

Article

A Comparative Study of Improvement of Phycoremediation Using a Consortium of Microalgae in Municipal Wastewater Treatment Pond Systems as an Alternative Solution to Africa's Sanitation Challenges

Paul J. Oberholster ¹, Maronel Steyn ² and Anna-Maria Botha ^{3,*} 

¹ Centre for Environmental Management, University of the Free State, Bloemfontein 9300, South Africa; OberholsterPJ@ufs.ac.za

² CSIR, Smart Places Unit, Water Centre, P.O. Box 320, Stellenbosch 7599, South Africa; msteyn@csir.co.za

³ Genetics Department, University of Stellenbosch, Private Bag X1, Matieland, Stellenbosch 7601, South Africa

* Correspondence: ambo@sun.ac.za

Abstract: The reuse of wastewater has been observed as a viable option to cope with increasing water stress in Africa. The present case studies evaluated the optimization of the process of phycoremediation as an alternative low-cost green treatment technology in two municipality wastewater treatment pond systems that make up the largest number of domestic sewage treatment systems on the African continent. A consortium of specific microalgae (*Chlorella vulgaris* and *Chlorella protothecoides*) was used to improve the treatment capacity of domestic wastewater at two operational municipality wastewater pond systems under different environmental conditions in South Africa. Pre- and post-phycoremediation optimization through mass inoculation of a consortium of microalgae, over a period of one year under different environmental conditions, were compared. It was evident that the higher reduction of total phosphates (74.4%) in the effluent, after treatment with a consortium of microalgae at the Motetema pond system, was possibly related to (1) the dominance of the algal taxa *C. protothecoides* (52%), and to a lesser extent *C. vulgaris* (36%), (2) more cloudless days, (3) higher air temperature, and (4) a higher domestic wastewater strength. In the case of the Brandwag pond treatment system, the higher reduction of total nitrogen can possibly be related to the dominance of *C. vulgaris*, different weather conditions, and lower domestic wastewater strength. The nutrient reduction data from the current study clearly presented compelling evidence in terms of the feasibility for use of this technology in developing countries to reduce nutrient loads from domestic wastewater effluent.

Keywords: consortia of microalgae; sustainable development goals; phycoremediation; pond treatment systems; domestic wastewater; Africa



Citation: Oberholster, P.J.; Steyn, M.; Botha, A.-M. A Comparative Study of Improvement of Phycoremediation Using a Consortium of Microalgae in Municipal Wastewater Treatment Pond Systems as an Alternative Solution to Africa's Sanitation Challenges. *Processes* **2021**, *9*, 1677. <https://doi.org/10.3390/pr9091677>

Academic Editor: Dimitris Zagklis

Received: 25 July 2021

Accepted: 9 September 2021

Published: 18 September 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Attention towards addressing sanitation and wastewater issues in Africa has grown in recent years [1]. While the aim of improving access to sanitation is included both in the Millennium Development Goals (MDGs) (2000–2015) and the Sustainable Development Goals (SDGs) (2015–2030), it is gaining prominence in the SDGs. Although overall service coverage increased, the target was missed by a margin of almost 700 million people, many living in sub-Saharan Africa. The new SDGs were endorsed by the UN General Assembly in September 2015, which included a commitment to provide universal access to sanitation and drinking water services by 2030 [2]. Population and urban growth are interlinked and the foremost challenge facing Africa's sanitation provision. Rapid urbanization poses major infrastructure, economic, environmental, and social problems. Africa's urban population will double by 2030, and the difficulties African cities currently face in providing sanitation

will be exacerbated. Rates of urbanization in Africa are the highest in the world (5.8% in sub-Saharan Africa) [1].

The capacity of insufficient wastewater treatment ponds (WWTPs) to cope with increasing wastewater loads due to an increase in population numbers is among the technical, social, economic, and environmental challenges facing governments in Africa. Another key challenge is to cope with variation in pollution loads caused by uncontrolled discharges into the sewage network (e.g., industrial discharge). Furthermore, high operational and maintenance costs lead to inappropriate sludge disposal and odor. Power cuts also play a major role in cases where energy is needed for the treatment process. Financial problems were observed to impact all the selected African countries, which negatively affects the operation, maintenance, or upgrade of WWTPs [3]. Release of insufficiently treated wastewater into the environment is also observed when treatment plants are dysfunctional or temporarily disconnected, which is common in countries such as Ghana and South Africa [3,4]. Workers and managers in charge of treatment plants also lack the skills to maintain them and are not motivated to maintain treatment plants due to low wages.

In a comparative study on wastewater practices of seven African countries, namely Algeria, Burkina Faso, Egypt, Ghana, Morocco, Senegal, and Tunisia [3], it was evident that at least seven out of ten WWTPs are either waste stabilization ponds or activated sludge. Table 1 shows an overview of the most used sanitation infrastructure in these countries. In all the selected countries, both activated sludge (AS) and waste stabilization pond systems presented 68–100% of all implemented units still in operation. AS systems are applied mostly by private entities (e.g., industry) in Ghana, while pond treatment systems are preferred by public entities. In Burkina Faso, only pond systems are used [3]. However, in the other five countries, both AS and pond systems have been used. Overall, waste stabilization pond systems are the most used treatment technology in Africa.

Table 1. Overview of the insufficient wastewater treatment plants (WWTPs) in operation at the seven selected countries (Algeria, Burkina Faso, Egypt, Ghana, Morocco, Senegal, and Tunisia) [3].

Country	Total Number	First Most Used Technology	Second Most Used Technology	Third Most Used Technology	Feed Flow Rate (m ³ /h)
Burkina Faso	2	100% pond systems	N/A	N/A	96 (for the largest pond system)
Ghana	19 ¹ (4 ponds under construction)	42% are pond systems	26% are activated sludge (AS) or aerated tanks	16% are anaerobic digesters	1–25
Senegal	9	56% are ponds	44% are AS	N/A	N/A
Algeria	123 (96 under construction, of which 60 are AS and 36 are pond systems)	55% are ponds	45% are AS	N/A	8–2750
Egypt	>99	Between 65% and 85% are AS	About 10% are pond systems	Others	–
Morocco	>100	>77% are pond systems	5% are AS	Trickling filters	12–4914
Tunisia	109	82% are AS	13% are pond systems	Trickling filters and wetlands	4–3250

¹ Among the WWTPs under construction, 60 are AS and 36 are pond systems.

Conventional domestic wastewater treatment systems (including AS reactors and trickling filters) [5] are labor- and energy-intensive, often requiring expensive infrastructure and regular maintenance, leaving the municipalities of rural communities in developing countries unable to treat the domestic inflow [6]. This led to the need and emergence of cost-effective wastewater treatment systems (also referred to as ecotechnologies), which

require less infrastructure and use little or no energy [7]. Among these ecotechnologies are wastewater stabilization pond (WSP) systems [7]. WSP systems are regarded as the wastewater treatment method of choice worldwide [8]. In cooler climates, including Europe, WSP systems are used in small rural communities of around 2000 people [9]. However, in warmer climate conditions (including Africa and Asia), WSP systems are used for larger communities of up to a million citizens [10]. WSP systems use a series of different pond types, namely anaerobic ponds, facultative ponds, oxidative ponds, and aerobic ponds, each creating its own specific microbial, chemical, and biochemical characteristics, based on the oxygen level and nutrient content of the respective environments [11,12]. Each pond is dedicated to addressing a specific aspect of wastewater through the metabolic activities of the algae and microorganisms, while the organic and chemical content of the wastewater is metabolized and removed from the liquid via the cycling of nutrients [13].

Sustainable development and the global move towards a more circular use of resources where waste is reduced and resources are recycled, have driven a paradigm shift within the scientific community with regard to wastewater solutions [14]. Furthermore, nutrient recovery from domestic wastewater extends beyond direct economic benefits to human health and also environmental benefits, reducing eutrophication [14].

Phycoremediation is a biological cleanup technology involving the use of micro- or macro-algae for the biological transformation of contaminants, including nutrients such as inorganic and organic carbon, phosphorus, nitrogen, sulfates, and heavy metals [12,15,16]. During the process of phycoremediation, algae utilize nitrogen, phosphorus, carbon, and other salts from the wastewater for their growth [17]. This cleanup process has been a research subject for many decades [18]. Although a vast amount of literature related to phycoremediation and domestic wastewater treatment is available from developing countries, little is known of this technology on the African continent.

There are only a few known published case studies in Africa from the literature on phycoremediation and domestic wastewater treatment. One of these case studies is the Belmont Valley integrated algae pond system (IAPS) using natural occurring algae in the algae raceway. The IAPS is a derivation from the Oswald designed algal integrated wastewater pond system. The IAPS technology was introduced to South Africa in 1996 and a pilot plant was designed and commissioned at the Belmont Valley WWTW in Grahamstown. The IAPS primary treatment of domestic wastewater makes use of an advanced facultative pond which houses an in-pond anaerobic digester, followed by high-rate algae oxidation ponds with paddlewheel mixing. The IAPS technology makes use of gravity, solar energy, and natural occurring algae in the high-rated algae ponds [19]. Other places in Africa where IAPS has been used are Morocco and Zimbabwe [20]. However, in the case study by Oberholster et al. [12], the authors used specific phycoremediation to improve the treatment capacity of domestic wastewater in existing pond systems in South Africa, by mass culturing and inoculation of a consortium of selected algae.

The objective of the current study was to present comparative data of the nutrient treatment capacity of two municipal wastewater pond systems in South Africa, after implementing the phycoremediation technology process in maturation ponds by mass inoculation of a consortium of selected microalgae. Data were recorded before the technology was implemented at both plants and monitored after phycoremediation was introduced at the two wastewater treatment plants. The latter two municipal systems treat ≤ 0.5 and 4.5 mL day^{-1} of domestic wastewater and are located in two different locations with vastly different climate conditions (coastal and inland).

2. Materials and Methods

2.1. Background

The improvement of the process of phycoremediation in the two municipal pond treatment systems utilized a specific consortium of microalgal species (*Chlorella vulgaris* and *Chlorella protothecoides*) to remove nutrients through mass inoculation. Renuka [21] stated that *Chlorella* spp. is one of the most explored microalga genera in relation with nutrient

removal in different types of wastewater. The microalgae scaling-up from laboratory cultures to the outdoor photobioreactors was performed according to Oberholster et al. [12]. The intention was to implement a self-sustaining system that can be operated independently of electricity or expensive chemicals and can be effectively maintained within financial and capacity constraints of developing countries [12].

The Motetema WWTP system ($25^{\circ}6'3.87''$ S and $29^{\circ}28'6.78''$ E) is located inland in the Limpopo province of South Africa, in a temperate climatic zone, and treats 4.5 mL day^{-1} of domestic wastewater. This WWTP system consists of twelve earth ponds organized in two series of six each, parallel to one another. Of the twelve ponds, only six ponds are operated at a time, while the other six ponds are dried for sludge removal [12]. The Brandwag WWTP system, on the other hand, has a coastal location and is near the small town of Brandwag in the Western Cape, South Africa (34.0493° S and 22.0573° E). This WWTP system consists of a series of seven earth ponds, namely anaerobic ponds, primary facultative ponds, secondary facultative ponds, and maturation ponds, that naturally overflow gravitationally from one pond to another. The above-mentioned pond treatment system treats 0.5 mL day^{-1} of domestic effluent and is categorized as a micro-WWTP system (Figure 1).



Figure 1. The locations of the Motetema and Brandwag wastewater treatment pond (WWTP) systems in South Africa.

Five semi-transparent photobioreactor tanks with a capacity of 5000 L each, diameter of 1800 mm, and height of 2040 mm, were installed at the Motetema wastewater pond system (Figure 2A), and three at the Brandwag wastewater pond system (Figure 2B), also with a capacity of 5000 L each [12,22].

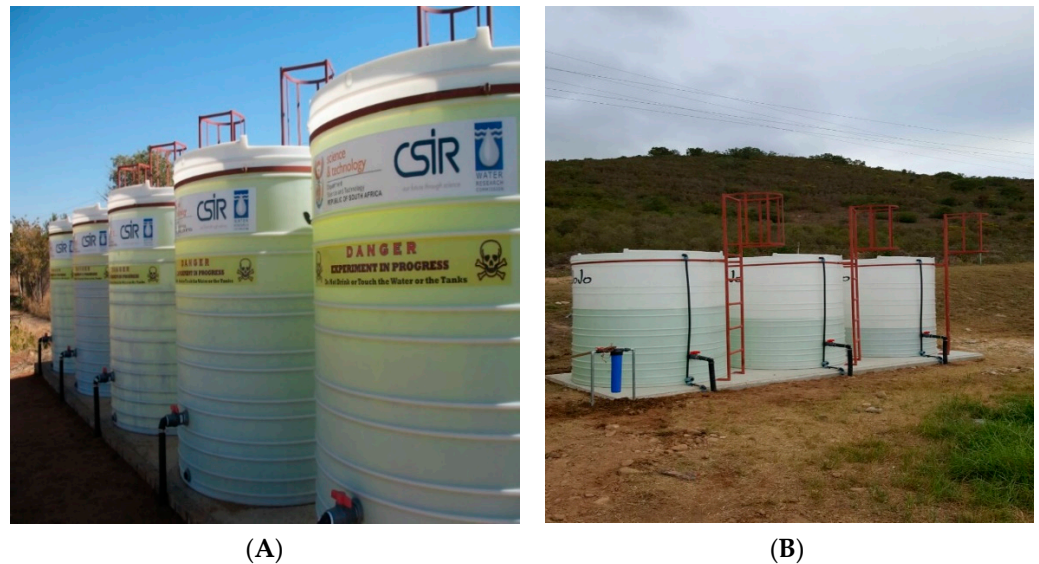


Figure 2. Photobioreactors for the outdoor mass culturing of the consortium of specific microalgae at the Motetema (A) and Brandwag (B) municipal WWTP systems.

The microalgae strains, *Chlorella vulgaris* (Beijerinck, ATCC: 30821) and *Chlorella protothecoides* (Kruger, ATCC: 30411), were acquired from American Type Culture Collection (ATCC) and cultured as described by the ATCC protocol and used as starting culture. In each of these outdoor bioreactors, a volume of 25 L of the two algal species (50:50%) was added to 1000 L of dechlorinated tap water and 10 g of synthetic fertilizer (10 phosphorus:20 nitrogen:10 potassium) for the start-up culture in the outdoor photobioreactors. This culture was upscaled to 5000 L in each photobioreactor and released in the selected different oxidation ponds on a 3 to 4 weekly basis, depending on the season. The photobioreactors' algae dry weights (wt.) during the different culturing phases were the following: lag phase (average dry wt. $1.12 (\pm 0.13) \text{ mg L}^{-1}$), exponential phase (average dry wt. $1.69 (\pm 0.15) \text{ mg L}^{-1}$), and in the stationary phase ($1.18 (\pm 0.13) \text{ mg L}^{-1}$) for the Motetema WWTP outdoor photobioreactors. In the Brandwag WWTP outdoor photobioreactors, it was: lag phase (average dry wt. $1.01 (\pm 0.18) \text{ mg L}^{-1}$), exponential phase (average dry wt. $1.55 (\pm 0.11) \text{ mg L}^{-1}$), and in the stationary phase ($1.10 (\pm 0.9) \text{ mg L}^{-1}$). Solar illuminance reached $1251 \mu\text{mol m}^{-2} \text{ s}^{-1}$ during midday in the Motetema WWTP outdoor photobioreactors and $1077 \mu\text{mol m}^{-2} \text{ s}^{-1}$ in the Brandwag WWTP outdoor photobioreactors. Temperature ranges from 29 to 31 °C during midday in the Motetema WWTP outdoor bioreactors and 27 to 28 °C in the Brandwag WWTP outdoor bioreactors. The pH in the Motetema WWTP outdoor photobioreactors was 9.1, and 8.8 in the Brandwag WWTP outdoor photobioreactors during midday (12h00–13h00). All the above data were generated in duplicate during the startup of the mass algae culturing in the photobioreactors. The cultured microalgae were release from the photobioreactors when they reached a chlorophyll-a value of 250 mg L^{-1} to prevent overshadowing and suspension in the photobioreactors [23]. The protocol of Porra et al. [24] was followed for the determination of suspended chl-a ($\mu\text{g L}^{-1}$) in the photobioreactors. The volume of cultured algae in the photobioreactors was stirred manually every four days with an oar by the wastewater plant operator. The total volume of cultured algae being released in the maturation ponds at one time (every three or four weeks) was 15,000 L at the Motetema pond treatment system and 12,000 L at the Brandwag pond treatment system. Of the 5000 L in each of the outdoor

photobioreactors, only 4000 L was released at each mass inoculation, while 1000 L was left in the photobioreactor for subculturing. The pond systems are based on natural overflow from one pond to another, using no electricity, and are gravity-fed. Residence times for both the Motetema and Brandwag pond treatment systems were between 26 and 29 days depending on environmental conditions, for example, rain conditions. The average depth of the maturation ponds in both systems was 1.5 m. Although the photobioreactors were connected to all the maturation ponds by a piping network, only the first three maturation ponds, after the facultative pond, were inoculated with the consortium of microalgae in both systems. Chemical data presented in the current study were related to the last two ponds, which include the effluents of the last pond over a period of one year.

2.2. Physical and Chemical Sampling Analyses

Water samples were taken before and after treatment (one year later) at the outflow of the last two maturation ponds at each of the case study locations and analyzed according to Oberholster et al. [12]. In short, the first water samples were taken two times ($n = 2$) before any occurrence of mass microalgae inoculation at the two pond treatment systems, while the last water samples were taken three times ($n = 3$), four weeks after the last mass microalgae inoculation over the period of one year. On-site water quality parameters, such as temperature, pH, and electrical conductivity (EC), were measured using a Hanna HI991300 handheld water quality meter. The sampled water from each sampling location was divided into two subsamples for the following analyses: (a) one liter for dissolved nutrient analyses, and (b) one liter which was kept for the analyses of chemical oxygen demand (COD) and sulfates. Samples were kept on ice and sent to an accredited chemical laboratory for analysis within 48 h of collection. The standard chemical analysis procedures for all the water samples followed the approved analytical methods detailed in “Standard Methods for the Analysis of Water and Wastewater” [25]. Data of the effluent of the last maturation pond of each of the pond systems were compared with the South African wastewater discharge standards [6]. The following methods were used for the analyses: American Public Health Association (APHA) 4500-N: total nitrogen; APHA 5310-B: total organic carbon; APHA 5220-D/HACH Method 8000: total COD; APHA 4500-P: total phosphorus; APHA 2540-D: suspended solids; APHA 4500, APHA 4500-SO₄ G: sulfate; APHA 4500-NH₃ H: ammonia; APHA 4500-PO₄ G: phosphate. Wastewater samples before and after microalgae treatment of the different ponds were filtered through 0.22 µm pore size Whatman GF filters to separate the microalgae from the treated water for the determination of nitrogen and phosphorus uptake by the microalgae.

2.3. Phytoplankton Sampling and Analyses

Phytoplankton sampling was performed at the outflow of the last two maturation ponds of each of the case studies before and one year after treatment. The water sample was divided into two subsamples: (a) preserved for microscope soft algae identification and (b) unpreserved for diatom identification. The soft algae subsample was fixed in 2.5% glutaraldehyde in the field and kept cold and in the dark until the laboratory analysis. Diatom samples of each sampling site were cleared of organic matter by heating in a potassium dichromate and sulfuric acid solution and the cleared material was rinsed, diluted, and mounted in a Pleurax medium for microscopic examination. All algae were identified using a compound microscope (Carl Zeis, Jena, Germany) at a 1250× magnification [26–29]. Samples were sedimented in a Sedgewick–Rafter sedimentation chamber and analyzed using the strip–count method [30]. The Berger–Parker dominance index [31] was used to measure the evenness or dominance of each algal species at each sampling pond using actual algae cell numbers:

$$D = N_{\max} / N \quad (1)$$

where N_{\max} = the number of individuals of the most abundant species present in each sample, and N = the total number of individuals collected at each pond site.

2.4. Weather Condition at Each WWTP Location

Weather data were generated from World Weather Online [32] to determine the influence of climate conditions on the treatment performance of the consortium of algae.

2.5. Statistical Analyses

Water quality data were captured in Microsoft Excel Spreadsheets for both wastewater treatment works. Simple error bar graphs for the water quality parameters were created in SigmaPlot (Version 14), and statistical analysis was performed using SigmaStat 14. The Mann–Whitney Rank Sum test was used to determine statistical significance for each parameter in each of the ponds for both wastewater treatment plants. Where data was normally distributed, the *t*-test was performed to determine significance. In all tests, the level of significance was adopted at = 0.05.

3. Results and Discussion

Chinnasamy et al. [33] envisaged that the use of a consortium of algae can be a promising alternative to increase the efficiency of wastewater treatment, but that further research is needed. They, however, speculated that competition between native and non-native algal strains may be a problematic factor for more effective treatment [33]. Furthermore, they also stated that environmental conditions such as climate and different types of wastewater may hamper the effective treatment of the consortia of algal strains [33]. Nevertheless, a study by Ruiz-Martinez et al. [34] showed that after a 42-day culturing period in WWTP effluent, a mixture of the microalgae *Chlorococcales* and cyanobacteria isolated from a submerged anaerobic bioreactor achieved reduction efficiencies for $\text{NH}_4\text{-N}$ and $\text{PO}_4\text{-P}$ of 67.2% and 97% respectively. Silva-Benavides and Torzillo [35] compared the removal efficiency of *Chlorella* and a *Chlorella–Planktothrix* co-culture grown in municipal wastewater and showed that co-culture of *Chlorella–Planktothrix* removed the highest nitrogen concentration (80%) over the two-day exposure period. In 2015, Renuka et al. [21] presented a comprehensive review on different microalgal consortia in various types of wastewater. Nonetheless, for all the above studies, a consortium of microalgae was used to test for their nutrient removal in batch flask cultures, small high-rated algal ponds, small algal race ways, or outdoor photobioreactors [21,36]. The scaling-up of microalgal cultures to outdoor conditions presents additional challenges, for example variable irradiance, temperature, and rainfall [37]. These challenges are not observed in the constant environment experience by small-scale laboratory cultures. To the authors' knowledge, the current study is the first report on a comparative study using two existing municipality pond treatment systems to treat domestic wastewater by improving the process of phycoremediation using a specific consortium of algae and mass inoculation over a period of one year. Table 2 summarizes the data, showing the percentage reduction after treatment as well as the standard deviation. Figures 3 and 4 shows the standard error and statistical significance of Brandwag wastewater treatment works and Motetema wastewater treatment works, respectively.

Table 2. Median of physical and chemical parameters measured before (day 0) and after treatment (one year later) with a specific consortium of microalgae at the Motetema (MT) wastewater pond system and Brandwag wastewater pond system.

Parameters	Motetema Wastewater Pond System						Brandwag Wastewater Pond System					
	Before Treatment Pond 6 (STDEV)	After Treatment Pond 6 (STDEV)	Reduction after Algae Treatment (%)	Before Treatment Pond 7 (Outflow) (STDEV)	After Treatment Pond 7 (Outflow) (STDEV)	Reduction after Algae Treatment (%)	Before Treatment Pond 5 (STDEV)	After treatment Pond 5 (STDEV)	Reduction after Algae Treatment (%)	Before Treatment Pond 6 (Outflow) (STDEV)	After Treatment Pond 6 (Outflow) (STDEV)	Reduction after Algae Treatment (%)
Total nitrogen (mgL)	50 (3)	24 (7)	52.00	41 (5)	11 (4)	73.1	70 (11)	45 (7)	43.0	28 (7)	17 (4)	35.4
Total organic carbon (mgL)	53 (10)	32 (12)	39.62	50 (4)	23 (3)	54.0	195 (18)	69 (9)	69.0	52 (11)	42 (8)	22.2
Total chemical oxygen demand (mgL)	140 (26)	145 (46)	−3.57	122 (72)	114 (42)	6.6	572 (83)	147 (23)	75.0	235 (39)	97 (11)	60.0
Total phosphorous (mgL)	17 (2)	19 (9)	11.76	12 (2)	6 (1)	50.0	9.5 (4)	2.7 (0.9)	74.5	9 (3)	2.2 (0.3)	74.4
Sulfate as SO ₄ dissolved (mgL)	195 (90)	73 (20)	62.56	184 (85)	78 (24)	58.0	81 (10)	113 (21)	−45.0	172 (13)	127 (11)	26.9
Ortho Phosphate as P (mgL)	12 (1)	2.36 (0.79)	80.33	8 (3)	1.36 (0.7)	83.0	5.8 (1.1)	2.1 (0.6)	77.0	3.7 (0.8)	0.74 (0.4)	87.0
Ammonia as N (mgL)	20 (13)	9 (4)	55.00	19 (2)	0.1 (0.85)	99.4	48 (11)	32 (7)	43.0	27 (9)	23 (8)	16.6
Electrical conductivity (mSm)	114 (10)	185 (33)	−62.28	118 (2)	160 (22)	−36.0	117 (11)	125 (21)	−7.1	112 (10)	128 (9)	−16.0
pH (Lab) 20 °C	8.1 (0.15)	8.4 (0.06)	−3.70	8.2 (0.06)	9.1 (0.49)	10.9	7.7 (0.03)	8.8 (0.03)	−14.1	8.2 (0.02)	8.7 (0.18)	−6.1

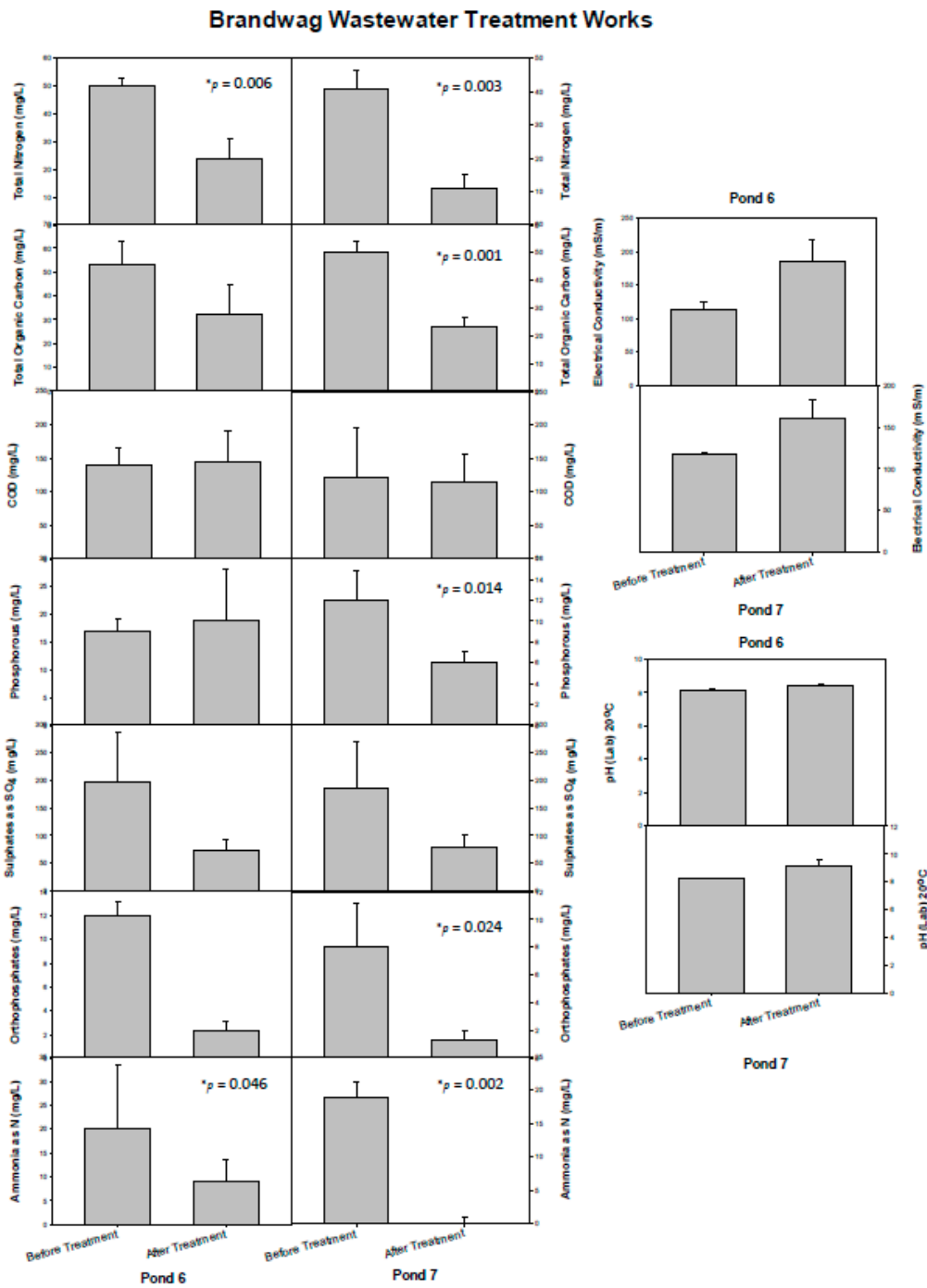


Figure 3. Water quality results from Brandwag wastewater pond treatment before and after phycoremediation treatment. * *p*-values shown for those parameters that were statistically significantly different before and after treatment.

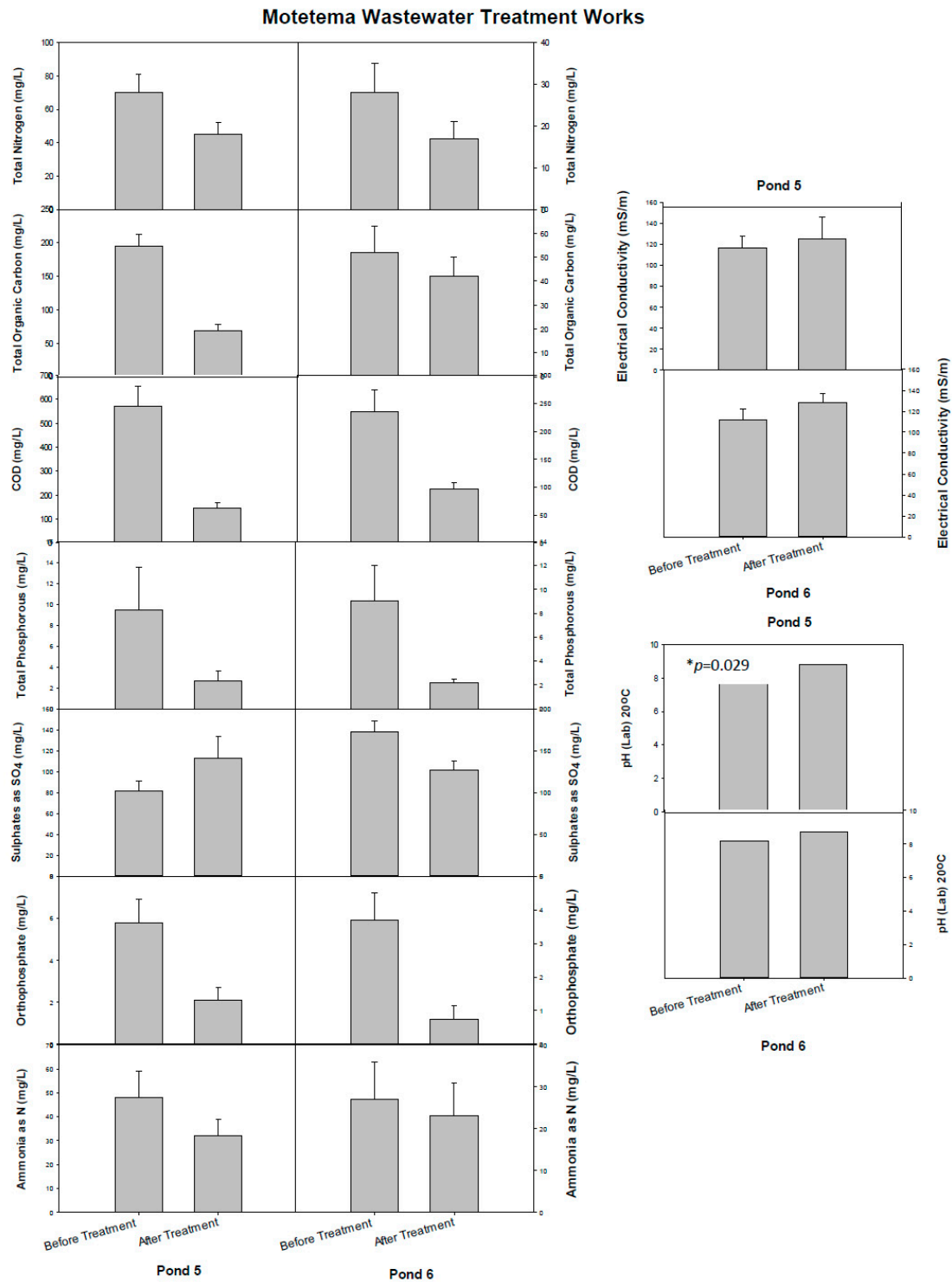


Figure 4. Water quality results from Motetema wastewater pond treatment plants before and after phycoremediation treatment. * p -values shown for those parameters that were statistically significantly different before and after treatment.

In the current study, the average pH of both pond treatment systems increased after treatment with the specific algae consortium. The average pH was determined from sampling data two times ($n = 2$) before any occurrence of mass microalgae inoculation at the two pond treatment systems, and three times ($n = 3$) one year after the mass microalgae inoculation. The increase in pH values after specific algae treatment suggested a higher photosynthetic rate, drawing more dissolved CO₂ from the water (Table 2). The pH values

in the two pond treatment systems during the study period did not reach a value of 11, which is required for the precipitation of phosphorus, indicating that the microalgae were the main mechanism for the removal of phosphorus in the two treatment pond systems [38] (Table 2). The highest reduction of total nitrogen (73.1%) occurred in the last maturation pond of the Brandwag pond treatment system from pre- to post-treatment. Nevertheless, the highest removal of total phosphorus (74.4%) from pre- to post-specific mass algae treatment occurred in the effluent of the last matured pond at the Motetema pond system. The uppermost removal of ammonia (99.4%) after specific algae mass treatment occurred at the effluent of the last matured pond of the Brandwag pond system (Table 2). The N:P ratios before mass algae treatment in the final effluent of the Brandwag and Motetema pond systems were 3.4:1 and 3.5:1. After one year of mass microalgae treatment at the Brandwag and Motetema pond systems, the N:P ratios were 1.8:1 and 10:1. Although both the systems displayed a reduction of unfiltered COD after specific algae treatment in the last pond (Motetema pond treatment system 92 mgL^{-1} (60% reduction); Brandwag pond treatment system 114 mgL^{-1} (6.6% reduction)), they were unable to reduce unfiltered COD levels to meet the South African effluent discharge standard of 75 mgL^{-1} (Table 2). However, a large portion of this residual COD was possibly related to the microalgae biomass that increased the chemical demand of dissolved oxygen levels [12]. The treatment performance of microalgae is strongly related to three primary nutrients: C, N, and P. The C (C as COD):N:P ratios before mass algae treatment in the final effluent at the Brandwag and Motetema pond systems were 1.4:4:15 and 1.9:30 [39]. After one year of mass microalgae treatment of the Brandwag and Motetema pond systems, the C:N:P ratios in the final effluent were 1.1:12:22 and 1.4:7:63. Comparing the water quality data of the final effluent of both systems with the South African general standards for wastewater effluent, it was evident that the following standard target concentrations were not met after one year's treatment with specific microalgae: ammonia (23 mgL^{-1}) in the final effluent of the Motetema pond treatment system (South African general effluent standard 6 mgL^{-1}), total nitrogen (17 mgL^{-1}) in the final effluent of the Motetema pond treatment system (South African general effluent standard 15 mgL^{-1}), and EC (160 mSm^{-1}) in the final effluent of the Brandwag pond treatment system (South African general effluent standard 150 mSm^{-1}) (Table 2). Overall, a higher reduction of all the selected parameters was observed in the last maturation pond of both pond treatment systems, except for EC values that increased from the second to last maturation pond to the last maturation pond (Table 2). To reach the latter nutrient target values, it is suggested to increase the number of algae photobioreactors.

Mahapatra et al. [40] reported on the treatment efficiency of a natural algae-based sewage treatment plant in India of 67.65 million liters per day treatment capacity, with a residence time of 14.3 days. Their study showed a moderate performance, with the removal of total COD (60%) and filterable COD (50%), as sewage travels from the inlet to the outlet [40]. Furthermore, nitrogen content showed sharp variations with total Kjeldahl nitrogen removal of 36%, ammonium removal efficiency of 18%, nitrate ($\text{NO}_3\text{-N}$) removal efficiency of 22%, and nitrite removal efficiency of 57.8%. The predominant algae classes were euglenoids (in facultative pond) and chlorophyceae in the maturation ponds. However, in the current study, the resident times of the two pond treatment systems were much longer with a much lower treatment capacity per day, as in the case of the study by Mahapatra et al. [40]. Furthermore, Mahapatra et al. [40] compared data generated from the inlet to the outlet with treatment of natural algae. In the current study, the authors evaluated the treatment capacity of the last two maturation ponds before and after mass treatment with a specific consortium of algae before and one year after treatment.

Before treatment with the specific consortium of algae (day 0), the dominant taxa consisted of 40% *Microcystis aeruginosa* and 24% *Micractinium pussillum* in the last maturation pond of the Brandwag pond treatment system (Figure 5A). Although *M. aeruginosa* is one of the most common species in freshwater ecosystems, it is not common in pond treatment systems [41,42]. However, after one year of algae inoculation, the algae assemblage changed, with 45% *C. vulgaris* as the dominant taxa, followed by *C. protothecoides* with 37%.

It must be mentioned that a natural strain of *C. vulgaris* occurred in relatively low numbers at the last pond of the Brandwag pond treatment system before the inoculation of the consortium of microalgae. These findings suggest that the combination of both *C. vulgaris* and *C. protothecoides* was able to suppress the dominance of *M. aeruginosa* after inoculation with the consortium of specific microalgae (Figure 5B).

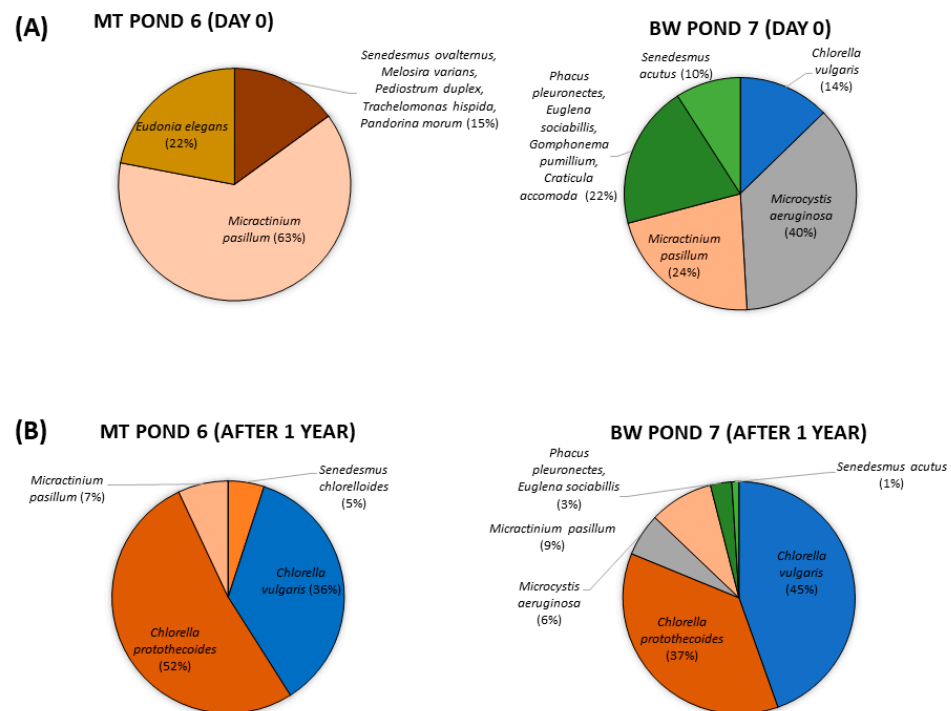


Figure 5. (A) Distribution of natural algae species in the last maturation pond before mass inoculation of the consortium of specific microalgae species (day 0) at the Motetema (MT) and Brandwag (BW) municipality WWTP systems. (B) Distribution of algae species in the last maturation pond 1 year after inoculation of the consortium of specific microalgae species at the Motetema (MT) and Brandwag (BW) municipality WWTP system.

At the Motetema pond treatment system, before the inoculation of the consortium of algae, *M. pussillum* was the dominant taxa (63%) in the last pond (Figure 5A), followed by the green algal *Eudorina elegans*. One year later, after the inoculation with the consortium of specific algae, the last pond was dominated by *C. protothecoides* (52%) and *C. vulgaris* (36%) (Figure 5B). No natural *Chlorella* spp. was observed at the Motetema pond treatment system before the inoculation, as in the case of the Brandwag pond treatment system.

Almomani and Örmeci [43] showed that the microalgae *C. vulgaris* can grow and perform well at 20 and 36 °C in 500 mL sterilized reactors. Zhang et al. [44] showed that *Chlorella* spp. can grow in surface water temperatures in the temperature range of 5–30 °C, while Shi and Shi [45] reported significant growth of *C. protothecoides* at 28 °C. However, Shi and Shi [45] showed that a lower algae growth was observed at 35 °C. A study by González-Camejo et al. [36] used two outdoor photobioreactors to evaluate the effects of ambient temperature on an indigenous microalgae-nitrifying bacteria culture dominated by *Chlorella*. The authors conducted four experiments over different seasons, maintaining the temperature-controlled photobioreactor at 25 °C, while the temperature in the non-temperature-controlled photobioreactors was allowed to vary with the surrounding environmental conditions [36]. The authors reported that temperature in the range of 15–30 °C had no significant effects on the microalgae cultivation performance; however, when the temperature rose to 30–35 °C, the microalgae viability was significantly reduced.

In the current study, the monthly air temperature did not increase above 31 °C at the two localities of the two treatment plants.

The different observations in the treatment capacity at the two pond treatment systems under study can possibly be related to the characteristics of the domestic wastewater as well as environmental conditions, for example weather patterns. The strength of wastewater refers to its organic matter content, where the higher the organic matter content, the 'stronger' the wastewater, which is measured by its biological oxygen demand (BOD₅) or COD value [46]. From the current study, it was evident that there was a large difference in the COD values between the two pond treatment systems before the treatment with a specific consortium of microalgae. The Brandwag WWTP system had a value of 140 mgL⁻¹ at Pond 6 and the Motetema WWTP system had a value of 576 mgL⁻¹ at Pond 5 (Table 2). The latter observation indicates that the community of the town of Brandwag had high water consumption that caused a weaker wastewater inflow into the Brandwag WWTP [46], which may have played a role in the results of the current study.

Borowitzka [37] stated that the number of sunny days and degree of cloud cover can determine the outcome of intensive outdoor algae culture systems. In the current study, the Motetema pond treatment system experienced more cloudless days than in the case of the Brandwag WWTP (Figure 6C,D). Cassidy [47] reported that the optimal growth temperature for *C. vulgaris* was between 25 and 30 °C, while Latala [48] observed poor or no growth at temperatures between 5 and 10 °C. Even though the minimum air temperatures measured at both localities (11 °C at the Brandwag pond treatment system and 4 °C at Motetema pond treatment system) were not optimal for the growth of *C. vulgaris*, a previous study by Oberholster et al. [12] showed that the temperature at the Motetema pond treatment system was not a major concern during the winter months, since these low air temperatures occur sporadically for two or three days during the winter season (Figure 6A,B). However, during the winter season, the microalgae culturing in the photobioreactors for the mass microalgae inoculation into the maturation ponds shift from a four-week cycle in the summer to a five-week cycle to reach the targeted chlorophyll-a value of 250 µgL⁻¹ before inoculation at both pond treatment localities. The latter shift was possibly due to slower growth by the cultured microalgae in the photobioreactors at lower air temperatures (Figure 6A,B).

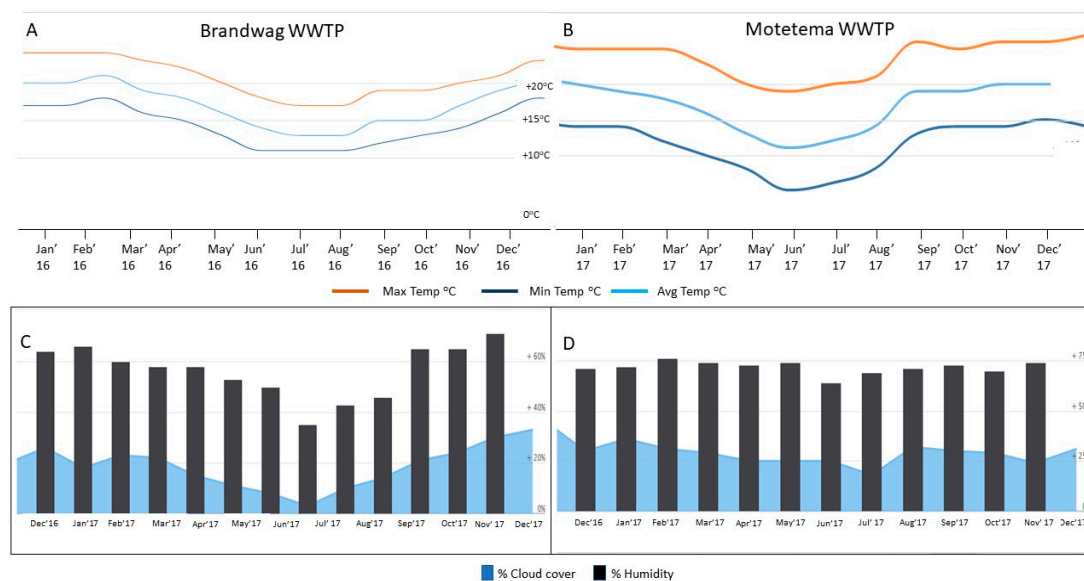


Figure 6. (A) Maximum, minimum, and average air temperature at the Brandwag municipality WWTP system during 2017. (B) Maximum, minimum, and average air temperature at the Motetema municipality WWTP system during 2016. (C) The percentages of cloud cover and humidity at the Brandwag municipality WWTP system in 2017. (D) The percentages of cloud cover and humidity at the Motetema municipality WWTP system in 2017.

It was evident from the data in the current study that the higher reduction of total phosphates (74.4%) in the effluent, after treatment with a consortium of microalgae at the Motetema WWTP system, was possibly related to some of the following factors: (1) the dominance of the algal taxa *C. protothecoides* (52%), and to a lesser extent, *C. vulgaris* (36%) in the microalgae consortium, (2) more cloudless days, (3) higher air temperature, and (4) a higher domestic wastewater strength. In the case of the Brandwag WWTP system, the higher reduction of total nitrogen can possibly be related to the dominance of *C. vulgaris* in the microalgae consortium, less cloudless days, lower air temperature, and a lower domestic wastewater strength. According to Mara [46], when comparing with other treatment methods, waste stabilization ponds are the most important method for wastewater treatment in developing countries, since space is available, and temperature is suitable for the process.

4. Conclusions

The algal diversity in wastewater stabilization systems is greatly influenced by the design parameters, environmental conditions, such as temperature, solar radiation, and domestic wastewater strength, as well as operation conditions. However, it was evident that more cloudless days, higher air temperature, and domestic wastewater strength may have played a role in the treatment capacity and dominance of certain algal species of the pond treatment systems of the two case studies, after mass inoculation with a specific consortium of microalgae. From the case studies presented, it was evident that improvement of phycoremediation through specific microalgae consortiums can play a major role in the removal of nutrients from domestic wastewater of WSP systems in Africa. The construction of the whole phycoremediation treatment systems (five photobioreactors, piping and installation), treating around 4.5 mL day⁻¹ at a cost of 13,800 USD, make it a feasible option for developing countries to improve their existing pond treatment systems to reduce eutrophic conditions in receiving waterbodies. The implementation of phycoremediation in maturation treatment pond systems in African countries could also effectively minimize greenhouse effects, since the algae mass culturing in maturation ponds is a carbon-absorbing process, which can be used by municipalities in the carbon trading market and to provide a subsidy to reward their contributions to environmental protection. Currently, further research is in progress for using the algae biomass in the circular economy as a biofertilizer in African countries with a dominant agricultural economy.

Author Contributions: Conceptualization, P.J.O. and M.S.; methodology, M.S.; software, A.-M.B.; validation, P.J.O., M.S. and A.-M.B.; formal analysis, A.-M.B.; investigation, P.J.O.; resources, M.S.; data curation, A.-M.B.; writing—original draft preparation, A.-M.B.; writing—review and editing, P.J.O. and M.S.; visualization, A.-M.B.; supervision, P.J.O. and A.-M.B.; project administration, M.S.; funding acquisition, M.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research contract PECG1/08/2019/141 was funded by the Climate Finance and Environment Division (PECG1) of the African Development Bank (AfDB).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Weather data is available from <https://weathersa.co.za>.

Acknowledgments: The authors express their gratitude to the African Development Bank for funding the current study. We also want to thank the municipalities involved and providing resources for the sampling of the Brandwag WWTP and Motetema WWTP.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Oberholster, P.; Munyayi, R.C.; Turton, A.; Du Plessis, A. Overview. In *Sanitation and Wastewater Atlas of Africa*; AfDB: Abidjan, Ivory Coast; UNEP: Nairobi, Kenya; GRID-Arendal: Arendal, Norway, 2020; Volume 283, pp. 9–35, ISBN 978-82-7701-194-3.
2. Weststrate, J.; Dijkstra, G.; Eshuis, J.; Gianoli, A.; Rusca, M. The sustainable development goal on water and sanitation: Learning from the Millennium Development Goals. *Soc. Indic. Res.* **2019**, *143*, 797–800. [CrossRef]
3. Nikiema, J.; Figoli, A.; Weissenbacher, N.; Langergraber, G.; Marrot, B.; Moulin, P. Wastewater treatment practices in Africa—Experiences from seven countries. *SSP* **2013**, *14*, 26–34. Available online: <https://hdl.handle.net/10568/40210> (accessed on 5 July 2010).
4. Oberholster, P.J.; Botha, A.-M.; Chamier, J.; De Klerk, A.J. Longitudinal trends in water chemistry and phytoplankton assemblage downstream of the Riverview WWTP in the Upper Olifants River. *Ecohydrol. Hydrobiol.* **2013**, *13*, 41–51. [CrossRef]
5. Sasongko, N.A.; Noguchi, R.; Ito, J.; Demura, M.; Ichikawa, S.; Nakajima, M.; Watanabe, M.M. Engineering Study of a Pilot Scale Process Plant for Microalgae-Oil Production Utilizing Municipal Wastewater and Flue Gases: Fukushima Pilot Plant. *Energies* **2018**, *11*, 1693. [CrossRef]
6. South Africa, Department of Water Affairs. *Revision of General Authorisations in Terms of Section 39 of the National Water Act, 1998 (Act No. 36 of 1998), No. 665 of 2013*; Department of Water Affairs: Pretoria, South Africa, 2013.
7. Gray, N.F. (Ed.) How nature deals with waste. In *Biology of Wastewater Treatment*, 2nd ed.; Series on Environmental Science and Management; Imperial College Press: London, UK, 2004; Volume 4, pp. 1–131. [CrossRef]
8. Mohammed, B. Design and performance evaluation of a wastewater treatment unit. *AU JT* **2006**, *9*, 193–198.
9. Bucksteeg, K. German experiences with sewage treatment ponds. *Water Sci. Technol.* **1987**, *19*, 17–23. [CrossRef]
10. Barceló, D.; Petrovic, M. Waste water treatment and reuse in the Mediterranean region. In *The Handbook of Environmental Chemistry*; Springer: Berlin/Heidelberg, Germany, 2011; Volume 14.
11. Bitton, G. Formula handbook for environmental engineers and scientists. In *Environmental Science and Technology*; A Wiley-Interscience Series of Texts and Monographs; Schoor, J.L., Zehnder, A., Eds.; John Wiley & Sons: Hoboken, NJ, USA, 1998; Volume 117.
12. Oberholster, P.J.; Cheng, P.-H.; Genthe, B.; Steyn, M. The environmental feasibility of low-cost algae-based sewage treatment as a climate change adaption measure in rural areas of SADC countries. *J. Appl. Phycol.* **2019**, *31*, 355–639. [CrossRef]
13. Anderson, C.R.; Condrón, L.M.; Clough, T.J.; Fiers, M.; Stewart, A.; Hill, R.A.; Sherlock, R.R. Biochar induced soil microbial community change: Implications for biogeochemical cycling of carbon, nitrogen and phosphorus. *Pedobiologia* **2011**, *54*, 309–320. [CrossRef]
14. Otoo, M.; Drechsel, P. (Eds.) *Resource Recovery from Waste: Business Models for Energy, Nutrient and Water Reuse in Low- and Middle-Income Countries*; Routledge—Earthscan: Oxon, UK, 2018. Available online: <https://hdl.handle.net/10568/93011> (accessed on 5 July 2010).
15. Kang, D.; Kim, K. Real Wastewater Treatment Using a Moving Bed and Wastewater-Borne Algal-Bacterial Consortia with a Short Hydraulic Retention Time. *Processes* **2021**, *9*, 116. [CrossRef]
16. Debowski, M.; Rusanowska, P.; Zieliński, M.; Dudek, M.; Romanowska-Duda, Z. Biomass Production and Nutrient Removal by *Chlorella vulgaris* from Anaerobic Digestion Effluents. *Energies* **2018**, *11*, 1654. [CrossRef]
17. Mohsenpour, S.F.; Hennige, S.; Willoughby, A.A.; Adeyoye, A.; Gutierrez, T. Integrating micro-algae into wastewater treatment: A review. *Sci. Total Environ.* **2021**, *752*, 142–168. [CrossRef]
18. Debowski, M.; Zieliński, M.; Kazimierowicz, J.; Kujawska, N.; Talbierz, S. Microalgae Cultivation Technologies as an Opportunity for Bioenergetic System Development—Advantages and Limitations. *Sustainability* **2020**, *12*, 9980. [CrossRef]
19. Mambo, P.M.; Westensee, D.K.; Zuma, B.M.; Cowan, A.K. The Belmont Valley integrated algae pond system in retrospect. *Water SA* **2014**, *40*, 385–394. [CrossRef]
20. Oswald, W.J. Ponds in the 21st century. *Water Sci. Technol.* **1995**, *31*, 1–8. [CrossRef]
21. Renuka, N.; Sood, A.; Prasanna, R.; Ahluwalia, A.S. Phycoremediation of wastewaters: A synergistic approach using microalgae for bioremediation and biomass generation. *Int. J. Environ. Sci. Technol.* **2015**, *12*, 1443–1460. [CrossRef]
22. Van den Berg, M.F.; Botha, A.M.; Bierman, A.; Oberholster, P. Assessing domestic wastewater effluent with a battery of bioassays after treatment with a specific consortium of microalgae and different flocculation methods. *Water Air Soil Pollut.* **2020**, *231*, 257. [CrossRef]
23. Masaraure, R. The Development of an Approach for the Production and Use of Algae to Treat Urban Wastewater. 2016. Available online: https://repository.up.ac.za/bitstream/handle/2263/57260/Masaraure_Development_2016.pdf?isAllowed=y&sequence=1 (accessed on 5 July 2010).
24. Porra, R.J.; Thompson, W.A.; Kriedemann, P.E. Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls *a* and *b* extracted with four different solvents: Verification of the concentration of chlorophyll standards by atomic absorption spectrometry. *Biochim. Biophys. Acta* **1989**, *975*, 384–394. [CrossRef]
25. American Public Health Association; American Water Works Association; Water Pollution Control Federation. *Standard Methods for the Examination of Water and Wastewater*, 19th ed.; APHA, AWWA, and WPCF: Washington, DC, USA, 1992.
26. Taylor, J.C.; Harding, W.R.; Archibald, C.G.M. *An Illustrated Guide to Some Common Diatom Species from South Africa*; WRC Report, No. TT 282/07; Water Research Commission: Pretoria, South Africa, 2007; pp. 1–178.

27. Truter, E. *An Aid to the Identification of the Dominant and Commonly Occurring Genera of Algae Observed in Some South African Impoundments*; Department of Water Affairs, Hydrological Research Institute: Pretoria, South Africa, 1987. Available online: https://www.dws.gov.za/iwqs/reports/tr/TR_135_Truter_1987_An_aid_to_the_identification_of_algae_in_South_African_impoundments_s.pdf (accessed on 5 July 2010).
28. Van Vuuren, S.; Taylor, J.; Gerber, A.; Van Ginkel, C. *Easy Identification of the Most Common Freshwater Algae: A Guide for the Identification of Microscopic Algae in South African Freshwaters*; North-West University and Department of Water Affairs and Forestry: Pretoria, South Africa, 2006. Available online: https://www.dws.gov.za/iwqs/eutrophication/NEMP/Janse_van_Vuuren_2006_Easy_identification_of_the_most_common_freshwater_algae.pdf (accessed on 5 July 2010).
29. Wehr, J.D.; Sheath, R.G. Freshwater habitats of algae. In *Freshwater Algae of North America: Ecology and Classification*; Wehr, J.D., Sheath, R.G., Eds.; Academic Press: Cambridge, MA, USA, 2003; pp. 11–57.
30. American Public Health Association. *Standard Methods for Examination of Water and Wastewater*, 20th ed.; American Public Health Association, American Water Works Association, Water Environment Federation: Washington, DC, USA, 2006.
31. Berger, W.H.; Parker, F.L. Diversity of planktonic foraminifera in deep-sea sediments. *Science* **1970**, *168*, 1345–1347. [[CrossRef](#)]
32. World Weather Online. 2021. Available online: <https://www.worldweatheronline.com/> (accessed on 17 July 2021).
33. Chinnaamy, S.; Bhatnagar, A.; Claxton, R.; Das, K.C. Biomass and bioenergy production potential of microalgae consortium in open and closed bioreactors using untreated carpet industry effluent as growth medium. *Bioresour. Technol.* **2010**, *101*, 3097–3105. [[CrossRef](#)]
34. Ruiz-Martinez, A.; Garcia, N.M.; Romero, I.; Seco, A.; Ferrer, J. Microalgae cultivation in wastewater: Nutrient removal from anaerobic membrane bioreactor effluent. *Bioresour. Technol.* **2012**, *126*, 247–253. [[CrossRef](#)]
35. Silva-Benavides, A.M.; Torzillo, G. Nitrogen and phosphorus removal through laboratory batch cultures of microalgae *Chlorella vulgaris* and cyanobacterium *Planktothrix isothrix* grown as monoalgal and as co-cultures. *J. Appl. Phycol.* **2011**, *24*, 267–276. [[CrossRef](#)]
36. González-Camejo, J.; Aparicio, S.; Ruano, M.V.; Borrás, L.; Barat, R.; Ferrer, J. Effect of ambient temperature variations on an indigenous microalgae-nitrifying bacteria culture dominated by *Chlorella*. *Bioresour. Technol.* **2019**, *290*, 121788. [[CrossRef](#)]
37. Borowitzka, M.A. Culturing microalgae in outdoor ponds. In *Algal Culturing Techniques*; Andersen, R.A., Ed.; Academic Press: Cambridge, MA, USA, 2005; Chapter 14, pp. 205–206.
38. Larsdotter, K. Wastewater treatment with microalgae—A literature review. *Vatten* **2006**, *62*, 31–38. Available online: https://www.tidskriftenvatten.se/wp-content/uploads/2017/04/48_article_2125.pdf (accessed on 5 July 2010).
39. Falkowski, P.G.; Raven, J.A. *Aquatic Photosynthesis*, 2nd ed.; Princeton University Press: Princeton, NJ, USA, 2007.
40. Mahapatra, D.M.; Chanakya, H.N.; Ramachandra, T.V. Treatment efficacy of algae-based sewage treatment plants. *Environ. Monit. Assess.* **2013**, *185*, 7145–7164. [[CrossRef](#)] [[PubMed](#)]
41. Ndlela, L.L.; Oberholster, P.J.; Van Wyk, J.H.; Cheng, P.H. An overview of cyanobacterial bloom occurrences and research in Africa over the last decade. *Harmful Algae* **2016**, *60*, 11–26. [[CrossRef](#)]
42. Vasconcelos, V.M. Toxic cyanobacteria (blue–green–algae) in Portuguese fresh-waters. *Arch. Hydrobiol.* **1994**, *130*, 449–451. [[CrossRef](#)]
43. AlMomani, F.; Örmeci, B. Assessment of algae-based wastewater treatment in hot climate regions: Treatment performance and kinetics. *Process Saf. Environ. Prot.* **2020**, *141*, 140–149. [[CrossRef](#)]
44. Zhang, Q.; Zhan, J.-J.; Hong, Y. The effects of temperature on the growth, lipid accumulation and nutrient removal characteristics of *Chlorella* sp. HQ. *Desalin. Water Treat.* **2016**, *57*, 10403–10408. [[CrossRef](#)]
45. Shi, C.; Shi, X. Characterization of three genes encoding the subunits of light-independent protochlorophyllide reductase in *Chlorella protothecoides* CS-41. *Biotechnol. Prog.* **2016**, *22*, 1050–1055. [[CrossRef](#)]
46. Mara, D. *Domestic Wastewater Treatment in Developing Countries*; Earthscan: London, UK, 2003. Available online: https://www.pseau.org/outils/ouvrages/earthscan_ltd_domestic_wastewater_treatment_in_developing_countries_2003.pdf (accessed on 10 February 2005).
47. Cassidy, K.O. Evaluating Algal Growth at Different Temperature. Master’s Thesis, University of Kentucky, Lexington, KY, USA, 2011. Available online: https://uknowledge.uky.edu/bae_etds/3 (accessed on 22 August 2021).
48. Latala, A. Effects of salinity, temperature and light on the growth and morphology of green planktonic algae. *Oceanologia* **1991**, *31*, 119–138. Available online: http://www.iopan.gda.pl/oceanologia/OC_31/OC_31_119-138.pdf (accessed on 22 August 2021).