coefficients were significant. Fig. 17 and Fig. 18 depicts that sampling points with low DO had high total dissolved solids content. According to a study done by Muigai et al. (2010), sampling points with low DO had high total dissolved solids content. On the other hand, the sampling sites with high DO registered low total dissolved solids content.

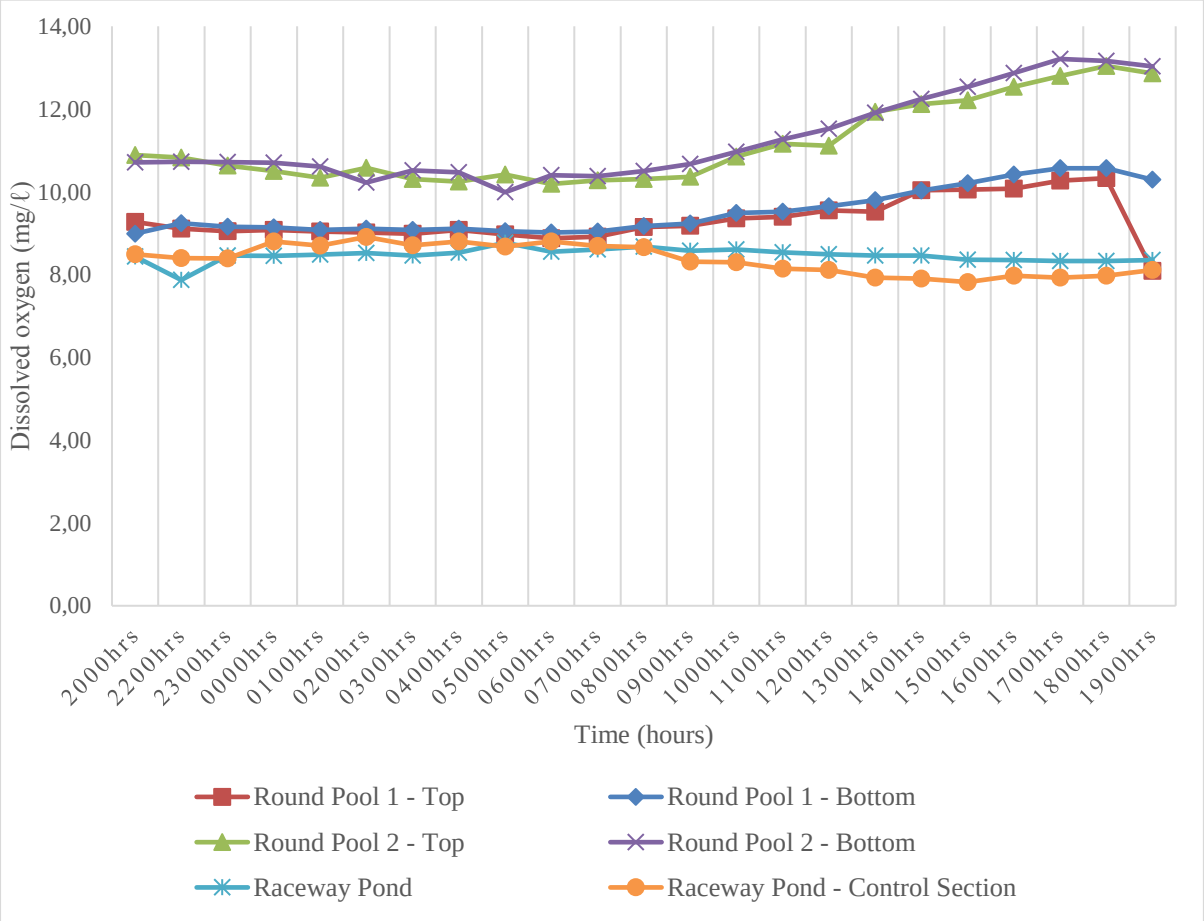


Figure 17: Dissolved oxygen recorded in the round pools and raceway pond on the 7th and 8th of August 2015

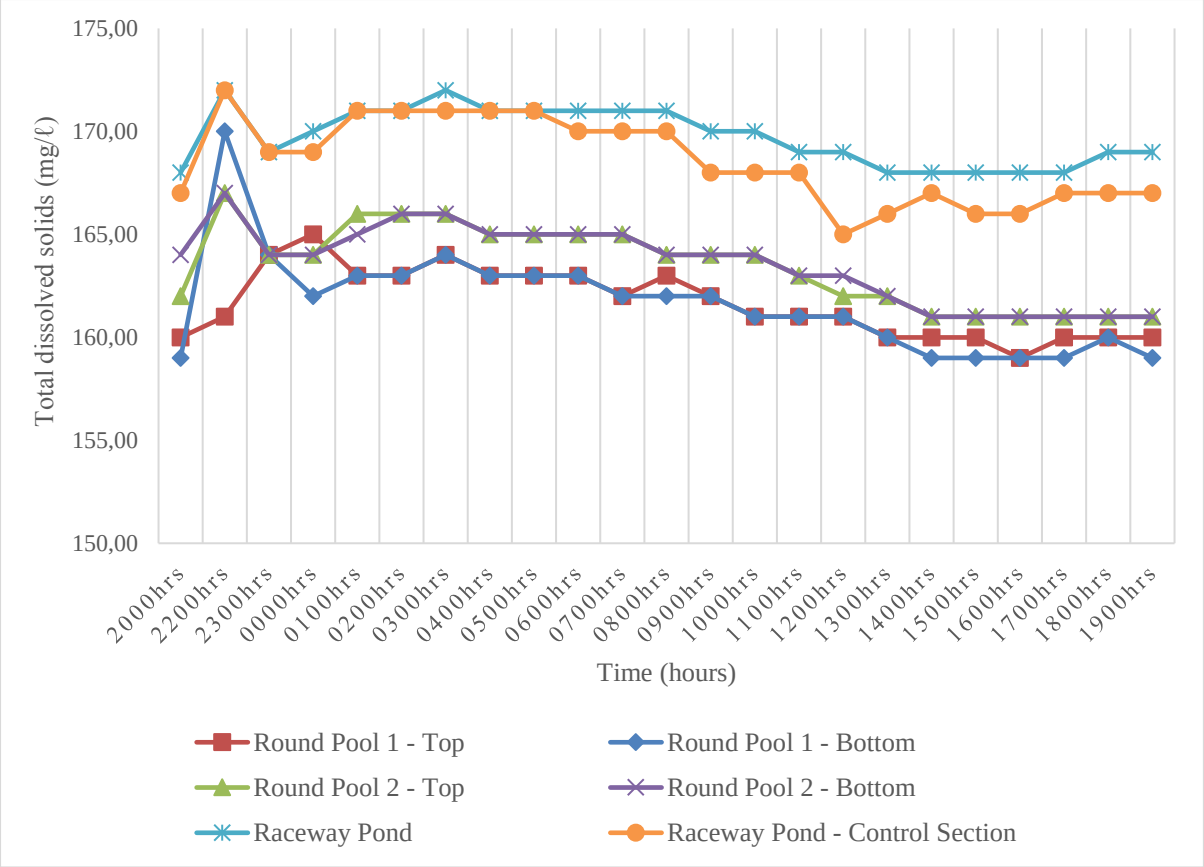


Figure 18: Total dissolved solids recorded in the round pools and raceway pond on the 7th and 8th of August 2015

4.1.4 pH

Initial pH values were 10.59 for round pool 1 and 10.16 for round pool 2 (Fig. 19). These values increased to 10.70 and 10.31 in round pool 1 and round pool 2 respectively. This was probably due to photosynthetic carbon dioxide assimilation. The pH values were recorded on the 25th, 26th and 27th of November 2015 in round pools only. The values remained stationary at 10.65 for round pool 1 from 4 pm to 6:30 pm on the 25th of November 2015 during the 24-hour sampling period but increased to 10.70 at 11 am on the 26th of November 2015. The pH values for round pool 2 remained stationary at 10.24 from 12 am to 5:30 am on the 27th of November 2015 but increased to 10.31 from 10:30 am on the same day. Round pool 1 recorded the highest pH values with a median value of 10.63 whilst the round pool 2 had low pH values as compared to round pool 1 with a median value of 10.24.

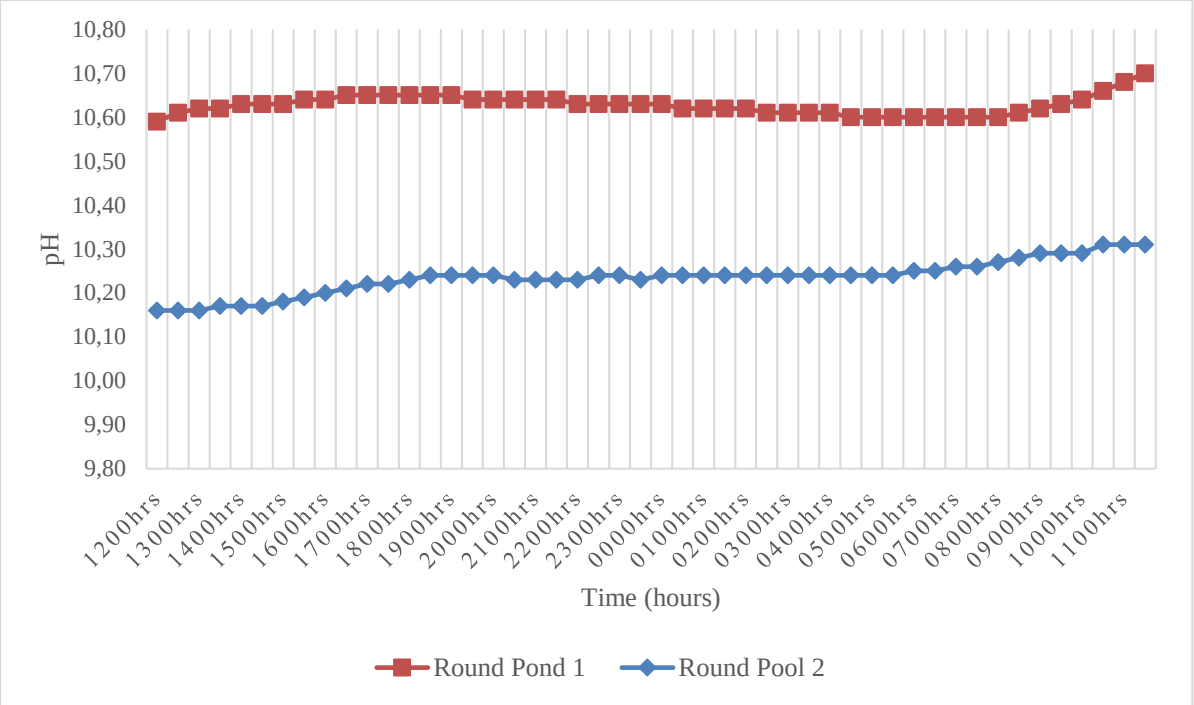


Figure 19: pH recorded in round pool 1 on the 25th and 26th of November 2015 and round pool 2 on the 26th and 27th of November 2015

4.1.5 Chlorophyll *a*, Ammonium and Nitrates

Chlorophyll *a* is often used as an indicator of algal biomass, with concentrations above 40 µg/ℓ estimated to indicate algal blooms (Stanley et al., 2003). The results depicts that in round pool 1 there was more chlorophyll *a* indicating more algae concentration (Fig. 20). The results indicated that *C. vulgaris* in round pool 1 grew much better than *C. prototheco*ides in round pool 2 under the same prevailing conditions. This was shown by the presence of high concentrations of chlorophyll *a* in round pool 1, with concentration value of 277.3 µg/ℓ as compared to round pool 2 that had maximum concentration value of 68.08 µg/ℓ.

Chlorophyll *a* concentration had marked fluctuations between 5 pm and 5 am in round pool 1. Both pools showed a remarkable decrease in chlorophyll *a* concentrations as from 5 am, this was probably due to decreasing temperatures. This indicates that water temperature is an important factor during algae culturing. Although light was not measured in this study, it is

important to reiterate the role it plays in algae growth during culturing. Depriving algae of optimum light will have an effect on their growth as they would invest a lot of energy in chlorophyll production at the expense of their growth. According to Friedman (1991), microalgae exposure to light must be monitored because high light intensities tend to increase polysaccharide production in the algal cell. The diurnal fluctuations resulting from alternating day and night also have a negative impact on the growth of algae. Light changes in quality and quantity on different time scales.

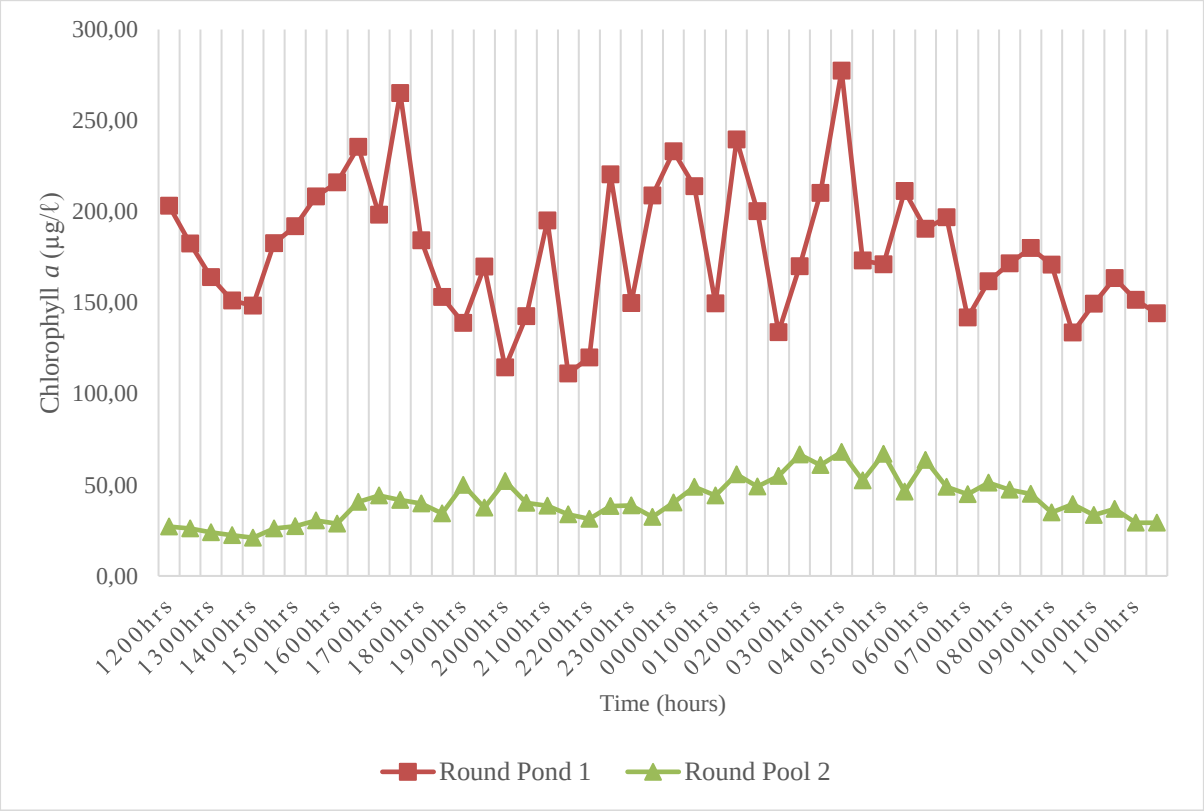


Figure 20: Chlorophyll *a* recorded in round pool 1 on the 25th and 26th of November 2015 and round pool 2 on the 26th and 27th of November 2015

Previous research (Bbalali et al., 2013) indicated a significant negative correlation between ammonium and chlorophyll *a* and this research had different outcomes between round pool 1 and round pool 2 (Fig. 20 and Fig. 21). The results showed that there is a weak negative correlation (r = - 0.0756) in round pool 1 between ammonium and chlorophyll *a*. The round

pool 1 had a p-value of 0.607666 (see Appendix L). Because the p-value for round pool 1 was greater than the significant level of 0.05, there is inconclusive evidence about the significance of association between these variables.

Round pool 2 gave a different picture about the correlation between ammonium and chlorophyll *a*. The results from round pool 2 showed that there is a moderate positive correlation (r = 0.4911) between ammonium and chlorophyll *a*. The round pool 2 had a p-value of 0.000394, which is a lot less than the significant level of 0.05. This indicates that there is significant positive correlation between ammonium and chlorophyll *a*. Average concentration of ammonium in round pool 2 was 104.91 mg/ℓ whilst round pool 1 had an average of 51.41 mg/ℓ. The results depicts that *C. prototheco*ides did not efficiently utilise ammonium for their growth as a result there were low levels of chlorophyll *a* concentration in round pool 2 as compared to round pool 1.

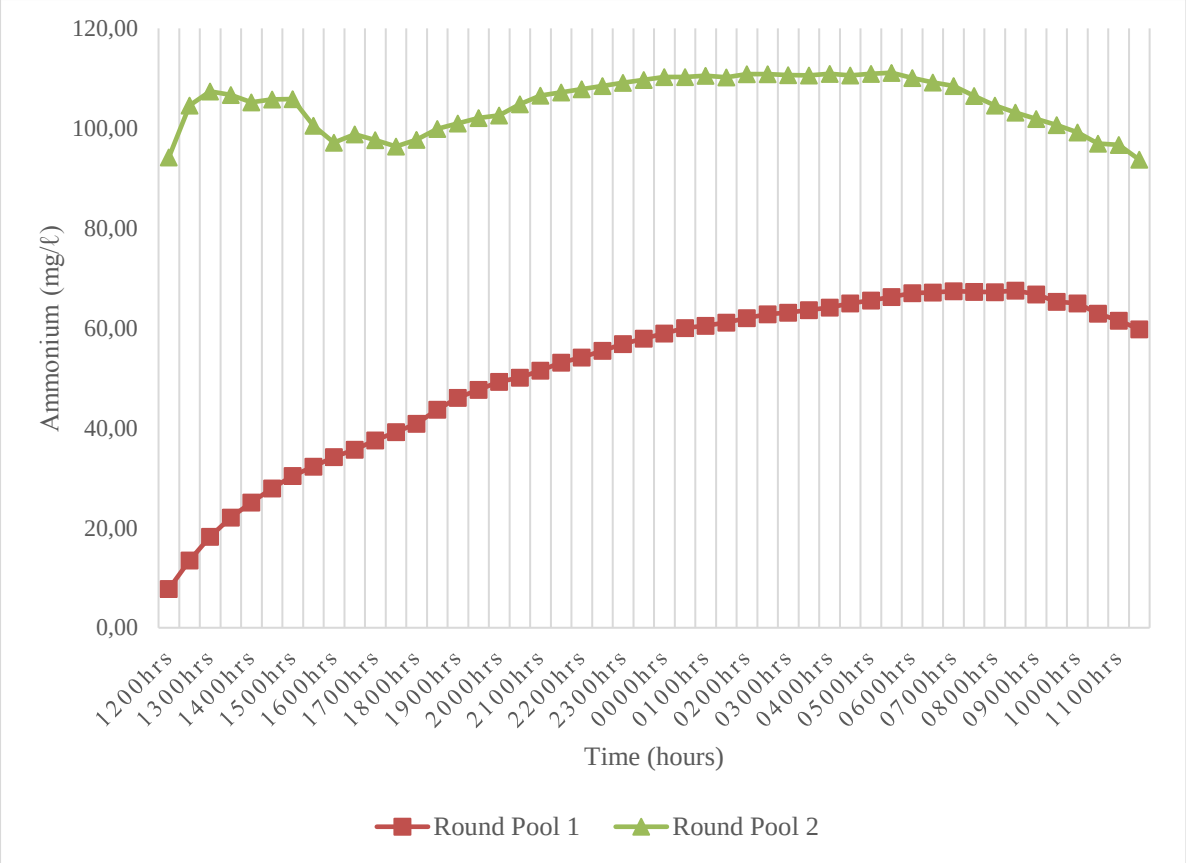


Figure 21: Ammonium recorded in round pool 1 on the 25th and 26th of November 2015 and round pool 2 on the 26th and 27th of November 2015

The ammonium levels for round pools were extremely high due to the absence of mixing in the pools. Mixing is essential to ensure that all algae cells are equally exposed to the air. Poor gas exchange between the culture medium and the air will result in low oxygen content. This can lead to anoxic conditions causing nitrate to be reduced to ammonium (Reynolds, 1984). According to Dortch and Postel (1989), once nitrate is absorbed by algae, it accumulates in the cell or is reduced to intermediate compounds, such as ammonium thereby increasing its level.

Nitrates level in the round pools showed a slight increase of 0.01 mg/ℓ, from 0.03 mg/ℓ to 0.04 mg/ℓ in round pool 1 and 0.04 mg/ℓ to 0.04 mg/ℓ in round pool 2. The results were different as compared to the stabilization ponds. After the introduction of cultured algae to stabilization ponds the nitrates levels decreased by 7.95 % from 0.88 mg/ℓ to 0.81 mg/ℓ. This indicates a slight uptake of nutrients by microalgae in the stabilization ponds.

4.2 Introduction of *C. vulgaris* and *C. protothecoides* to wastewater stabilization ponds at Motetema wastewater treatment works

Algae cultured at the Council for Scientific and Industrial Research (CSIR) premises was introduced to stabilization ponds at Motetema wastewater treatment works. As Table 4 indicates, the wastewater physicochemical properties changed from stabilization pond 1 to stabilization pond 5 (see Appendix J).

Table 4: Physicochemical parameters recorded on the 16th of January 2016 after the introduction of algae at Motetema wastewater treatment works stabilization ponds

Date (D/M/YYYY)	Time (Hours)	Pond Site No.	pH	Dissolved Oxygen (mg/ℓ)	Temperature (^{0}C)	Conductivity (µS/cm)	Total Dissolved Solids (mg/ℓ)	Nitrates (mg/ℓ)	Chlorophyll *a* (µg/ℓ)
16/01/2016	1000hrs	5	7,41	0,20	23,50	954,00	0,60	0,81	33,61
16/01/2016	1010hrs	4	7,39	0,17	23,34	943,00	0,60	0,90	46,40
16/01/2016	1030hrs	3	7,39	0,18	23,41	942,00	0,60	0,88	45,38
16/01/2016	1100hrs	2	7,39	0,16	23,41	942,00	0,60	0,88	45,38
16/01/2016	1120hrs	1	7,39	0,16	23,41	942,00	0,60	0,88	45,38

4.2.1 pH

An increase in pH values from 7.39 to 7.41 as shown in Fig. 22, was due to photosynthetic carbon dioxide assimilation. According to Hoham et al. (2007), the different species of algae can survive and grow well at similar ranges but the optimum pH will be different. Other studies have shown that *Chlorella* spp. can grow well in an optimum pH of 7.0 (Kim et al., 2012;

Martinez et al., 2011). pH values indicates whether the stabilization ponds are alkaline or acidic. In this case, the stabilization ponds were alkaline with pH values above 7.0. pH controls the environment for algae, with values of 5.5 and 9.5 considered general limit for effluent discharge (DWA, 2010).

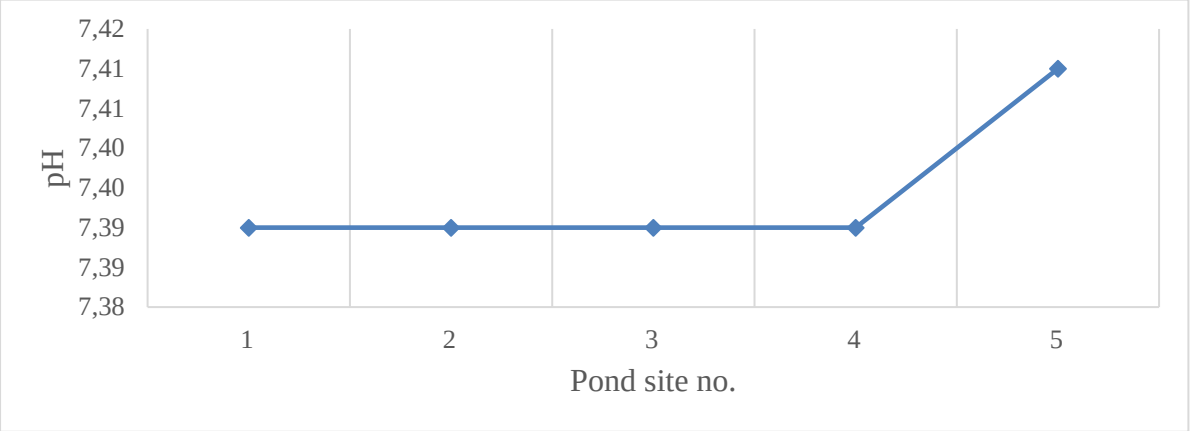


Figure 22: pH recorded in stabilization ponds at Motetema on the 16th of January 2016

4.2.2 Dissolved oxygen

The DO levels in the five stabilization ponds were found to be slightly increasing from stabilization pond 1 (0.16 mg/ℓ) to stabilization pond 5 (0.20 mg/ℓ) as shown in Fig. 23. Increased DO levels may be an indication of less bacteria and reduced amount of BOD, which use up oxygen. However, stabilization pond 3 was notably higher than stabilization ponds 1, 2 and 4. Temporal patterns of DO levels that were present in these stabilization ponds were generally influenced by multiple factors that include temperature, ground water exchange, heterotrophic respiration and algal metabolism. Although the DO levels showed an increase from stabilization pond 1 to stabilization pond 5, still the DO levels in these stabilization ponds were very low. This indicates poor maintenance, high BOD load, low retention time, poor light penetration and possibly toxic industrial waste.

These presence of duckweed showed that the stabilization ponds were poorly maintained. The duckweed shields off light and gas exchange leading to anoxic conditions. High BOD loading affects the availability of oxygen and the growth of algae. High BOD loading was also an indication of low retention time in these stabilization ponds. The retention time was too little

for the treatment process to be effective. BOD removal depends on sufficient retention time, adequate oxygen supply and absence of high concentration of chemical pollutants. The previous study done by Mara (1976) indicated that BOD_5 removal increase as the retention time increase. The retention time of 1 day, 2.5 days and 5 days showed BOD_5 removal of 50 %, 60 % and 70 % respectively.

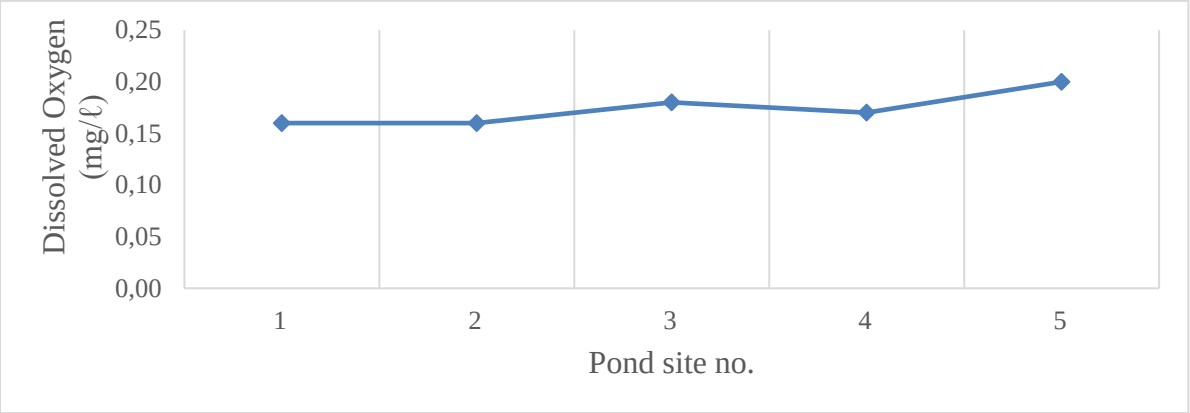


Figure 23: Dissolved oxygen recorded in stabilization ponds at Motetema on the 16th of January 2016

4.2.3 Chlorophyll *a* and Nitrates

The quality of wastewater showed an improvement since the introduction of *Chlorella* spp. This was indicated by the reduction in the concentration of nitrates and chlorophyll *a* from stabilization pond 1 to stabilization pond 5 (Fig. 24 and Fig. 25). The stabilization pond 5 contains the final effluent to be discharged into the river. Reduction in the concentration of chlorophyll *a* by 25.94 % from 45.38 mg/ℓ to 33.61 mg/ℓ showed an improvement in the quality of water discharged into the river. The amount of nitrates also showed a slight decrease from 0.88 mg/ℓ to 0.81 mg/ℓ (Fig. 25). The results from stabilization ponds showed that there is a strong positive correlation (r = 0.9855) between nitrates and chlorophyll *a*. The stabilization ponds had a p-value of 0.002091, which is a lot less than the significant level of 0.05 (see Appendix L). This indicates that there is significant positive correlation between nitrates and chlorophyll *a*. The results depicts a reduction in the concentration of both chlorophyll *a* and nitrates in the final effluent stabilization pond 5 as compared to the influent in stabilization

pond 1. The concentration of nitrates was below the general limit for effluent discharge of 15 mg/ℓ as required by the Department of Water Affairs (DWA, 2010).

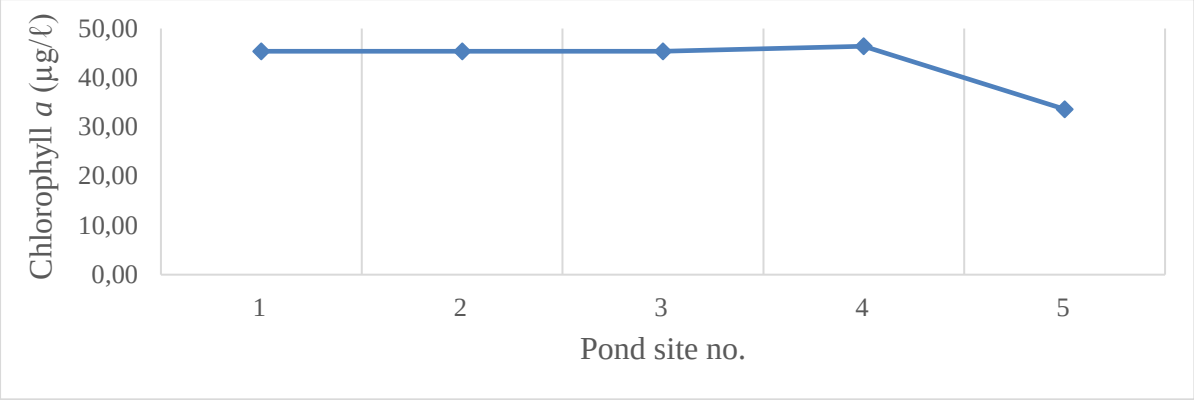


Figure 24: Chlorophyll *a* recorded in stabilization ponds at Motetema on the 16th of January 2016

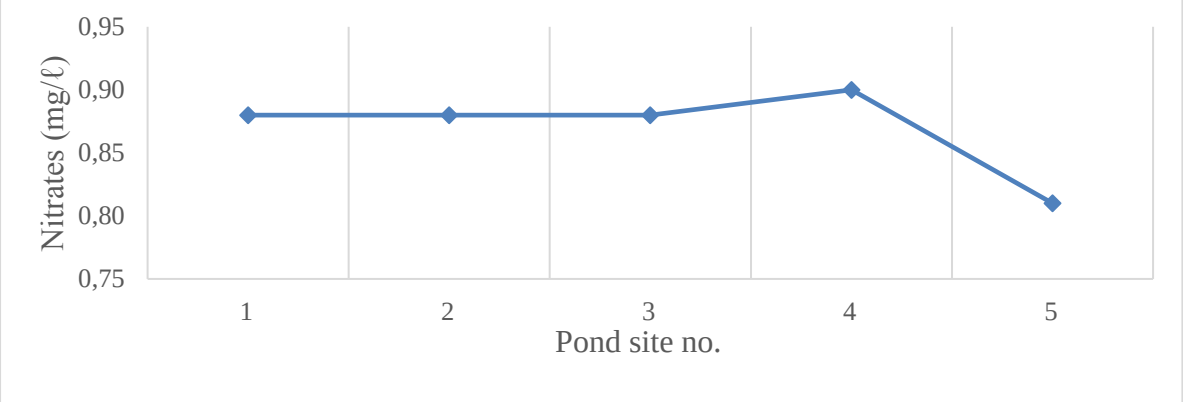


Figure 25: Nitrates recorded in stabilization ponds at Motetema on the 16th of January 2016

4.2.4 Temperature and Conductivity

Temperature values were not that different for all the stabilization ponds, averaging 23.41 ºC (Fig. 26). The temperatures recorded in these stabilization ponds appeared favourable for algae growth and survival. Li (1980) indicated that the microalgae species are capable of photosynthesising over a wide range of temperature generally stated between 15 and 30 ºC. Stabilization pond 5 recorded the highest temperature of 23.5 ^{0}C. The results showed that there is a strong positive correlation (r = 0.7992) in the stabilization ponds between temperature and

conductivity. The stabilization ponds had a p-value of 0.1047 (see Appendix L). Because the p-value was greater than the significant level of 0.05, there is inconclusive evidence about the significance of association between temperature and conductivity (Fig. 26 and Fig. 27).

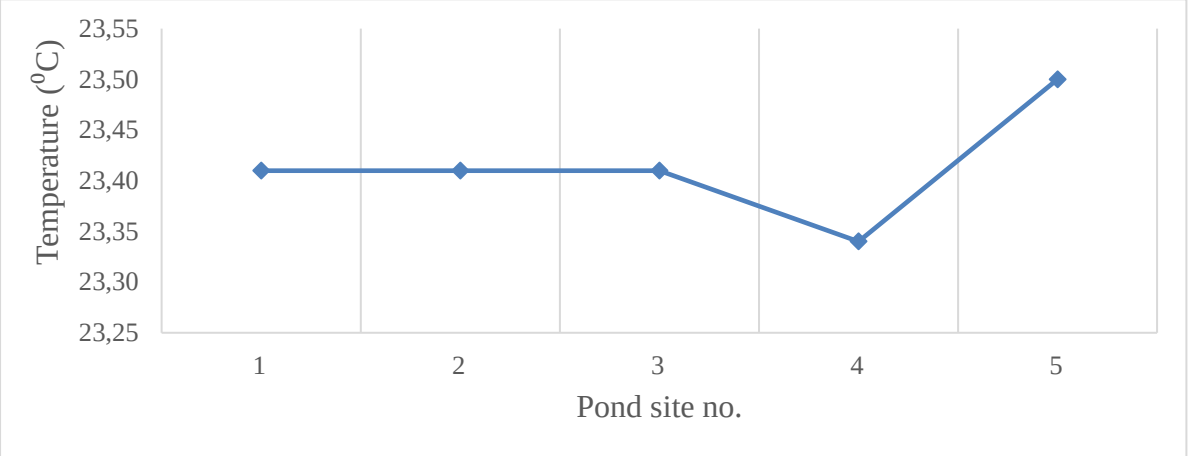


Figure 26: Temperatures recorded in stabilization ponds at Motetema on the 16th of January 2016

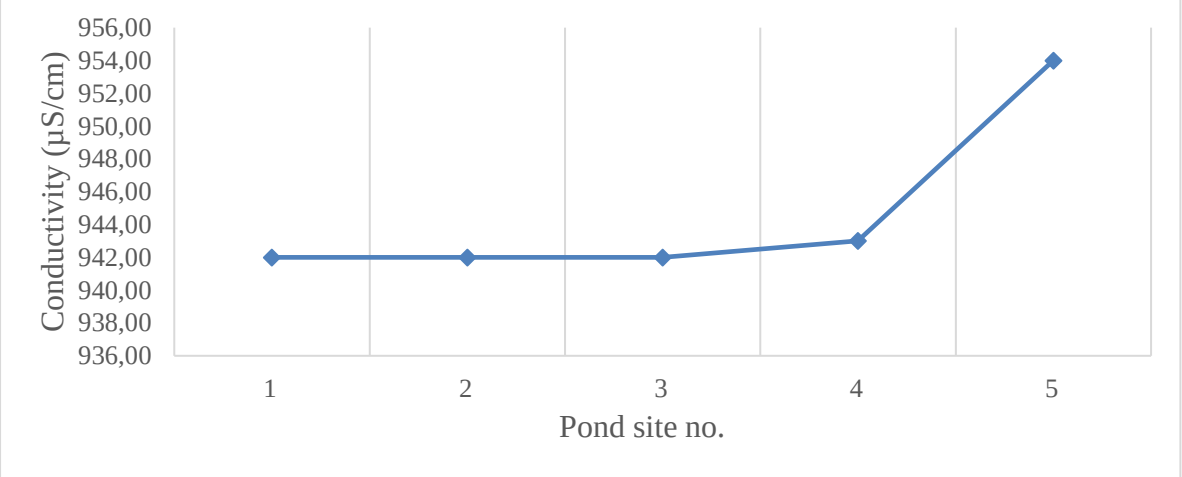


Figure 27: Conductivity recorded in stabilization ponds at Motetema on the 16th of January 2016

CHAPTER 5

Conclusions

This study was aimed at developing an approach for microalgae culturing and their use for pycoremediation of nutrients in urban wastewater. Two species of microalgae, *C. vulgaris* and *C. protothecoides* were cultured in round pools and in a raceway pond. The algae was first cultured in separate round pools in order to eliminate inter-species competition. The analysis was to evaluate the culturing conditions for both algae species in round pools and in a raceway pond, and this was done in a sequential process. As soon as the concentrations were high, they were mixed in the raceway with mixing to further increase the concentrations for dosing in the stabilization ponds. This research has revealed that these two *Chlorella* spp. can grow within certain ranges of physicochemical parameters and they showed potential for treating wastewater. The conclusions for this research were:

- The effects of different physicochemical variables were observed in the round pools and raceway pond. The minimum temperature of 10.6 °C was recorded in the raceway pond on the 8th of August 2015. The sampling period done on the 25th, 26th and 27th of November 2015 recorded the highest temperatures from the round pools ranging from 22.4 °C and 27.27 °C. The raceway pond recorded the lowest values for DO, with a difference of 15 % comparing to the maximum values. The minimum value for DO was 7.82 mg/ℓ and the maximum was 10.57 mg/ℓ. Conductivity showed a remarkable variability with minimum recordings of 292 µS/cm and maximum recordings of 361 µS/cm. The 24-hour sampling for conductivity was done on the 25th, 26th and 27th of November 2015 in the round pools only. The pH values recorded on the 25th, 26th and 27th of November 2015 did not show much difference. The lowest value recorded was 10.16 and the maximum being 10.7. According to McCarty (1964), the optimal pH is between 7.0 and 7.2 to avoid odour problems and harming the bacteria. Based on these major findings above, this study can therefore conclude that the physicochemical parameter ranges for the production of *C. vulgaris* and *C. protothecoides* are indicated in the Table 5 below:

Table 5: The physicochemical parameters range for microalgae culturing

Physicochemical Parameters	**Range**
Temperature (°C)	10.6 – 27.27
DO (mg/ℓ)	7.82 – 10.57
pH (units)	10.16 – 10.7
Conductivity (µS/cm)	292 - 361

- The algae culture cultivated from the round pools and raceway pond were used for treatment of urban wastewater at Motetema stabilization ponds. The stabilization ponds showed a constant value for total dissolved solids. The concentration of total dissolved solids remained stationary at 0.60 mg/ℓ from stabilization pond 1 to stabilization pond 5. The concentration of nitrates was constant from stabilization pond 1 to stabilization pond 3 at 0.88 mg/ℓ and it increased by 0.2 % from 0.88 mg/ℓ to 0.90 mg/ℓ in stabilization pond 4. Stabilization pond 5 showed a remarkable decrease of 10 % in the concentration of nitrates from 0.90 mg/ℓ to 0.81 mg/ℓ. DO concentration was 0.16 mg/ℓ for stabilization ponds 1 and 2 and it increased by 12.5 % from 0.16 to 0.18 mg/ℓ in stabilization pond 3. Stabilization pond 4 recorded a reduction in DO by 5.6 % from 0.18 to 0.17 mg/ℓ. This indicates that there was less algal photosynthesis in stabilization pond 4 as compared to stabilization ponds 3 and 5 because the generated oxygen was utilised by organic carbon mineralization. Stabilization pond 4 also experienced the highest nitrates concentration as compared to the rest of the stabilization ponds. Algal photosynthesis increased as evidenced in stabilization pond 5 with DO concentration of 0.20 mg/ℓ, which was an increase of 17.6 % from stabilization pond 4. This showed a reduction in the organic content of the final effluent in stabilization pond 5. Due to photosynthetic carbon dioxide assimilation, the pH values increased. The recordings for stabilization ponds 1 to 4 and stabilization pond 5 were 7.39 and 7.41 respectively. pH values were constant at 7.39 for stabilization ponds 1 to 4 and increased by 0.27 % in stabilization pond 5. Introduction of algae in wastewater stabilization ponds showed a decrease in the concentration of nitrates and chlorophyll *a* in the effluent from 0.90 mg/ℓ to 0.81 mg/ℓ and 46.40 µg/ℓ to 33.61 µg/ℓ respectively. The quality of wastewater showed a remarkable improvement in stabilization pond 5 as compared to other stabilization ponds. This was indicated by the concentration of chlorophyll *a*,

which remained constant at 45.38 µg/ℓ for stabilization ponds 1 to 3. Stabilization pond 4 remained the worst pond with highest recordings of nitrates and chlorophyll *a* of 0.90 mgℓ and 46.40 µg/ℓ respectively. However, stabilization pond 5 recorded a decrease in chlorophyll *a* concentration by 27.6 % from 46.40 µg/ℓ to 33.61 µg/ℓ. The results above showed that microalgae is capable of effective removal of nutrients from wastewater and has the potential to be implemented on a large scale.

The specific conclusions of this research were:

- The growth conditions for culturing *C. vulgaris* and *C. protothecoides* in the round pools and in a raceway pond were different.
- There was 7.95% decrease in the amount of nitrates in the stabilisation ponds after the introduction of algae.
- The *Chlorella* spp. had different uptake rates of ammonium with *C. vulgaris* proving to be more efficient than *C. protothecoids*.
- Microalgae is capable of effective removal of nutrients from wastewater and has the potential to be implemented on a large scale.

CHAPTER 6

6.1 Recommendations for future research

The following recommendations are made for future research:

- Much of the research work that has been conducted for algae culture has been done on a small scale, especially in the laboratories. Conducting such a research outdoors poses many challenges especially the contamination of the algae culture that will have a detrimental effect on algae growth. The round pools and raceway pond must be constructed in areas where minimal interference with algae culture is achieved in order to avoid contamination.

- Based on the results and conclusions indicated in Chapter 4 and 5 respectively, it is recommended that further research on the optimal conditions for algal growth be carried out on a lengthy period like one year or more to necessitate the collection of more data. This would make it easier to do statistical comparison and fully analyse the physicochemical parameters (such as pH, DO and temperature) in order to determine their extent of effect on algae culturing over a long period.

- Since this was a pilot project done on a small scale, further research is needed to be done on a large scale and the introduction of algae to wastewater treatment plants be monitored for a long period and be carried out on more than one wastewater treatment plant.

- Other factors that might affect the growth and survival of algae in wastewater stabilization ponds such as pesticides, fungicides, industrial influent and herbicides must be monitored. It becomes imperative that research be carried out to monitor the tolerance of algae to these chemicals.

- Measurement of light intensity was also important in this research as it a key parameter when studying algae growth. Unfortunately this requires specialised equipment that was not available for the study therefore it is recommended for future studies.

6.2 Limitations of the study

This research had some methodological limitations, which includes:

- Although this research used Pearson correlation analyses, more statistical analyses were essential if more data was collected. The data collected was insufficient to conduct a lot of statistical analysis hence charts were used.

- During data collection, problems were encountered with the instruments, resulting in pH not being recorded in August. This somehow affected the pH range as only November recordings were considered.

- More time was needed for data collection and analysis after the introduction of microalgae to stabilization ponds. It was essential to know the quality and type of influent, what was happening inside the stabilization ponds and the type and concentration of effluent. This could give a clear picture as to the efficiency of microalgae in wastewater treatment. Therefore, three major points of measurements were required, which were the influent, the pond and the effluent. In this research, the samples were collected from the stabilization ponds and the effluent without considering influent samples, making it so difficult to analyse the results.

- More time was needed to monitor the algae cell counts in the round pools, raceway pond and stabilization ponds. That was going to give a better insight of the growth of microalgae in relation to physicochemical parameters.

REFERENCES

ABU-REZQ TS, AL-MUSALLAM L, AL-SHIMMARI J and DIAS P (1999) Optimum production conditions for different high-quality marine algae. *Hydrobiologia* **403** (0) 97-107.

ACHARA N (2012) Biofuel from algae. *Journal of American Science* **8** (1) 240-244.

AHLUWALIA SS and GOYAL D (2007) Microbial and plant derived biomass for removal of heavy metals from wastewater. *Bioresource Technology* **98** (12) 2243-2257.

AKSU Z (2005) Application of biosorption for the removal of organic pollutants: a review. *Process Biochemistry* **40** (3-4) 997-1026.

ALSULL M and OMAR WMW (2012) Responses of *Tetraselmis* sp. and *Nannochloropsis* sp. isolated from Penang National Park coastal waters, Malaysia, to the combined influences of salinity, light and nitrogen limitation. *International Conference on Chemical, Ecology and Environmental Sciences* (ICEES'2012), March 17-18, Bangkok. 142-145.

AMENGUAL-MORRO C, MOYA-NIELL G and MARTINEZ-TABERNER A (2012) Phytoplankton as bioindicator for wastewater stabilization ponds. *Journal of Environmental Management* **95** S71-S76.

AN JY, SIM SJ, LEE JS and KIM BW (2003) Hydrocarbon production from secondarily treated piggery wastewater by green algae *Botryococcus braunii.* *Journal of Applied Phycology* **15** (2-3) 185-191.

ANDERSEN RA (ed.) (2005) Algal culturing techniques. Elsevier Academic Press, San Diego.

ANESIO AM, HODSON AJ, FRITZ A, PSENNER R and SATTLER B (2009) High microbial activity on glaciers: importance to the global carbon cycle. *Global Change Biology* **15** (4) 955-960.

AZOV Y (1982) Effect of pH on inorganic carbon uptake in algal cultures. *Applied and Environmental Microbiology* **43** (6) 1300-1306.

AZOV Y, SHELEF G, MORAINE R and LEVY A (1980) Controlling algal genera in high rates wastewater oxidation ponds. In *Algae Biomass*, (ed.) G. Shelef & C. Soeder, Elsevier/North Holland Biomedical Press, Amsterdam. 245-253 pp.

BARSANTI L and GUALTIERI P (2006) Algae: Anatomy, Biochemistry and Biotechnology. CRC Press, Taylor and Francis Group, New York. 2 pp.

BBALALI S, HOSEINI SA, GHORBANI R and KORDI H (2013) Relationships between nutrients and chlorophyll *a* concentration in the international Alma Gol wetland, Iran. *Journal of Aquaculture Research & Development* **4** 173 doi:10.4172/2155-9546.1000173.

BERMAN T and CHAVA S (1999) Algal growth on organic compounds as nitrogen sources. *Journal of Plankton Research* **21** (8) 1423-1437.

BHATT NC, PANWAR A, BISHT TS and TAMTA S (2014) Coupling of algal biofuel production with wastewater. *Scientific World Journal* 2014:210504. doi: 10.1155/2014/210504.

BLAIR MF, KOKABIAN B and GUDE VG (2014) Light and growth medium effect on *Chlorella vulgaris* biomass production. *Journal of Environmental Chemical Engineering* **2** (1) 665-674.

BOROWITZKA MA (1999) Commercial production of microalgae: ponds, tanks, and fermenters. *Progress in Industrial Microbiology* **35** 313-321.

BOROWITZKA MA (2005) Culturing microalgae in outdoor ponds. In: Anderson (ed) Algal culturing techniques. Elsevier Academic Press, San Diego. 205-218 pp.

BOUALI M, ZRAFI I, MOUNA F and BAKHROUF A (2012) Pilot study of constructed wetlands for tertiary wastewater treatment using duckweed and immobilized microalgae. *African Journal of Microbiology Research* **6** (31) 6066-6074.

BRENNAN L and OWENDE P (2010) Biofuels from microalgae: a review of technologies for production, processing and extractions of biofuels and co-products. *Renewable and Sustainable Energy Reviews* **14** (2) 557-577.

BRZEZINSKI MA (1985) The Si-C-N ration of marine diatoms: interspecific variability and the effect of some environmental variables. *Journal of Phycology* **21** (3) 347-357.

BUTTERWICK C, HEANEY SI and TALLING JF (2005) Diversity in the influence of temperature on the growth rates of freshwater algae, and its ecological relevance. *Freshwater Biology* **50** (2) 291-300.

CARVALHO AP, MEIRELES LA and MALCATA FX (2006) Microagal reactors: a review of enclosed system designs and performances. *Biotechnology Progress* **22** (6) 1490-1506.

CHAUMONT D (1993) Biotechnology of algal biomass production: a review of systems for outdoor mass culture. *Journal of Applied Phycology* **5** (6) 593-604.

CHISTI Y (2007) Biodiesel from microalgae. *Biotechnology Advances* **25** (3) 294-306.

CHO DY, LEE ST, PARK SW and CHUNG AS (1994) Studies on biosorption of heavy metals onto *Chlorella vulgaris*. *Journal of Environmental Science and Health* **29** (2) 389-409.

CHOI HJ and LEE SM (2013) Performance of *Chlorella vulgaris* for the removal of ammonia-nitrogen from wastewater. *Environmental Engineering Research* **18** (4) 235-239.

CHORUS I and BARTRAM J (1999) Toxic cyanobacteria in water: a guide to their public health consequences, monitoring, and management. St. Edmundsbury Press, Suffolk.

CONVERTI A, CASAZZA AA, ORTIZ EY, PEREGO P and DEL BORGHI M. (2009) Effect of temperature and nitrogen concentration on the growth and lipid content of *Nannochloropsis oculata* and *Chlorella vulgaris* for biodiesel production. *Chemical Engineering and Processing* **48** (6) 1146-1151.

CORRELL DL (1998) Role of phosphorus in the eutrophication of receiving waters: a review. *Journal of Environmental Quality* **27** (2) 261-266.

DE LA NOUE J, LALIBERTE G and PROULX D (1992) Algae and wastewater. *Journal of Applied Phycology* **4** (3) 247-254.

DEPARTMENT FOR INTERNATIONAL DEVELOPMENT (DFID) (1998) Guidance manual on water supply and sanitation programmes, London: Water Engineering and Development Centre (WEDC) for the Department for International Development.

DEPARTMENT OF WATER AFFAIRS (DWA) (2010) Water risk abatement plan. *A guideline to plan and manage towards safe and compliant wastewater collection and treatment in South Africa.* Water Research Commission, Draft 1. Republic of South Africa.

DORTCH Q and POSTEL JR (1989) Phytoplankton-Nitrogen interactions. In: Coastal Oceanography of Washington and Oregon (M.R. Landry & B.M. Hickey, eds.). Elsevier Science Publishers, Amsterdam. 139-173 pp.

FALLOWFIELD HJ and GARRETT MK (1985) The treatment of wastes by algae culture. *Journal of Applied Microbiology* **59** (s14) 187S-205S.

FARHADIAN M, VACHELARD C, DUCHEZ D and LARROCHE C (2008) In situ bioremediation of monoaromatic pollutants in the groundwater: a review. *Bioresource Technology* **99** (13) 5296-5308.

FRIEDMAN O, DUBINSKY Z and ARAD S (1991) Effect of light intensity on growth and polysaccharide production in red and blue-green rhodophyta unicells. *Bioresource Technology* **38** 105-110.

GANTAR M, OBREHT Z and DALMACIJA B (1991) Nutrient removal and algal succession during the growth of *Spirulina platensis* and *Scenedesmus quadricauda* on swine wastewater. *Bioresource Technology* ***36*** (2) 167-171.

GAST RJ, SANDERS RW and CARON DA (2009) Ecological strategies of protists and their symbiotic relationships with prokaryotic microbes. *Trends in Microbiology* **17** (12) 563-569.

GOOGLE MAP (2016) *Motetema A* 25° 6' 3.87" S; 29° 28' 6.78" E

URL: https://www.google.co.za/maps/place/Motetema-A (Accessed 18 March 2016).

GRAHAM LE, WILCOX LW and GRAHAM JM (2009) Algae. Pearson/Benjamin Cummings, San Francisco.

GRIMA EM, BELARBI EH, FERNÁNDEZ FGA, MEDINA AR and CHISTI Y (2003) Recovery of microagal biomass and metabolites: process options and economics. *Biotechnology Advances* **20** (7-8) 491-515.

GROBBELAAR JU (2006) Photosynthetic response and acclimation of microalgae to light fluctuations. In: Algal cultures analogues of blooms and applications. Subba Rao DV (ed.) Science Publishers Enfield (NH), USA, Plymouth, UK. 671-683 pp.

HAMZEH R and PONCE VM (2016) Design and performance of waste stabilization ponds. URL: http://ponce.sdsu.edu/ramadan/stabilizationponds.html (Accessed 05 April 2016).

HANAGATA N, TAKEUCHI T, FUKUJU Y, BARNES, DJ and KARUBE I (1992) Tolerance of microalgae to high CO_2 and high temperature. *Phytochemistry* **31** (10) 3345-3348.

HII YS, SOO CL, CHUAH TS, MOHD-AZMI A and ABOL-MUNAFI AB (2011) Interactive effect of ammonia and nitrate on the nitrogen uptake by *Nannochloropsis* sp. *Journal of Sustainability Science and Management* **6** (1*)* 60-68.

HOFFMANN JP (1998) Wastewater treatment with suspended and non-suspended algae. *Journal of Phycology* **34** (5) 757-763.

HOHAM RW, FILBIN RW, FREY FM, PUSACK TJ, RYBA JB, McDERMOTT PD and FIELDS RA (2007) The optimum pH of the green snow algae, *Chloromonas tughillensis* and *Chloromonas chenangoensis*, from upstate New York. *Arctic Antarctic and Alpine Research* **39** (1) 65-73.

JOB GM, KAVERIAMMAL S and ELAYARASI M (2014) Phytoremediation of domestic waste water of Gudiyatham town by microalgae *Chlorella vulgaris*. *International Journal of Environmental Biology* **4** (4) 243-247.

KAPLAN D, RICHMOND AE, DUBINSKY Z and AARONSON S (1986) Algal nutrition. In: Richmond A (ed.). Handbook for microalgal mass culture. CRC Press. 147-198 pp.

KESSLER E (1985) Upper limits of temperature for growth in *Chlorella (Chlorophyceae). Plant Systematics Evolution* **151** (1) 67-71.

KIM J, LINGARAJU BP, RHEAUME R, LEE JY and SIDDIQUI KF (2010) Removal of ammonia from wastewater effluent by *Chlorella vulgaris*. *Tshighua Science and Technol*ogy **15** (4) 391-396.

KIM W, PARK JM, GIM GH, JEONG SH, KANG CM, KIM DJ and KIM SW (2012) Optimization of culture conditions and comparison of biomass productivity of three green algae. *Bioprocess Biosystems Engineering* **35** (1-2) 19-27.

KNUD-HANSEN CF, MCELWEE K, BAKER J and CLAIR D (1998) Pond fertilization: ecological approach and practical application. *Pond dynamics/aquaculture collaborative research support program*. Oregon State University, Oregon.

KONOPKA A and BROCK TD (1978) Effect of temperature on blue-green algae (Cyanobacteria) in Lake Mendota. *Applied and Environmental Microbiology* **36** (4) 572-576.

KUDO I, MIYAMOTO M, NOIRI Y and MAITA Y (2000) Combined effects of temperature and iron on the growth and physiology of marine diatom *Phaeodactylum tricornutum* (Bacillariophyceae). *Journal of Phycology* **36** (6) 1096-1102.

KUMAR A, ERGAS S, YUAN X, SAHU A, ZHANG Q, DEWULF J, MALCATA XF and LANGENHOVE HV (2010) Enhanced CO_2 fixation and biofuel production via microalgae: recent developments and future directions. *Trends in Biotechnology* **28** (7) 371-380.

LAU PS, TAM NFY and WONG YS (1997) Wastewater nutrients (N and P) removal by carrageenan and alginate immobilized *Chlorella vulgaris. Environmental Technology* **18** (9) 945-951.

LAVENS P and SORGELOOS P (eds.) (1996) Manual on the production and use of live food for aquaculture. *FAO Fisheries Technical Paper*. No. 361. Rome, FAO. 295 pp.

LEAVITT PR, FINDLAY DL, HALL RI and SMOL JP (1999) Algal responses to dissolved organic carbon loss and pH decline during whole-lake acidification: evidence from paleolimnology. *American Society of Limnology and Oceanography* **44** (3, part 2) 757-773.

LEE JH, LEE JS, SHIN CS, PARK SC and KIM SW (2000) Effects of NO and SO_2 on growth of highly-CO_2-tolerant microalgae. *Journal of Microbiology and Biotechnology* **10** (3) 338-343.

LEE K and LEE CG (2001) Effect of light/dark cycles on wastewater treatments by microalgae. *Biotechnology Bioprocess Engineering* **6** (3) 194-199.

LEE RE (1980) Phycology. Cambridge University Press, New York. 3 pp.

LEE YK (2001) Microalgal mass culture system and methods: their limitation and potential. *Journal of Applied Phycology* **13** (4) 307-315.

LI WKW (1980) Temperature adaptation in phytoplankton: cellular and photosynthetic characteristics. In: Falkowski PG (ed.) Primary productivity in the sea. Plenum Press, New York. 259-279 pp.

LI Y, HORSMAN M, WU N, LAN CQ and DUBOIS-CALERO N (2008) Biofuels from microalgae. *Biotechnology Progress* **24** (4) 815-820.

LIM SL, CHU WL and PHANG SM (2010) Use *Chlorella vulgaris* for bioremediation of textile wastewater. *Bioresource Technology* **101** (19) 7314-7322.

LUNDQUIST TJ, WOERTZ IC, QUINN NWT and BENEMANN JR (2010) A realistic technology and engineering assessment of algae biofuel production. Energy and Biosciences Institute. Berkeley, California.

MAHAPATRA DM, CHANAKYA HN and RAMACHANDRA TV (2013) Treatment efficacy of algae-based sewage treatment plants. *Environmental Monitoring and Assessment* **185** (9) 7145-7164.

MALLICK N (2002) Biotechnological potential of immobilized algae for wastewater N, P and metal removal: a review. *BioMetals* **15** (4) 377-390.

MARA DD (1976) Sewage treatment in hot climates. John Wiley & Sons, London.

MARTINEZ L, REDONDAS V, GARCIA AI and MORAN A (2011) Optimization of growth operational conditions for CO_2 biofixation by native *Synechocystis* sp. *Journal of Chemical Technology and Biotechnology* **86** (5) 681-690.

MATA TM, MARTINS AA and CAETANO NS (2010) Microalgae for biodiesel production and other applications: a review. *Renewable and Sustainable Energy Reviews* **14** (1) 217-232.

MAYO AW (1997) Effects of temperature and pH on the kinetic growth of unialgal *Chlorella vulgaris* cultures containing bacteria. *Water Environment Research* **69** (1) 64-72.

McCARTY PL (1964) Anaerobic waste treatment fundamentals. *Public Works* **95 (**9-12) 95-126.

MOEENI SA and KUMAR JLG (2014) Wastewater quality analysis and design of waste stabilization ponds of Shiats. *International Journal of Engineering and Advanced Technology* **4** (2) 2249-8958.

MUIGAI PG, SHIUNDU PM, MWAURA FB and KAMAU GN (2010) Correlation between dissolved oxygen and total dissolved solids and their role in the eutrophication of Nairobi Dam, Kenya. *International Journal of BioChemiPhysics* **18** 38-46.

MUÑOZ R and GUIEYSSE B (2006) Algal-bacterial processes for the treatment of hazardous contamination: a review. *Water Research* **40** (15) 2799-2815.

NIJBOER RC and VERDONSCHOT PFM (2004) Variable selection for modelling effects of eutrophication on stream and river ecosystems. *Ecological Modelling* **177** (1-2) 17-39.

OBERHOLSTER PJ (2014) The piloting of algae-based wastewater treatment (phycoremediation) to achieve a significant improvement of the quality of effluent discharged at two municipal wastewater treatment works. *Work Plan, Deliverables, Schedule and Capital expenditure.* Council for Scientific and Industrial Research (CSIR).

ONO E and CUELLO JL (2003) Selection of optimal microalgae species for CO_2 sequestration. Proceedings of second annual conference on carbon sequestration. Alexandria, Virginia.

ÓRPEZ R, MARTINEZ ME, HODAIFA G, YOUSFI FE, JBARI N and SANCHEZ S (2009) Growth of the microalga *Botryococcus braunii* in secondarily treated sewage. *Desalination* **246** (1-3) 625-630.

OSWALD WJ (1988) Microalgae and wastewater treatment. In: Borowitzka MA, Borowitzka LJ (Eds.). Micro-algal Biotechnology. Cambridge University Press, Cambridge. 305-328 pp.

OSWALD WJ (2003) My sixty years in applied algology. *Journal of Applied Phycology* **15** (2) 99-106.

OSWALD WJ, GOTAAS HB, GOLUEKE CG, KELLEN WR, GLOYNA EF and HERMANN ER (1957) Algae in waste treatment. *Sewage and Industrial Wastes* **29** (4) 437-457.

PALIWAL R (2012) Phycoremediation – a clean technology for water pollution abatement. URL: http://www.slideshare.net/Paliwal23/phycoremediation-a-clean-technology-for-water-pollution-abatement (Accessed 05 April 2016).

PEDERSEN MF and HANSEN PJ (2003) Effects of high pH on the growth and survival of six marine heterotrophic protists. *Marine Ecology Progress Series* **260** 33-41.

PEDRONI P, DAVISON J, BECKERT H, BERGMAN P and BENEMANN J (2001) A proposal to establish an international network on biofixation of CO_2 and greenhouse gas abatement with microalgae. *Journal of Energy and Environmental Research* **1** (1) 136-150.

PITTMAN JK, DEAN AP and OSUNDEKO O (2011) The potential of sustainable algal biofuel production using wastewater resources. *Bioresource Technology* **102** (1) 17-25.

QIANG H and RICHMOND A (1996) Productivity and photosynthetic efficiency of *Spirulina platensis* as affected by light intensity, algal density and rate of mixing in a flat plate photobioreactor. *Journal of Applied Phycology* **8** (2) 139-145.

RAMARAJ R, TSAI DDW and CHEN PH (2010) Algae growth in natural water resources. *Journal of Soil and Water Conservation* **42** (4) 439-450.

RAN N, AGAMI M and ORON G (2004) A pilot study of constructed wetlands using duckweed (*Lemna gibba L.*) for treatment of domestic primary effluent in Israel. *Water Research* **38** (9) 2241-2248.

REYNOLDS CS (1984) The ecology of freshwater phytoplankton. Cambridge studies in ecology. Cambridge University Press, Cambridge.

ROUND FE (1984) The ecology of algae. Cambridge University Press, New York.

SEKHUKHUNE DISTRICT MUNICIPALITY (SDM) (2013) Operating manual for Motetema stabilization ponds. Infrastructure and Water Services. Inhlakanipho Consultants. Republic of South Africa.

SHARMA GK and KHAN SA (2013) Bioremediation of sewage wastewater using selective algae for manure production. *International Journal of Environmental Engineering and Management* **4** (6) 573 – 580.

SHEEHAN J, DUNAHAY T, BENEMANN J and ROESSLER P (1998) A look back at the U.S. Department of Energy's aquatic species program: biodiesel from algae. NREL/TP-580–24190. National Renewable Energy Laboratory (NREL). Golden, Colorado, USA. 2 pp.

SHEN Y, YUAN W, PEI ZJ, WU Q and MAO E (2009) Microalgae mass production methods. *Transactions of the ASABE* **52** (4) 1275-1287.

SINGH SK, BANSAL A, JHA MK and JAIN R (2013) Production of biodiesel from wastewater grown *Chlorella minutissima*. *Indian Journal of Chemical Technology* **20** (5) 341-345.

STANLEY CD, CLARKE RA, MCNEAL BL and MACLEOD BW (2003) Relationship of chlorophyll *a* concentration to seasonal water quality in Lake Manatee, Florida. In: Publication of the soil and water science department, Florida Cooperative Extension Service, IFAS, University of Florida, EDIS.

SUDHAKAR K and PREMALATHA M (2012) Micro-algal technology for sustainable energy production: state of the art. *Journal of Sustainable Energy and Environment* **3** 59-62.

SUZUKI Y and TAKAHASHI M (1995) Growth responses of several diatom species isolated from various environments to temperature. *Journal of Phycology* **31** (6) 880-888.

TAM NFY, LAU PS and WONG YS (1994) Wastewater inorganic N and P removal by immobilized *Chlorella vulgaris. Water Science and Technology* **30** (6) 369-374.

TCHOBANOGLOUS G and BURTON FL (1991) Wastewater engineering: treatment disposal reuse. McGraw-Hill, New York.

UDUMAN N, QI Y, DANQUAH MK, FORDE GM and HOADLEY A (2010) Dewatering of microalgal cultures: a major bottleneck to algae-based fuels. *Journal of Renewable and Sustainable Energy* **2** (1) 012701.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY (USEPA) (2002). Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms, Fourth Edition. EPA-821-R-02-013. Washington, DC.

URBAN MAIN PLACE (UMP) (2011) The population development of Motetema. URL: http://www.statisticssouthafrica (web)/adrianfrith.com (Accessed 23 March 2016).

VACCA G, WAND H, NIKOLAUSZ M, KUSCHK P and KÄSTNER M (2005) Effect of plants and filter materials on bacteria removal in pilot-scale constructed wetlands. *Water Research* **39** (7) 1361-1373.

VALDERRAMA LT, DEL CAMPO CM, RODRIGUEZ CM, DE-BASHAN LE and BASHAN Y (2002) Treatment of recalcitrant wastewater from ethanol and citric acid production using the microalga *Chlorella vulgaris* and the macrophyte *Lemna minuscula. Water Research* **36** (17) 4185-4192.

WANG C, YU X, LV H and YANG J (2013) Nitrogen and phosphorus removal from municipal wastewater by the green alga *Chlorella* sp. *Journal of Environmental Biology* **34** (2 Spec No) 421-425.

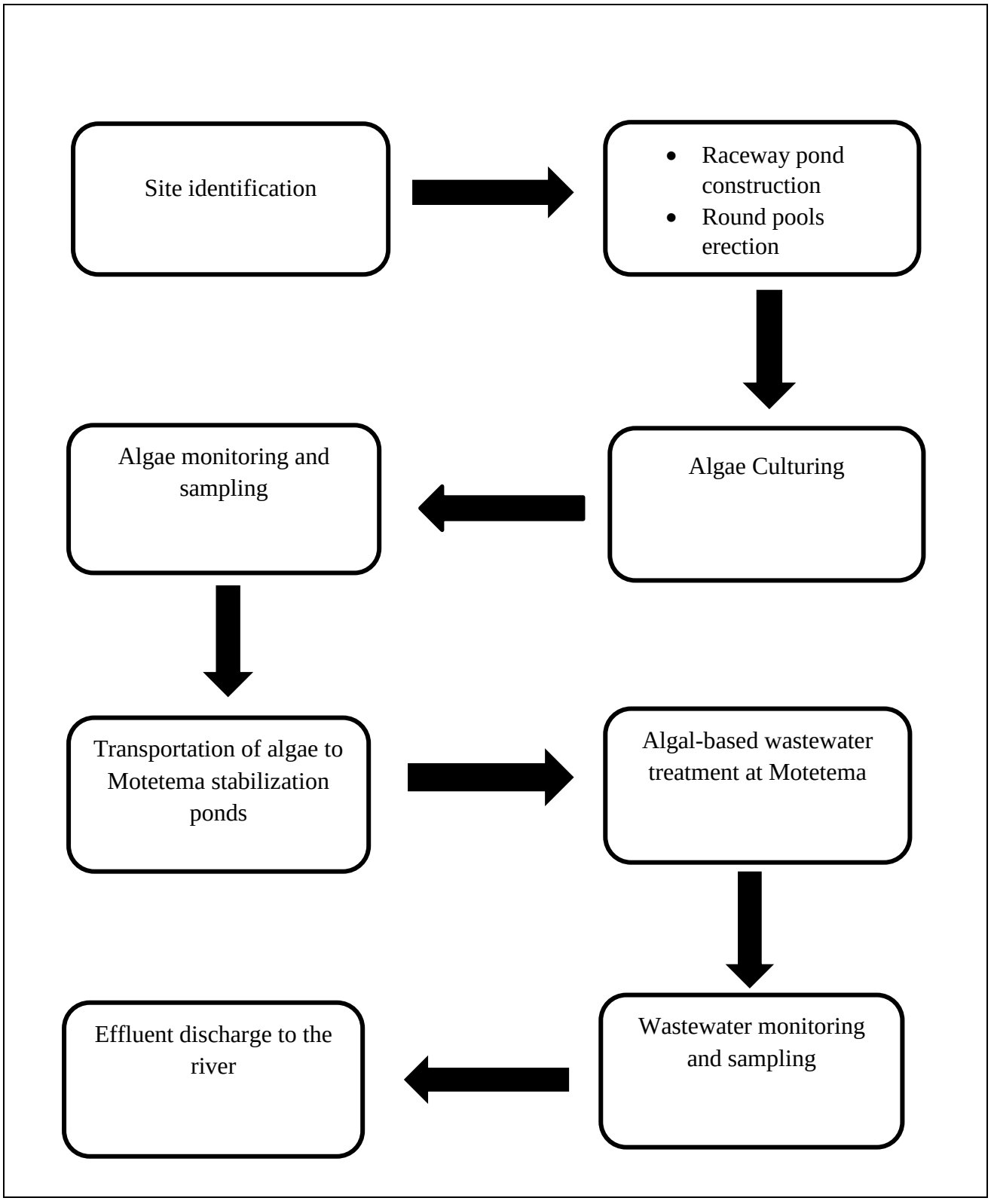
WILEY PE, BRENNEMAN KJ and JACOBSON AE (2009) Improved algal harvesting using suspended air flotation. *Water Environment Research* **81** (7) 702-708.

YAAKOB Z, KAMARUDIN KF, RAJKUMAR R, TAKRIFF MS and BADAR SN (2014) The current methods for the biomass production of the microalgae from wastewaters: an overview. *World Applied Sciences Journal* **31** (10) 1744-1758.

ZENG ZL, MENZIES NW and KERVEN G (2009) Employing *Chlorella protothecoides* for metal bioavailability studies under acidic conditions. *Journal of Plankton Research* **31** (3) 325-336.

APPENDICES

Appendix A: A flow diagram of the whole approach



Appendix B: Schematic layout of the algae culture in round pools and raceway pond

Round Pool 1: *C. vulgaris*

Round Pool 2: *C. protothecoides*

Rectangular shaped raceway pond with algae culture

Paddlewheel mixing algae culture

Appendix C: Hydrolab DS 5 used during the data collection

Parameters measured: temperature (°C), conductivity (µS/cm), pH (units), chlorophyll *a* (µg/ℓ) and nitrate (mg/ℓ)

Appendix D: Data collected from round pools and raceway pond on the 7th and 8th of August 2015

Data collected from Round Pool 1 containing *C. vulgaris* – Top Section

Date (D/M/YYYY)	Time (Hours)	Temp (^{0}C)	Conductivity (µS/cm)	TDS (mg/ℓ)	Oxygen (mg/ℓ)	Oxygen (%)	Samples in 50ml Falcon tube	Samples in 100ml bottle
7/8/2015	2000hrs	15,00	326,00	160,00	9,27	109,30		
7/8/2015	2200hrs	15,20	329,00	161,00	9,11	106,40		
7/8/2015	2300hrs	14,90	335,00	164,00	9,05	106,00	yes	yes
8/8/2015	0000hrs	14,50	336,00	165,00	9,08	105,10		
8/8/2015	0100hrs	14,50	334,00	163,00	9,04	104,40		yes
8/8/2015	0200hrs	14,20	333,00	163,00	9,02	103,50		
8/8/2015	0300hrs	13,90	334,00	164,00	8,99	102,60	yes	yes
8/8/2015	0400hrs	13,70	333,00	163,00	9,08	103,10		
8/8/2015	0500hrs	13,50	333,00	163,00	8,97	101,50		yes
8/8/2015	0600hrs	13,30	332,00	163,00	8,88	100,10		
8/8/2015	0700hrs	13,00	332,00	162,00	8,92	99,90	yes	yes
8/8/2015	0800hrs	12,60	332,00	163,00	9,15	101,20		
8/8/2015	0900hrs	12,70	331,00	162,00	9,18	101,50		yes
8/8/2015	1000hrs	12,90	329,00	161,00	9,36	103,90		
8/8/2015	1100hrs	13,40	329,00	161,00	9,40	105,90	yes	yes
8/8/2015	1200hrs	14,10	328,00	161,00	9,55	109,40		
8/8/2015	1300hrs	14,90	327,00	160,00	9,52	110,80		yes
8/8/2015	1400hrs	15,50	326,00	160,00	10,04	118,90		
8/8/2015	1500hrs	15,90	326,00	160,00	10,05	120,20	yes	yes
8/8/2015	1600hrs	16,30	325,00	159,00	10,08	121,90		
8/8/2015	1700hrs	16,30	326,00	160,00	10,27	123,30		yes
8/8/2015	1800hrs	16,20	326,00	160,00	10,33	123,80		
8/8/2015	1900hrs	16,00	326,00	160,00	8,09	96,20	yes	yes

Data collected from Round Pool 1containing *C. vulgaris* – Bottom Section

Date (D/M/YYYY)	Time (Hours)	Temp (^{0}C)	Conductivity (µS/cm)	TDS (mg/ℓ)	Oxygen (mg/ℓ)	Oxygen (%)	Samples in 50ml Falcon tube	Samples in 100ml bottle
7/8/2015	2000hrs	15,50	323,00	159,00	8,99	105,90		
7/8/2015	2200hrs	14,80	347,00	170,00	9,24	107,80		
7/8/2015	2300hrs	14,70	335,00	164,00	9,16	106,60	yes	yes
8/8/2015	0000hrs	14,70	331,00	162,00	9,14	106,00		
8/8/2015	0100hrs	14,50	333,00	163,00	9,08	104,90		yes
8/8/2015	0200hrs	14,20	333,00	163,00	9,11	104,60		
8/8/2015	0300hrs	14,00	334,00	164,00	9,08	103,80	yes	yes
8/8/2015	0400hrs	13,80	332,00	163,00	9,11	103,40		
8/8/2015	0500hrs	13,50	333,00	163,00	9,05	102,20		yes
8/8/2015	0600hrs	13,20	333,00	163,00	9,02	101,40		
8/8/2015	0700hrs	13,00	331,00	162,00	9,04	101,00	yes	yes
8/8/2015	0800hrs	12,80	330,00	162,00	9,17	101,80		
8/8/2015	0900hrs	12,80	330,00	162,00	9,23	102,20		yes
8/8/2015	1000hrs	12,90	329,00	161,00	9,49	105,30		
8/8/2015	1100hrs	13,50	329,00	161,00	9,52	107,10	yes	yes
8/8/2015	1200hrs	14,10	328,00	161,00	9,65	110,40		
8/8/2015	1300hrs	14,80	326,00	160,00	9,80	113,90		yes
8/8/2015	1400hrs	15,50	326,00	159,00	10,03	118,50		
8/8/2015	1500hrs	16,00	325,00	159,00	10,21	122,00	yes	yes
8/8/2015	1600hrs	16,40	325,00	159,00	10,42	125,60		
8/8/2015	1700hrs	16,40	325,00	159,00	10,57	127,40		yes
8/8/2015	1800hrs	16,20	326,00	160,00	10,57	127,10		
8/8/2015	1900hrs	16,10	325,00	159,00	10,29	123,10	yes	yes

Data collected from Round Pool 2 containing *C. protothecoides* – Top Section

Date (D/M/YYYY)	Time (Hours)	Temp (^{0}C)	Conductivity (µS/cm)	TDS (mg/ℓ)	Oxygen (mg/ℓ)	Oxygen (%)	Samples in 50ml Falcon tube	Samples in 100ml bottle
7/8/2015	2000hrs	15,50	331,00	162,00	10,89	128,50		
7/8/2015	2200hrs	15,10	341,00	167,00	10,83	127,00		
7/8/2015	2300hrs	14,90	335,00	164,00	10,63	123,80	yes	yes
8/8/2015	0000hrs	14,80	336,00	164,00	10,50	121,90		
8/8/2015	0100hrs	14,50	338,00	166,00	10,34	119,20		yes
8/8/2015	0200hrs	14,30	338,00	166,00	10,58	121,60		
8/8/2015	0300hrs	14,00	338,00	166,00	10,31	117,70	yes	yes
8/8/2015	0400hrs	13,70	337,00	165,00	10,25	116,30		
8/8/2015	0500hrs	13,50	337,00	165,00	10,42	117,60		yes
8/8/2015	0600hrs	13,20	337,00	165,00	10,19	114,10		
8/8/2015	0700hrs	13,00	336,00	165,00	10,28	114,60	yes	yes
8/8/2015	0800hrs	12,80	335,00	164,00	10,31	114,40		
8/8/2015	0900hrs	12,70	335,00	164,00	10,36	114,80		yes
8/8/2015	1000hrs	12,90	334,00	164,00	10,85	120,60		
8/8/2015	1100hrs	13,60	333,00	163,00	11,16	126,40	yes	yes
8/8/2015	1200hrs	14,30	331,00	162,00	11,11	127,30		
8/8/2015	1300hrs	15,10	330,00	162,00	11,93	139,40		yes
8/8/2015	1400hrs	15,60	329,00	161,00	12,12	143,50		
8/8/2015	1500hrs	16,20	329,00	161,00	12,21	147,10	yes	yes
8/8/2015	1600hrs	16,50	328,00	161,00	12,54	151,90		
8/8/2015	1700hrs	16,60	328,00	161,00	12,80	155,10		yes
8/8/2015	1800hrs	16,40	328,00	161,00	13,04	157,20		
8/8/2015	1900hrs	16,20	329,00	161,00	12,86	154,30	yes	yes

Data collected from Round Pool 2 containing *C. protothecoides* – Bottom Section

Date (D/M/YYYY)	Time (Hours)	Temp (^{0}C)	Conductivity (µS/cm)	TDS (mg/ℓ)	Oxygen (mg/ℓ)	Oxygen (%)	Samples in 50ml Falcon tube	Samples in 100ml bottle
7/8/2015	2000hrs	15,60	334,00	164,00	10,71	124,70		
7/8/2015	2200hrs	15,20	341,00	167,00	10,73	124,70		
7/8/2015	2300hrs	14,90	334,00	164,00	10,72	124,60	yes	yes
8/8/2015	0000hrs	14,80	335,00	164,00	10,70	124,40		
8/8/2015	0100hrs	14,50	337,00	165,00	10,61	122,60		yes
8/8/2015	0200hrs	14,30	338,00	166,00	10,22	117,00		
8/8/2015	0300hrs	14,00	338,00	166,00	10,52	120,10	yes	yes
8/8/2015	0400hrs	13,70	337,00	165,00	10,47	118,90		
8/8/2015	0500hrs	13,50	337,00	165,00	9,99	112,80		yes
8/8/2015	0600hrs	13,20	337,00	165,00	10,40	116,60		
8/8/2015	0700hrs	13,00	336,00	165,00	10,38	115,90	yes	yes
8/8/2015	0800hrs	12,80	335,00	164,00	10,50	116,60		
8/8/2015	0900hrs	12,70	336,00	164,00	10,67	118,20		yes
8/8/2015	1000hrs	12,90	335,00	164,00	10,97	121,90		
8/8/2015	1100hrs	13,50	333,00	163,00	11,27	127,00	yes	yes
8/8/2015	1200hrs	14,20	332,00	163,00	11,52	131,90		
8/8/2015	1300hrs	14,90	330,00	162,00	11,91	138,70		yes
8/8/2015	1400hrs	15,40	329,00	161,00	12,24	144,80		
8/8/2015	1500hrs	16,10	329,00	161,00	12,54	150,30	yes	yes
8/8/2015	1600hrs	16,40	329,00	161,00	12,87	155,50		
8/8/2015	1700hrs	16,60	328,00	161,00	13,21	159,90		yes
8/8/2015	1800hrs	16,40	328,00	161,00	13,16	158,80		
8/8/2015	1900hrs	16,20	329,00	161,00	13,03	156,60	yes	yes

Data collected from Raceway Pond containing *C. vulgaris* and *C. protothecoides*

Date (D/M/YYYY)	Time (Hours)	Temp (^{0}C)	Conductivity (µS/cm)	TDS (mg/ℓ)	Oxygen (mg/ℓ)	Oxygen (%)	Samples in 50ml Falcon tube	Samples in 100ml bottle
7/8/2015	2000hrs	13,50	343,00	168,00	8,44	95,60		
7/8/2015	2200hrs	13,40	351,00	172,00	7,87	96,10		
7/8/2015	2300hrs	12,90	345,00	169,00	8,46	94,70	yes	yes
8/8/2015	0000hrs	12,80	346,00	170,00	8,45	94,00		
8/8/2015	0100hrs	12,50	349,00	171,00	8,48	93,60		yes
8/8/2015	0200hrs	12,20	349,00	171,00	8,52	93,30		
8/8/2015	0300hrs	11,80	350,00	172,00	8,46	92,00	yes	yes
8/8/2015	0400hrs	11,60	349,00	171,00	8,53	92,30		
8/8/2015	0500hrs	11,50	348,00	171,00	8,77	95,40		yes
8/8/2015	0600hrs	11,00	349,00	171,00	8,55	91,10		
8/8/2015	0700hrs	10,70	349,00	171,00	8,61	91,20	yes	yes
8/8/2015	0800hrs	10,60	348,00	171,00	8,68	92,10		
8/8/2015	0900hrs	11,00	347,00	170,00	8,58	91,40		yes
8/8/2015	1000hrs	11,50	346,00	170,00	8,61	92,70		
8/8/2015	1100hrs	12,20	346,00	169,00	8,54	93,40	yes	yes
8/8/2015	1200hrs	12,90	345,00	169,00	8,49	94,70		
8/8/2015	1300hrs	13,60	344,00	168,00	8,46	96,00		yes
8/8/2015	1400hrs	14,30	343,00	168,00	8,46	97,70		
8/8/2015	1500hrs	14,70	343,00	168,00	8,36	97,30	yes	yes
8/8/2015	1600hrs	14,60	344,00	168,00	8,35	97,20		
8/8/2015	1700hrs	14,50	344,00	168,00	8,33	96,80		yes
8/8/2015	1800hrs	14,40	344,00	169,00	8,33	96,40		
8/8/2015	1900hrs	14,20	344,00	169,00	8,35	96,00	yes	yes

Data collected from Raceway Pond containing *C. vulgaris* and *C. protothecoides* – Control Section

Date (D/M/YYYY)	Time (Hours)	Temp (^{0}C)	Conductivity (µS/cm)	TDS (mg/ℓ)	Oxygen (mg/ℓ)	Oxygen (%)	Samples in 50ml Falcon tube	Samples in 100ml bottle
7/8/2015	2000hrs	13,50	341,00	167,00	8,49	95,80		
7/8/2015	2200hrs	13,20	351,00	172,00	8,40	94,20		
7/8/2015	2300hrs	12,90	345,00	169,00	8,39	93,40	yes	yes
8/8/2015	0000hrs	13,00	345,00	169,00	8,80	98,20		
8/8/2015	0100hrs	12,60	348,00	171,00	8,70	96,20		yes
8/8/2015	0200hrs	12,40	348,00	171,00	8,91	98,80		
8/8/2015	0300hrs	12,10	348,00	171,00	8,71	95,40	yes	yes
8/8/2015	0400hrs	11,80	348,00	171,00	8,80	96,10		
8/8/2015	0500hrs	11,20	349,00	171,00	8,68	93,30		yes
8/8/2015	0600hrs	11,20	348,00	170,00	8,80	94,20		
8/8/2015	0700hrs	10,90	348,00	170,00	8,69	92,70	yes	yes
8/8/2015	0800hrs	10,90	346,00	170,00	8,66	92,40		
8/8/2015	0900hrs	11,40	343,00	168,00	8,31	90,00		yes
8/8/2015	1000hrs	11,80	342,00	168,00	8,30	91,10		
8/8/2015	1100hrs	12,80	343,00	168,00	8,14	91,40	yes	yes
8/8/2015	1200hrs	13,60	337,00	165,00	8,11	92,60		
8/8/2015	1300hrs	14,00	338,00	166,00	7,93	92,10		yes
8/8/2015	1400hrs	14,50	341,00	167,00	7,90	94,10		
8/8/2015	1500hrs	15,10	339,00	166,00	7,82	93,00	yes	yes
8/8/2015	1600hrs	14,90	339,00	166,00	7,97	94,40		
8/8/2015	1700hrs	14,80	341,00	167,00	7,93	93,60		yes
8/8/2015	1800hrs	14,80	340,00	167,00	7,97	94,00		
8/8/2015	1900hrs	14,40	342,00	167,00	8,11	94,10	yes	yes

Appendix E: Data collected from round pool 1 on the 25th and 26th of November 2015 and round pool 2 on the 26th and 27th of November 2015

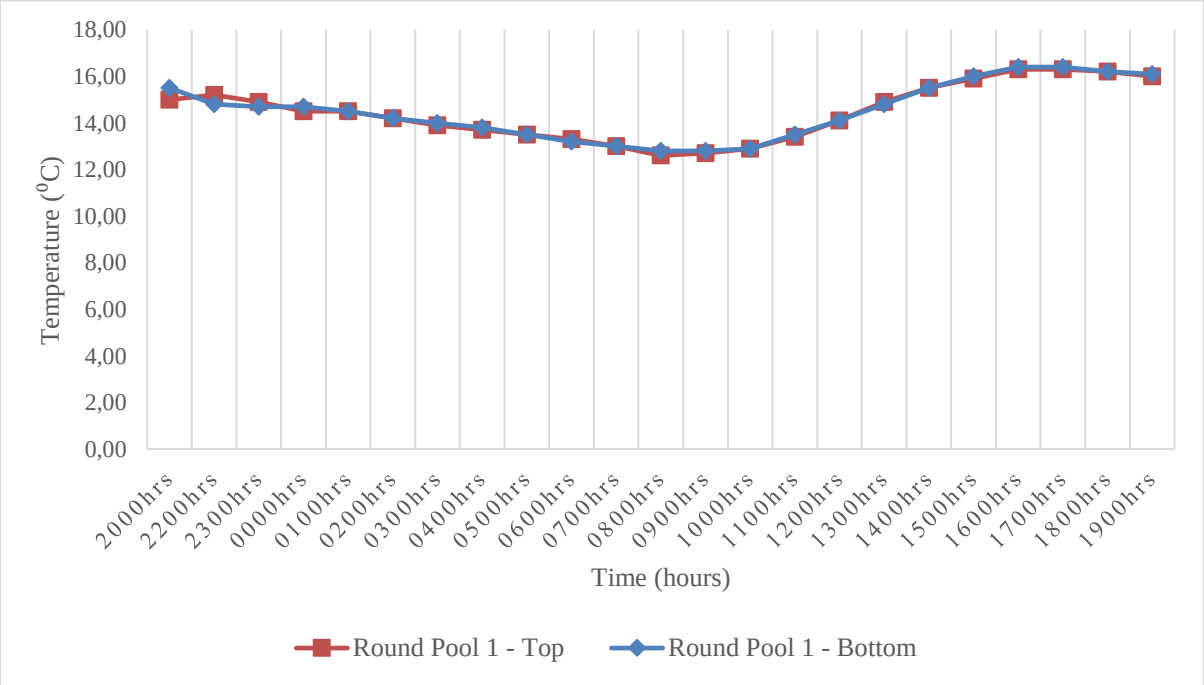
Data collected from Round Pool 1 containing *C. vulgaris*

Date	Time	Temp	pH	SpCond	Sal	TDS	NH4+	NO3-	CHL *a*
M/D/YYYY	Hours	^{0}C	Units	µS/cm	ppt	g/l	mg/l-N	mg/l-N	µg/l
11/25/2015	1130hrs	23,44	10,59	324,00	0,16	0,20	7,78	0,03	203,17
11/25/2015	1200hrs	23,96	10,61	328,00	0,16	0,20	13,46	0,03	182,47
11/25/2015	1230hrs	24,46	10,62	330,00	0,16	0,20	18,25	0,03	164,07
11/25/2015	1300hrs	24,95	10,62	338,00	0,17	0,20	22,08	0,03	151,10
11/25/2015	1330hrs	25,46	10,63	341,00	0,17	0,20	25,11	0,03	148,32
11/25/2015	1400hrs	25,91	10,63	345,00	0,17	0,20	27,91	0,03	182,58
11/25/2015	1430hrs	26,31	10,63	351,00	0,17	0,20	30,38	0,03	192,03
11/25/2015	1500hrs	26,65	10,64	357,00	0,18	0,20	32,26	0,03	208,28
11/25/2015	1530hrs	26,97	10,64	359,00	0,18	0,20	34,19	0,03	216,00
11/25/2015	1600hrs	27,17	10,65	362,00	0,18	0,20	35,67	0,03	235,46
11/25/2015	1630hrs	27,27	10,65	362,00	0,18	0,20	37,55	0,03	198,22
11/25/2015	1700hrs	27,13	10,65	362,00	0,18	0,20	39,19	0,03	265,08
11/25/2015	1730hrs	26,91	10,65	361,00	0,18	0,20	40,84	0,03	184,29
11/25/2015	1800hrs	26,70	10,65	362,00	0,18	0,20	43,66	0,03	153,03
11/25/2015	1830hrs	26,51	10,65	360,00	0,18	0,20	46,02	0,03	138,80
11/25/2015	1900hrs	26,24	10,64	358,00	0,18	0,20	47,68	0,03	169,78
11/25/2015	1930hrs	26,08	10,64	358,00	0,18	0,20	49,28	0,03	114,50
11/25/2015	2000hrs	25,93	10,64	355,00	0,17	0,20	50,08	0,03	142,58
11/25/2015	2030hrs	25,73	10,64	353,00	0,17	0,20	51,49	0,03	195,15
11/25/2015	2100hrs	25,57	10,64	352,00	0,17	0,20	53,10	0,03	111,19
11/25/2015	2130hrs	25,39	10,63	350,00	0,17	0,20	54,12	0,03	119,97
11/25/2015	2200hrs	25,19	10,63	348,00	0,17	0,20	55,49	0,04	220,38
11/25/2015	2230hrs	25,06	10,63	346,00	0,17	0,20	56,80	0,03	149,73
11/25/2015	2300hrs	24,89	10,63	344,00	0,17	0,20	57,90	0,03	208,76
11/25/2015	2330hrs	24,67	10,63	342,00	0,17	0,20	58,94	0,04	233,02
11/26/2015	0000hrs	24,51	10,62	340,00	0,17	0,20	59,99	0,04	213,91
11/26/2015	0030hrs	24,35	10,62	339,00	0,17	0,20	60,49	0,04	149,51
11/26/2015	0100hrs	24,12	10,62	338,00	0,17	0,20	61,09	0,04	239,50
11/26/2015	0130hrs	23,92	10,62	336,00	0,16	0,20	61,99	0,04	200,30
11/26/2015	0200hrs	23,74	10,61	334,00	0,16	0,20	62,77	0,04	133,77
11/26/2015	0230hrs	23,57	10,61	333,00	0,16	0,20	63,08	0,04	170,03
11/26/2015	0300hrs	23,40	10,61	331,00	0,16	0,20	63,58	0,04	210,29
11/26/2015	0330hrs	23,25	10,61	329,00	0,16	0,20	64,14	0,04	277,30
11/26/2015	0400hrs	23,08	10,60	329,00	0,16	0,20	64,98	0,04	173,21
11/26/2015	0430hrs	22,91	10,60	327,00	0,16	0,20	65,55	0,04	171,09
11/26/2015	0500hrs	22,75	10,60	326,00	0,16	0,20	66,27	0,04	211,27
11/26/2015	0530hrs	22,60	10,60	325,00	0,16	0,20	67,00	0,04	190,54
11/26/2015	0600hrs	22,43	10,60	324,00	0,16	0,20	67,12	0,04	196,80
11/26/2015	0630hrs	22,30	10,60	324,00	0,16	0,20	67,40	0,04	141,89
11/26/2015	0700hrs	22,24	10,60	324,00	0,16	0,20	67,25	0,04	161,80
11/26/2015	0730hrs	22,20	10,60	324,00	0,16	0,20	67,22	0,04	171,51
11/26/2015	0800hrs	22,14	10,61	324,00	0,16	0,20	67,51	0,04	179,96
11/26/2015	0830hrs	22,18	10,62	326,00	0,16	0,20	66,77	0,04	170,78
11/26/2015	0900hrs	22,23	10,63	328,00	0,16	0,20	65,29	0,04	133,60
11/26/2015	0930hrs	22,34	10,64	330,00	0,16	0,20	64,93	0,04	149,38
11/26/2015	1000hrs	22,52	10,66	333,00	0,16	0,20	62,91	0,04	163,41
11/26/2015	1030hrs	22,78	10,68	336,00	0,16	0,20	61,49	0,04	151,48
11/26/2015	1100hrs	23,17	10,70	340,00	0,17	0,20	59,79	0,04	144,17

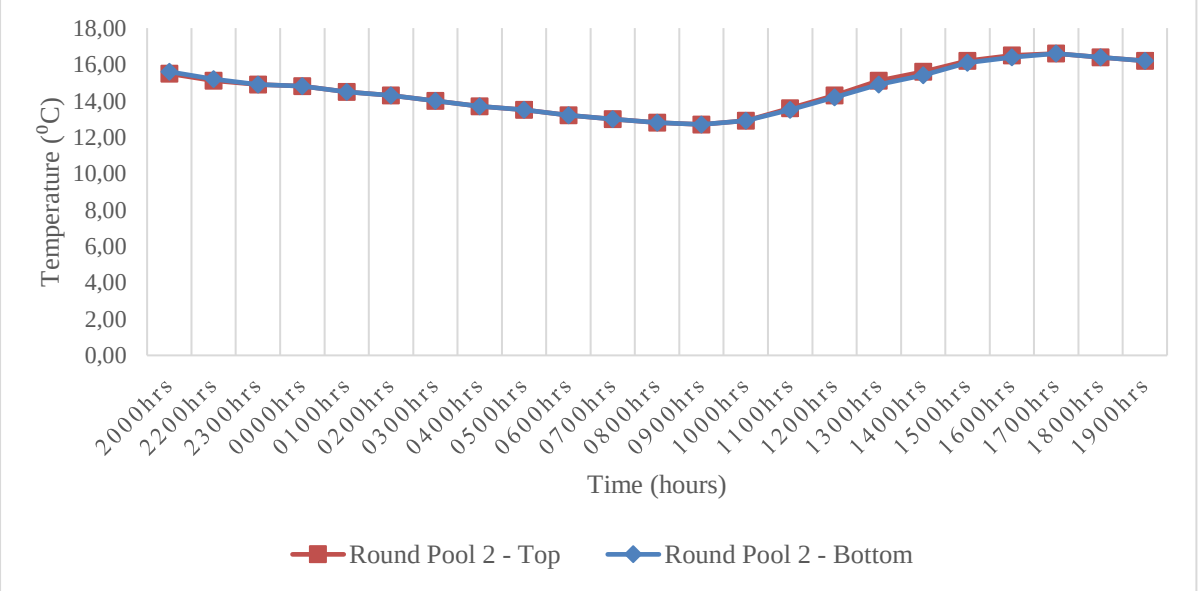
Data collected from Round Pool 2 containing *C. protothecoides*

Date	Time	Temp	pH	SpCond	Sal	TDS	NH4+	NO3-	CHL *a*
M/D/YYYY	Hours	^{0}C	Units	µS/cm	ppt	g/l	mg/l-N	mg/l-N	µg/l
11/26/2015	1200hrs	24,70	10,16	296,00	0,14	0,20	94,17	0,04	27,05
11/26/2015	1230hrs	25,28	10,16	298,00	0,14	0,20	104,57	0,04	25,98
11/26/2015	1300hrs	25,82	10,16	299,00	0,15	0,20	107,40	0,04	23,91
11/26/2015	1330hrs	26,34	10,17	300,00	0,15	0,20	106,71	0,04	22,30
11/26/2015	1400hrs	26,83	10,17	301,00	0,15	0,20	105,20	0,04	20,98
11/26/2015	1430hrs	27,14	10,17	301,00	0,15	0,20	105,80	0,04	26,05
11/26/2015	1500hrs	27,18	10,18	302,00	0,15	0,20	105,83	0,04	27,19
11/26/2015	1530hrs	27,34	10,19	305,00	0,15	0,20	100,51	0,04	30,36
11/26/2015	1600hrs	27,52	10,20	306,00	0,15	0,20	97,15	0,04	28,71
11/26/2015	1630hrs	27,44	10,21	307,00	0,15	0,20	98,82	0,04	40,61
11/26/2015	1700hrs	27,34	10,22	308,00	0,15	0,20	97,63	0,04	44,10
11/26/2015	1730hrs	27,14	10,22	307,00	0,15	0,20	96,37	0,04	41,70
11/26/2015	1800hrs	26,99	10,23	308,00	0,15	0,20	97,69	0,04	39,68
11/26/2015	1830hrs	26,78	10,24	307,00	0,15	0,20	99,88	0,04	34,30
11/26/2015	1900hrs	26,58	10,24	307,00	0,15	0,20	100,98	0,04	49,94
11/26/2015	1930hrs	26,38	10,24	306,00	0,15	0,20	102,06	0,04	37,49
11/26/2015	2000hrs	26,17	10,24	305,00	0,15	0,20	102,55	0,04	52,09
11/26/2015	2030hrs	26,05	10,23	304,00	0,15	0,20	104,84	0,04	40,14
11/26/2015	2100hrs	25,86	10,23	303,00	0,15	0,20	106,52	0,04	38,55
11/26/2015	2130hrs	25,67	10,23	303,00	0,15	0,20	107,17	0,04	33,71
11/26/2015	2200hrs	25,51	10,23	302,00	0,15	0,20	107,86	0,04	31,27
11/26/2015	2230hrs	25,30	10,24	301,00	0,15	0,20	108,47	0,04	38,40
11/26/2015	2300hrs	25,10	10,24	301,00	0,15	0,20	109,12	0,04	38,70
11/26/2015	2330hrs	24,90	10,23	300,00	0,15	0,20	109,72	0,05	32,43
11/27/2015	0000hrs	24,67	10,24	300,00	0,15	0,20	110,24	0,05	40,25
11/27/2015	0030hrs	24,48	10,24	299,00	0,14	0,20	110,27	0,05	48,85
11/27/2015	0100hrs	24,23	10,24	298,00	0,14	0,20	110,51	0,05	44,15
11/27/2015	0130hrs	24,02	10,24	298,00	0,14	0,20	110,18	0,05	55,66
11/27/2015	0200hrs	23,82	10,24	297,00	0,14	0,20	110,85	0,05	49,07
11/27/2015	0230hrs	23,65	10,24	296,00	0,14	0,20	110,83	0,05	54,90
11/27/2015	0300hrs	23,43	10,24	296,00	0,14	0,20	110,64	0,05	66,62
11/27/2015	0330hrs	23,18	10,24	295,00	0,14	0,20	110,62	0,05	60,87
11/27/2015	0400hrs	22,94	10,24	295,00	0,14	0,20	110,93	0,05	68,08
11/27/2015	0430hrs	22,71	10,24	294,00	0,14	0,20	110,61	0,05	52,37
11/27/2015	0500hrs	22,52	10,24	294,00	0,14	0,20	110,90	0,05	67,05
11/27/2015	0530hrs	22,28	10,24	293,00	0,14	0,20	111,12	0,05	46,34
11/27/2015	0600hrs	22,08	10,25	293,00	0,14	0,20	110,08	0,05	63,73
11/27/2015	0630hrs	21,88	10,25	292,00	0,14	0,20	109,17	0,05	48,82
11/27/2015	0700hrs	21,70	10,26	292,00	0,14	0,20	108,47	0,05	44,86
11/27/2015	0730hrs	21,56	10,26	292,00	0,14	0,20	106,50	0,05	51,20
11/27/2015	0800hrs	21,48	10,27	292,00	0,14	0,20	104,56	0,05	47,26
11/27/2015	0830hrs	21,53	10,28	293,00	0,14	0,20	103,13	0,05	45,04
11/27/2015	0900hrs	21,62	10,29	294,00	0,14	0,20	101,88	0,05	34,76
11/27/2015	0930hrs	21,80	10,29	294,00	0,14	0,20	100,66	0,05	39,33
11/27/2015	1000hrs	22,10	10,29	295,00	0,14	0,20	99,19	0,05	33,44
11/27/2015	1030hrs	22,35	10,31	296,00	0,14	0,20	96,94	0,05	36,78
11/27/2015	1100hrs	22,65	10,31	298,00	0,14	0,20	96,65	0,05	29,24
11/27/2015	1130hrs	23,16	10,31	299,00	0,14	0,20	93,71	0,05	29,23

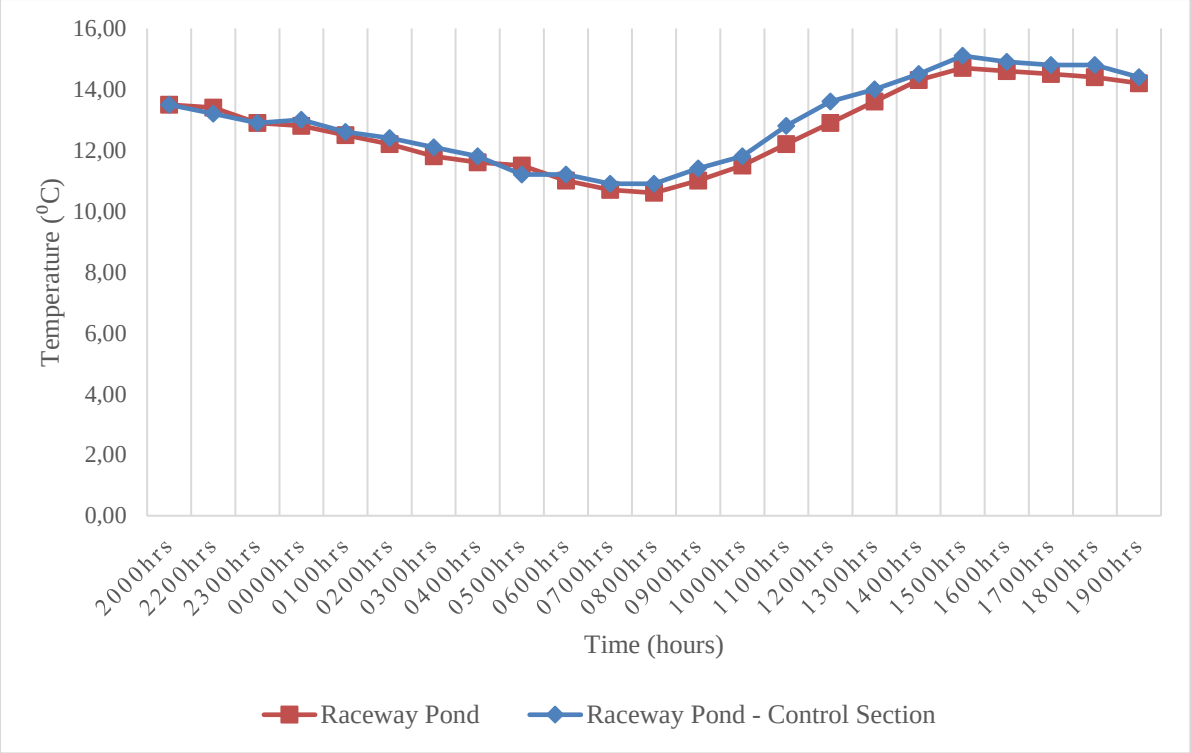
Appendix F: Analysis of temperatures recorded in round pools and raceway pond on the 7th and 8th of August 2015



Temperatures recorded in round pool 1

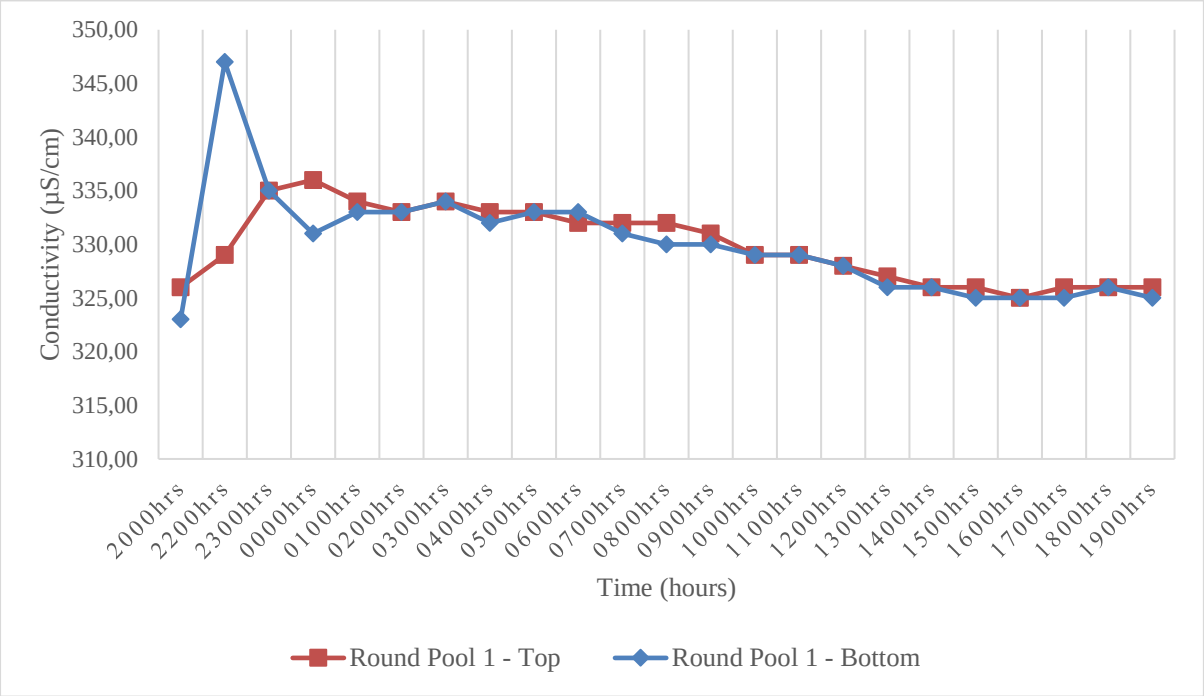


Temperatures recorded in round pool 2

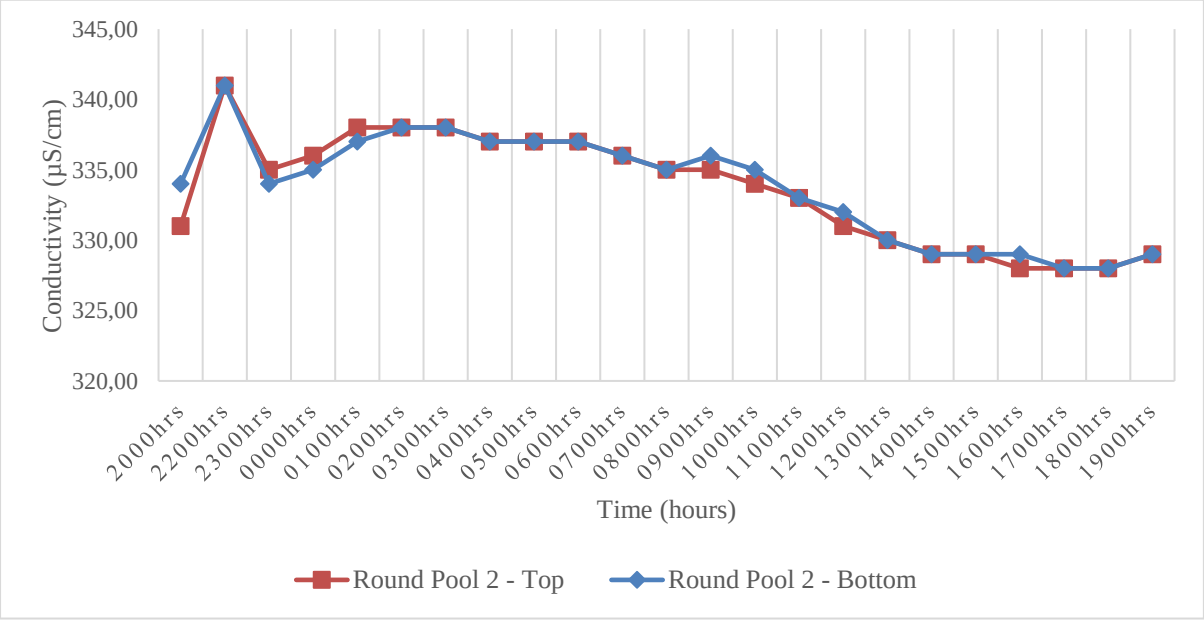


Temperatures recorded in the raceway pond

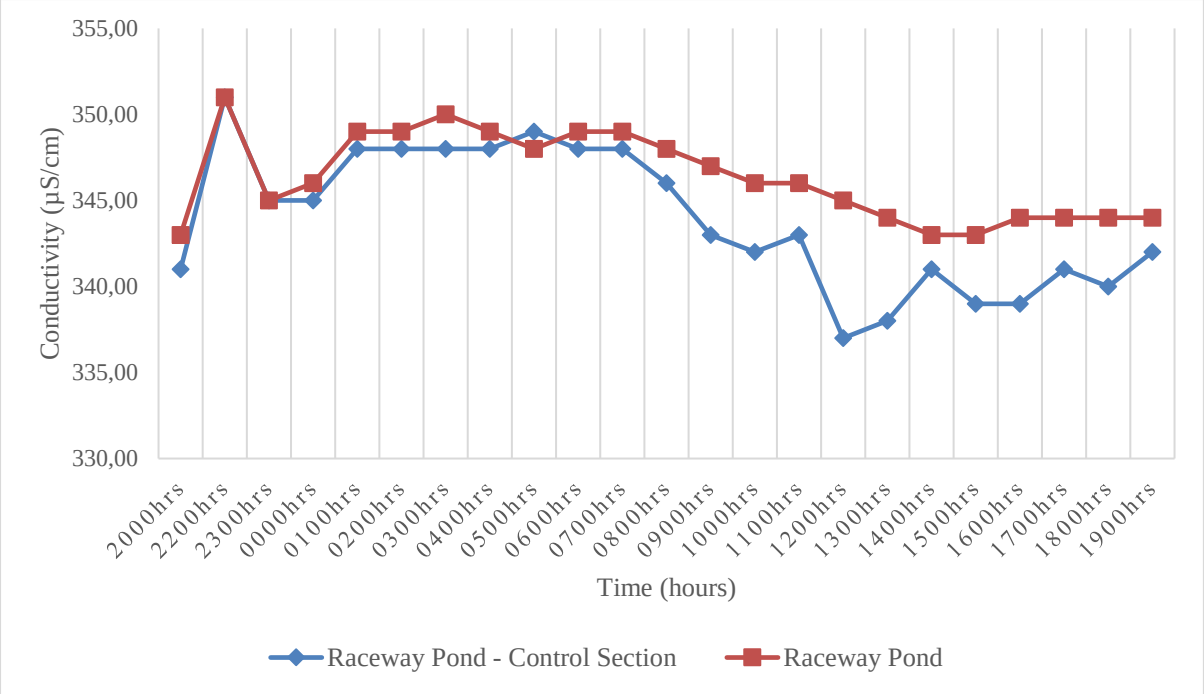
Appendix G: Analysis of conductivity recorded in round pools and raceway pond on the 7th and 8th of August 2015



Conductivity recorded in round pool 1

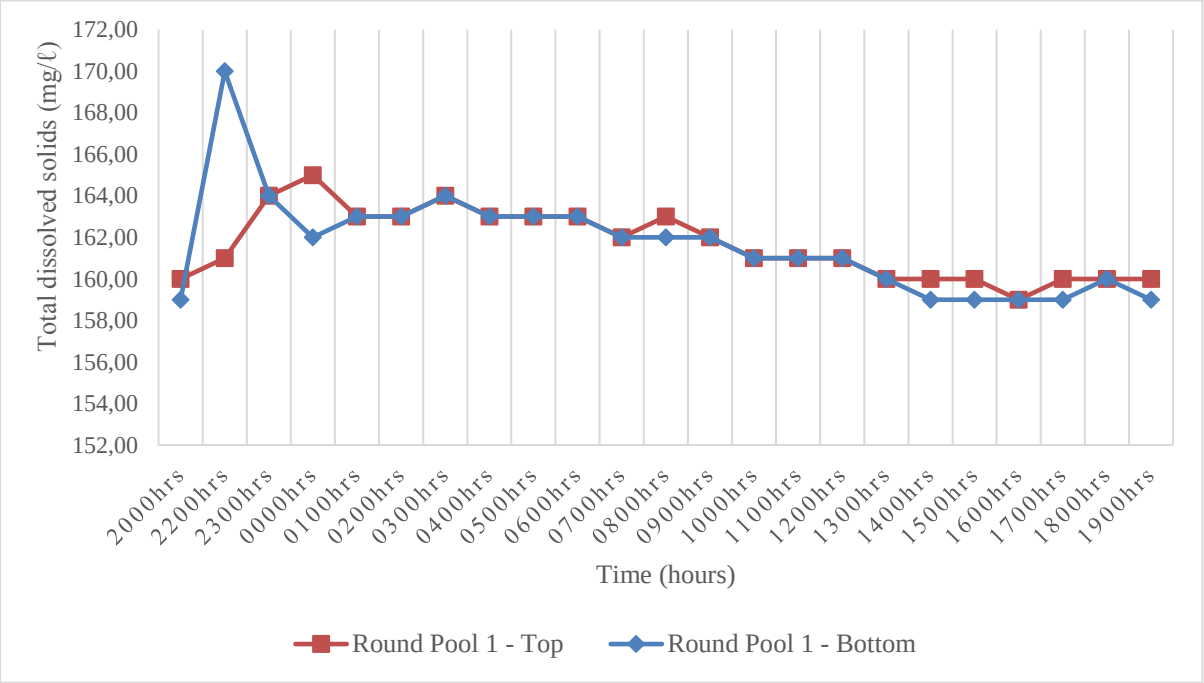


Conductivity recorded in round pool 2

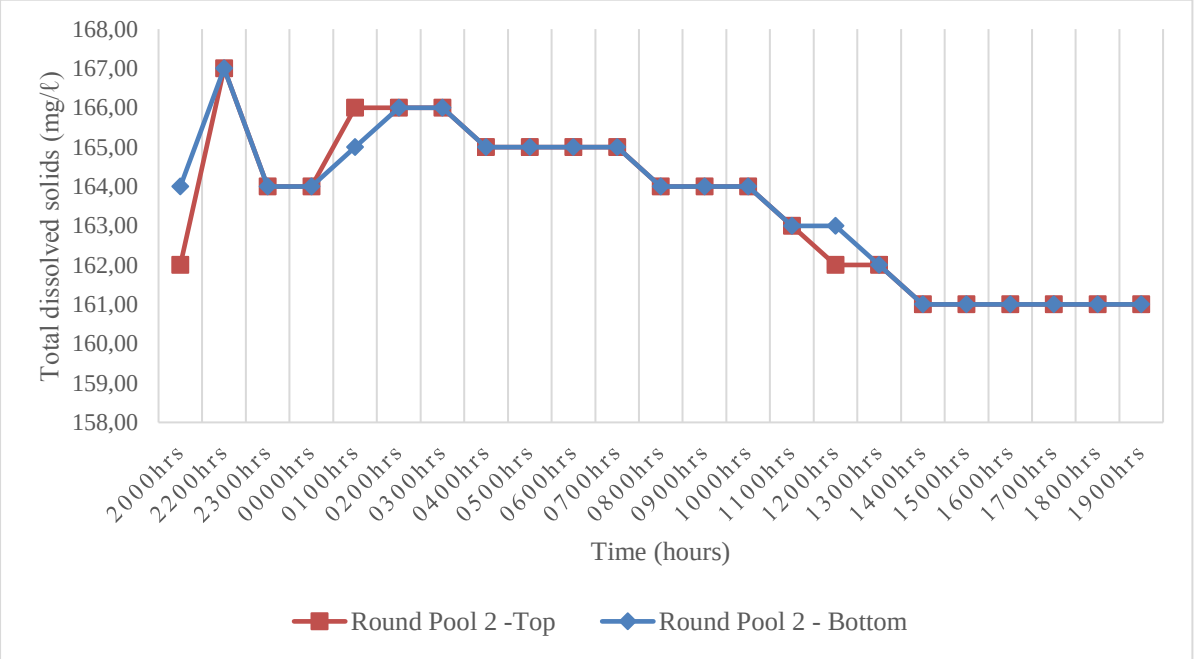


Conductivity recorded in the raceway pond

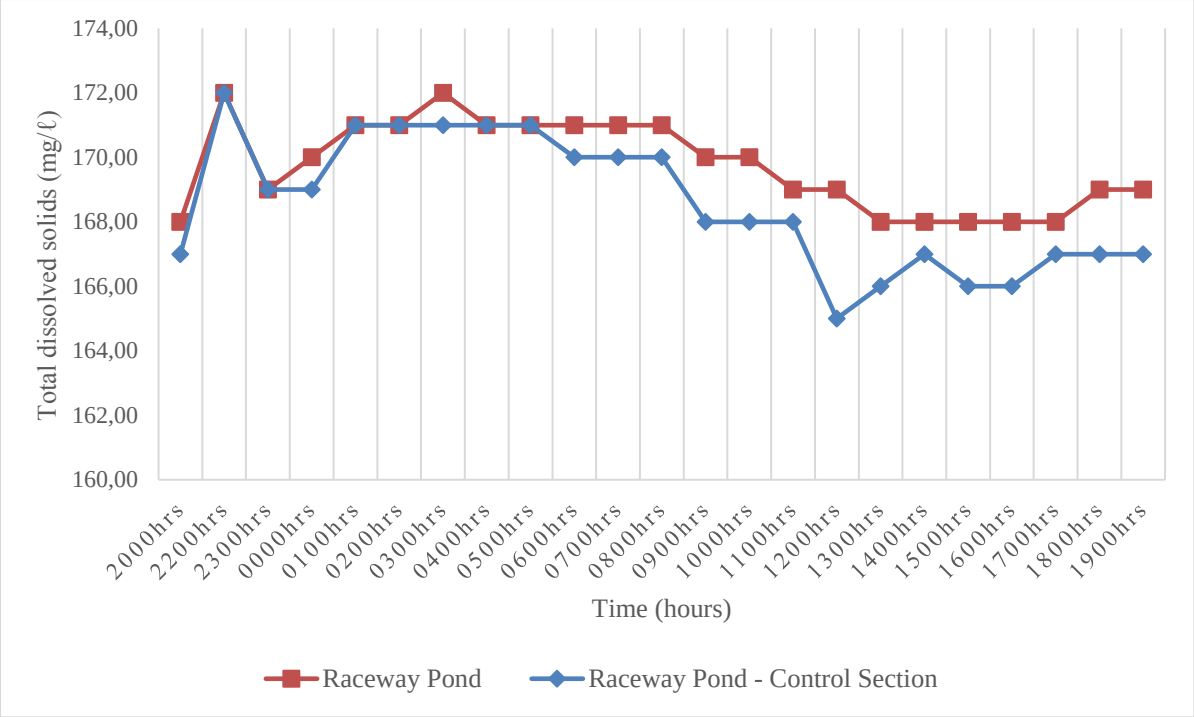
Appendix H: Analysis of total dissolved solids recorded in round pools and raceway pond on the 7th and 8th of August 2015



Total dissolved solids recorded in round pool 1

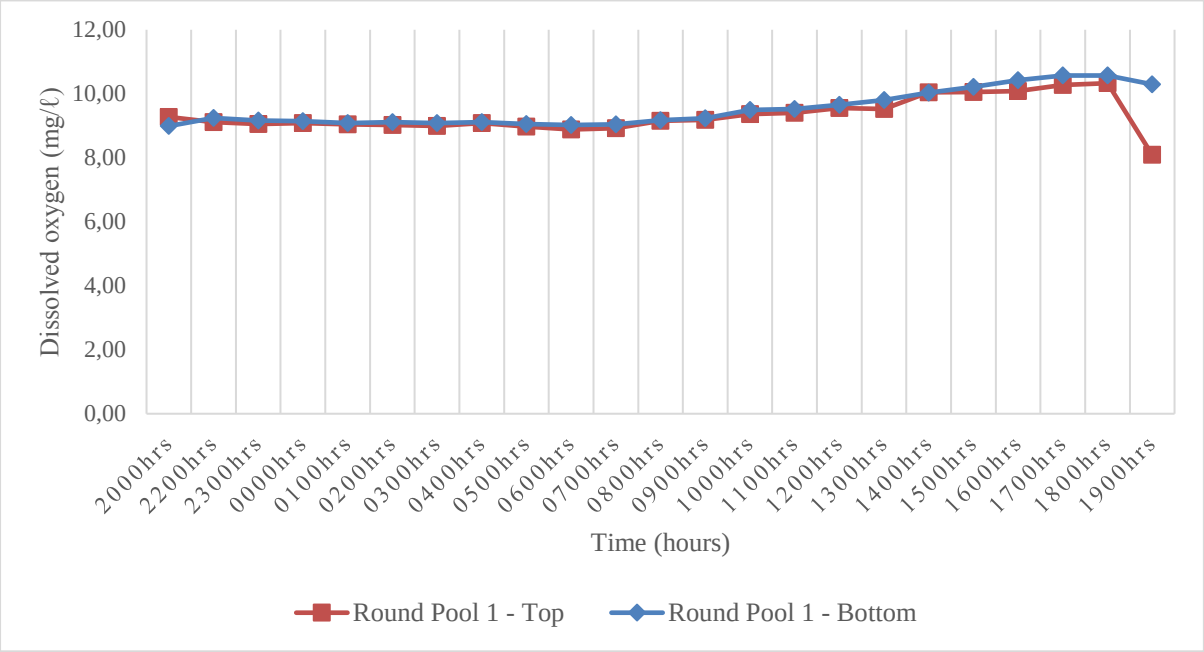


Total dissolved solids recorded in round pool 2

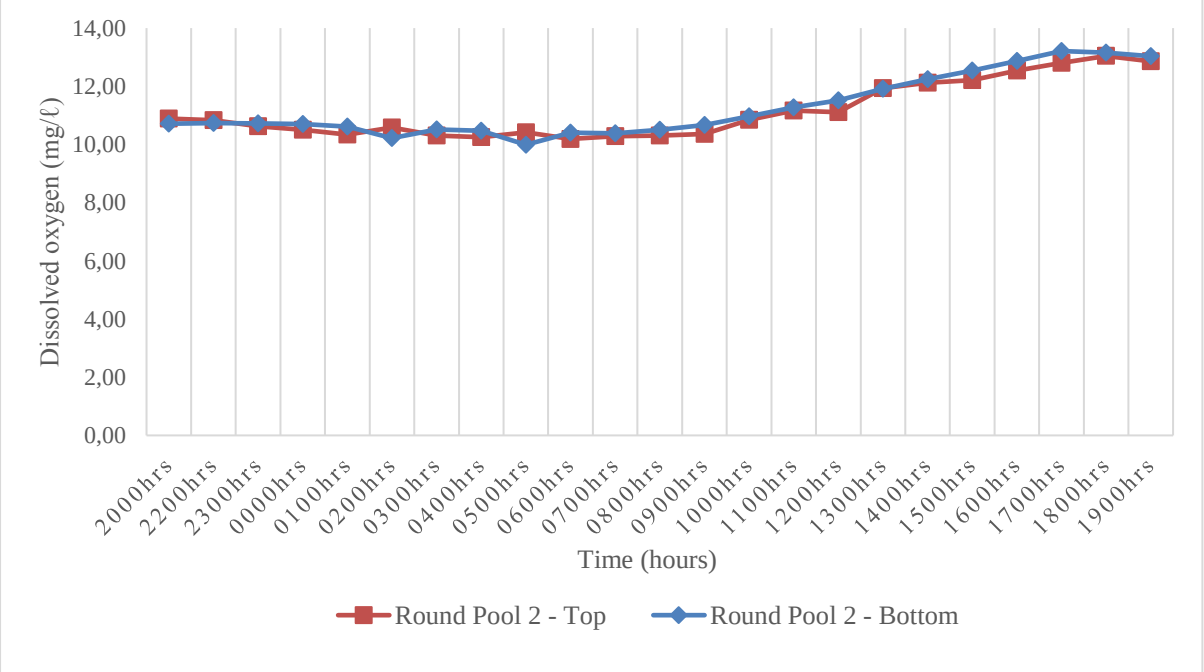


Total dissolved solids recorded in the raceway pond

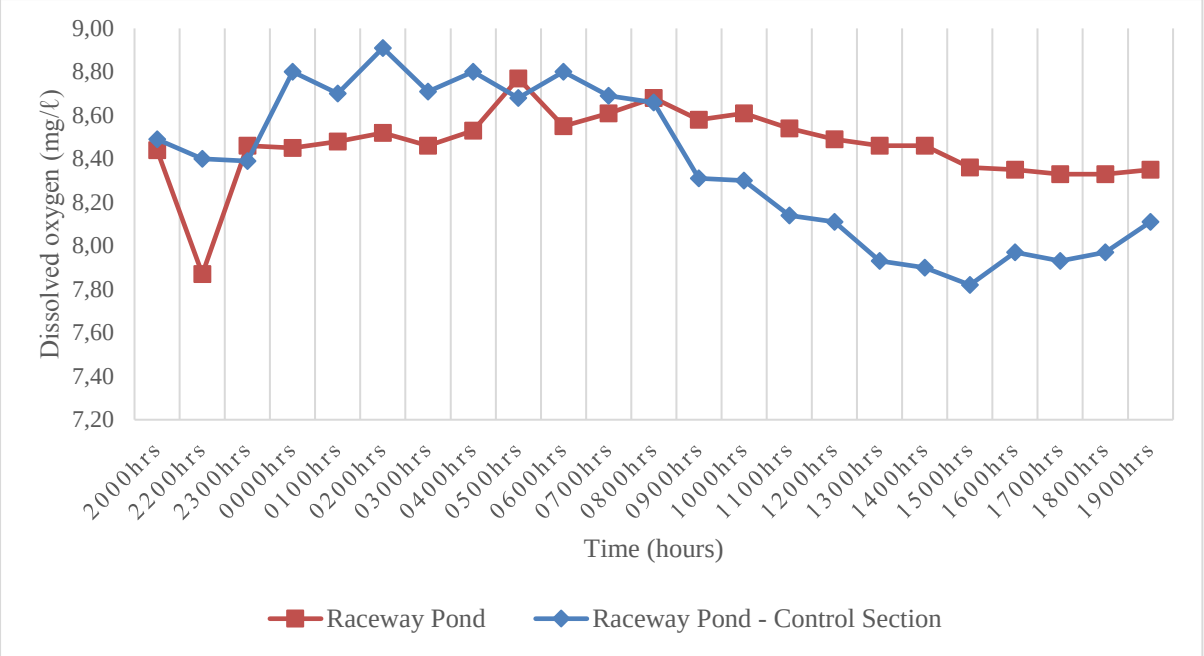
Appendix I: Analysis of dissolved oxygen recorded in round pools and raceway pond on the 7th and 8th of August 2015



Dissolved oxygen recorded in round pool 1

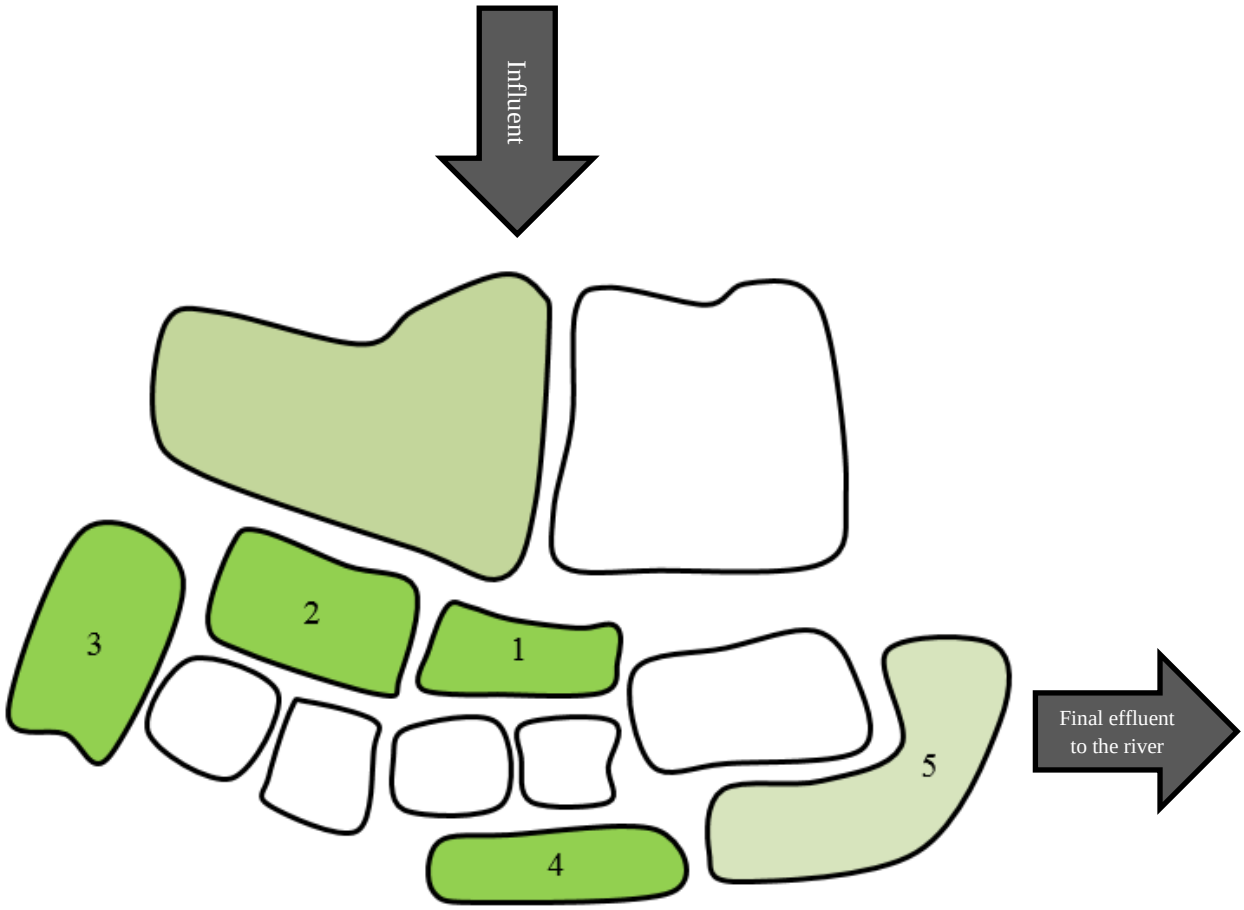


Dissolved oxygen recorded in round pool 2



Dissolved oxygen recorded in the raceway pond

Appendix J: Aerial view of the stabilization ponds at Motetema wastewater treatment works



Stabilization Pond Site No.	pH	Dissolved Oxygen (mg/ℓ)	Temperature (^{0}C)	Conductivity (µS/cm)	Total Dissolved Solids (mg/ℓ)	Nitrates (mg/ℓ)	Chlorophyll *a* (µg/ℓ)
1	7,39	0,16	23,41	942,00	0,60	0,88	45,38
2	7,39	0,16	23,41	942,00	0,60	0,88	45,38
3	7,39	0,18	23,41	942,00	0,60	0,88	45,38
4	7,39	0,17	23,34	943,00	0,60	0,90	46,40
5	7,41	0,20	23,50	954,00	0,60	0,81	33,61

Appendix K: Stabilization ponds pictures at Motetema wastewater treatment works

Microalgae wastewater treatment in a stabilization pond

Dried up stabilization pond

Appendix L: Pearson Correlation Analyses calculations and scatter plots

Total dissolved solids and Dissolved oxygen: Round Pool 1 bottom section (*C. vulgaris)*

Result Details & Calculation

X Values
Σ = 3718
Mean = 161.652
$\Sigma(X - M_x)^2 = SS_x = 139.217$

Y Values
Σ = 218.97
Mean = 9.52
$\Sigma(Y - M_y)^2 = SS_y = 6.705$

X and Y Combined
N = 23
$\Sigma(X - M_x)(Y - M_y) = -18.997$

R Calculation
$r = \Sigma((X - M_y)(Y - M_x)) / \sqrt{((SS_x)(SS_y))}$

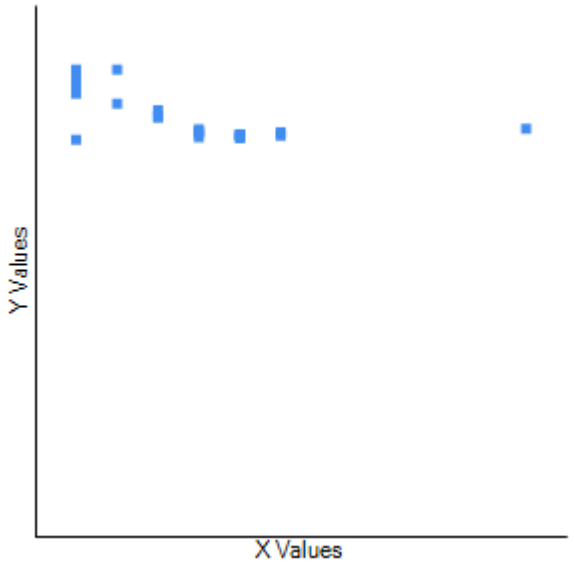
$r = -18.997 / \sqrt{((139.217)(6.705))} = -0.6218$

Meta Numerics (cross-check)
r = -0.6218

Key

X: X Values
Y: Y Values
M_x: Mean of X Values
M_y: Mean of Y Values
$X - M_x$ & $Y - M_y$: Deviation scores
$(X - M_x)^2$ & $(Y - M_y)^2$: Deviation Squared
$(X - M_x)(Y - M_y)$: Product of Deviation Scores

The value of R is -0.6218. This is a moderate negative correlation, which means there is a tendency for high X variable scores to go with low Y variable scores (and vice versa).



X Values = TDS (mg/ℓ)
Y Values = DO (mg/ℓ)

The p-value is 0.001531. The result is significant at $p < 0.05$.

Total dissolved solids and Dissolved oxygen: Round Pool 2 bottom section *C. protohecoides)*

Result Details & Calculation

X Values
Σ = 3762
Mean = 163.565
$\Sigma(X - M_x)^2 = SS_x = 77.652$

Y Values
Σ = 259.34
Mean = 11.276
$\Sigma(Y - M_y)^2 = SS_y = 24.222$

X and Y Combined
N = 23
$\Sigma(X - M_x)(Y - M_y) = -39.483$

R Calculation
$r = \Sigma((X - M_y)(Y - M_x)) / \sqrt{((SS_x)(SS_y))}$

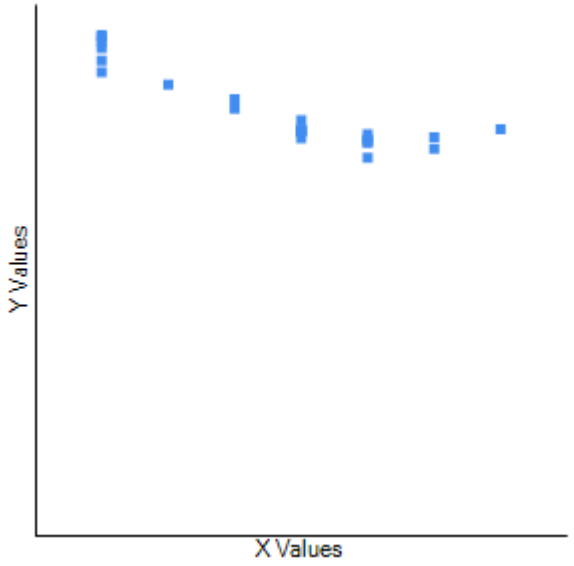
$r = -39.483 / \sqrt{((77.652)(24.222))} = -0.9104$

Meta Numerics (cross-check)
r = -0.9104

Key

X: X Values
Y: Y Values
M_x: Mean of X Values
M_y: Mean of Y Values
$X - M_x$ & $Y - M_y$: Deviation scores
$(X - M_x)^2$ & $(Y - M_y)^2$: Deviation Squared
$(X - M_x)(Y - M_y)$: Product of Deviation Scores

The value of R is -0.9104. This is a strong negative correlation, which means that high X variable scores go with low Y variable scores (and vice versa).



X Values = TDS (mg/ℓ)

Y Values = DO (mg/ℓ)

The p-value is < 0.00001. The result is significant at $p < 0.05$.

Ammonium and Chlorophyll *a*: Round Pool 1 (*C. vulgaris)*

Result Details & Calculation

X Values
Σ = 2467.84
Mean = 51.413
$\Sigma(X - M_x)^2 = SS_x = 12581.09$

Y Values
Σ = 8593.46
Mean = 179.03
$\Sigma(Y - M_y)^2 = SS_y = 66028.686$

X and Y Combined
N = 48
$\Sigma(X - M_x)(Y - M_y) = -2178.139$

R Calculation
$r = \Sigma((X - M_y)(Y - M_x)) / \sqrt{((SS_x)(SS_y))}$

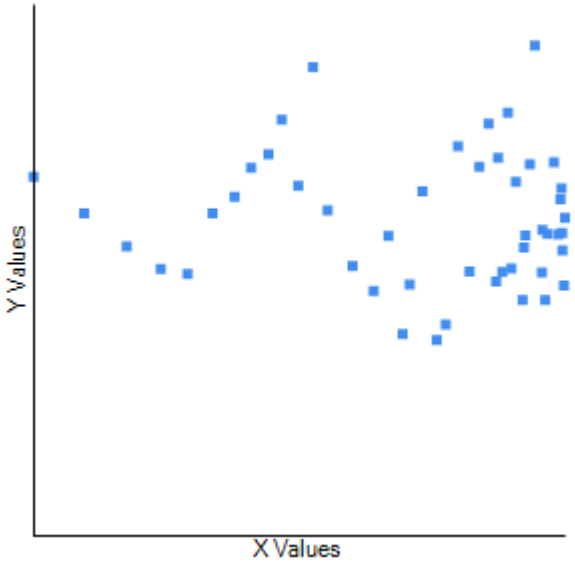
$r = -2178.139 / \sqrt{((12581.09)(66028.686))} = -0.0756$

Meta Numerics (cross-check)
r = -0.0756

Key

X: X Values
Y: Y Values
M_x: Mean of X Values
M_y: Mean of Y Values
$X - M_x$ & $Y - M_y$: Deviation scores
$(X - M_x)^2$ & $(Y - M_y)^2$: Deviation Squared
$(X - M_x)(Y - M_y)$: Product of Deviation Scores

The value of R is -0.0756. Although technically a negative correlation, the relationship between your variables is only weak (*nb.* the nearer the value is to zero, the weaker the relationship).



X Values = Ammonium (mg/ℓ)

Y Values = Chlorophyll *a* (µg/ℓ)

The p-value is 0.607666. The result is *not* significant at $p < 0.05$.

Ammonium and Chlorophyll *a*: Round Pool 2 (*C. protothecoides)*

Result Details & Calculation

X Values
Σ = 5035.66
Mean = 104.91
$\Sigma(X - M_x)^2 = SS_x = 1321.461$

Y Values
Σ = 1983.54
Mean = 41.324
$\Sigma(Y - M_y)^2 = SS_y = 6960.427$

X and Y Combined
N = 48
$\Sigma(X - M_x)(Y - M_y) = 1489.365$

R Calculation
$r = \Sigma((X - M_y)(Y - M_x)) / \sqrt{((SS_x)(SS_y))}$

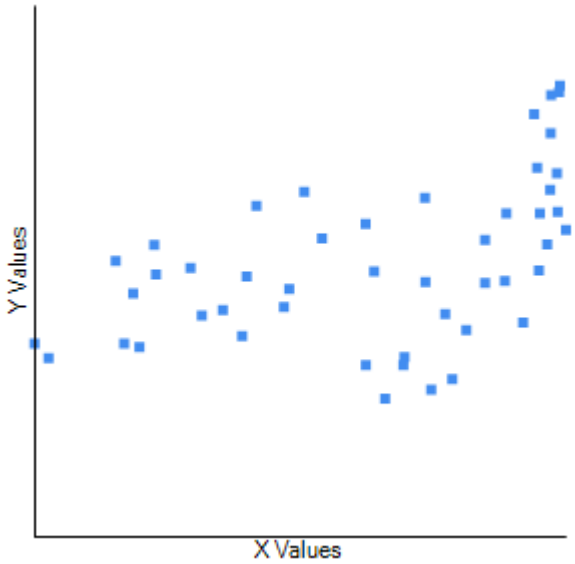
$r = 1489.365 / \sqrt{((1321.461)(6960.427))}$
$= 0.4911$

Meta Numerics (cross-check)
r = 0.4911

Key

X: X Values
Y: Y Values
M_x: Mean of X Values
M_y: Mean of Y Values
$X - M_x$ & $Y - M_y$: Deviation scores
$(X - M_x)^2$ & $(Y - M_y)^2$: Deviation Squared
$(X - M_x)(Y - M_y)$: Product of Deviation Scores

The value of R is 0.4911. Although technically a positive correlation, the relationship between your variables is weak (*nb.* the nearer the value is to zero, the weaker the relationship).



X Values = Ammonium (mg/ℓ)

Y Values = Chlorophyll *a* (µg/ℓ)

The p-value is 0.000394. The result is significant at $p < 0.05$.

Temperature and Conductivity: Motetema stabilization ponds

Result Details & Calculation

X Values
Σ = 117.07
Mean = 23.414
$\Sigma(X - M_x)^2 = SS_x = 0.013$

Y Values
Σ = 4723
Mean = 944.6
$\Sigma(Y - M_y)^2 = SS_y = 111.2$

X and Y Combined
N = 5
$\Sigma(X - M_x)(Y - M_y) = 0.958$

R Calculation
$r = \Sigma((X - M_y)(Y - M_x)) / \sqrt{((SS_x)(SS_y))}$

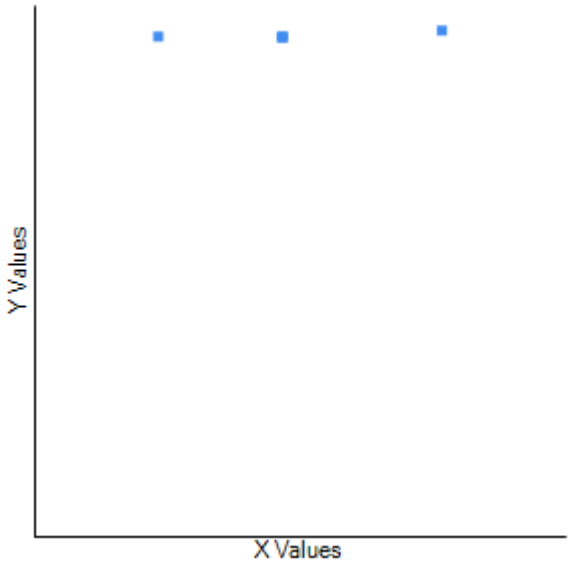
$r = 0.958 / \sqrt{((0.013)(111.2))} = 0.7992$

Meta Numerics (cross-check)
r = 0.7992

Key

X: X Values
Y: Y Values
M_x: Mean of X Values
M_y: Mean of Y Values
$X - M_x$ & $Y - M_y$: Deviation scores
$(X - M_x)^2$ & $(Y - M_y)^2$: Deviation Squared
$(X - M_x)(Y - M_y)$: Product of Deviation Scores

The value of R is 0.7992. This is a strong positive correlation, which means that high X variable scores go with high Y variable scores (and vice versa).



X Values = Temperature (°C)

Y Values = Conductivity (µS/cm)

The p-value is 0.1047. The result is *not* significant at $p < 0.05$.

Nitrates and Chlorophyll *a*: Motetema stabilization ponds

Result Details & Calculation

X Values
Σ = 4.35
Mean = 0.87
$\Sigma(X - M_x)^2 = SS_x = 0.005$

Y Values
Σ = 216.15
Mean = 43.23
$\Sigma(Y - M_y)^2 = SS_y = 116.461$

X and Y Combined
N = 5
$\Sigma(X - M_x)(Y - M_y) = 0.737$

R Calculation
$r = \Sigma((X - M_y)(Y - M_x)) / \sqrt{((SS_x)(SS_y))}$

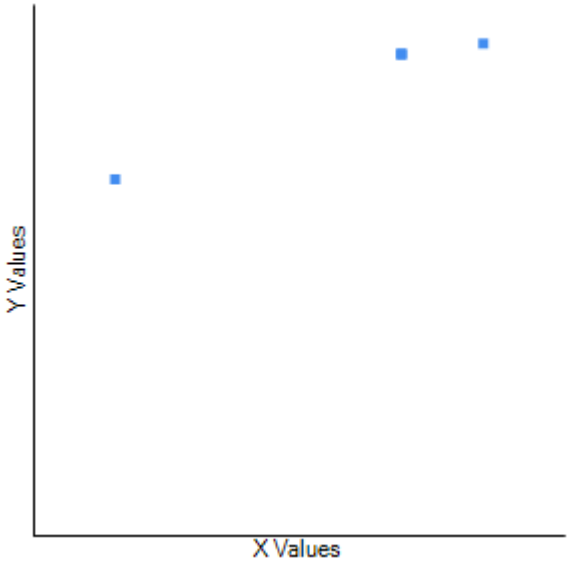
$r = 0.737 / \sqrt{((0.005)(116.461))} = 0.9855$

Meta Numerics (cross-check)
r = 0.9855

Key

X: X Values
Y: Y Values
M_x: Mean of X Values
M_y: Mean of Y Values
$X - M_x$ & $Y - M_y$: Deviation scores
$(X - M_x)^2$ & $(Y - M_y)^2$: Deviation Squared
$(X - M_x)(Y - M_y)$: Product of Deviation Scores

The value of R is 0.9855. This is a strong positive correlation, which means that high X variable scores go with high Y variable scores (and vice versa).



X Values = Nitrates (mg/ℓ)

Y Values = Chlorophyll *a* (µg/ℓ)

The p-value is 0.002091. The result is significant at $p < 0.05$.