The environmental feasibility of low cost algae-based sewage treatment as a climate change adaption measure in rural areas of SADC countries

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Abstract

Employing specific algae treatment to treat municipal domestic waste~~-~~water effluent presents an alternative practice to improving water quality effluent of existing rural pond systems in Southern Africa. In the present study domestic waste~~-~~water was treated by using existing infrastructure and inoculated specific selected algae strains in a pond system treatment plant. The objective was to determine through a field pilot study if algae nutrient treatment efficiencies in current traditional water~~ste~~-~~ ~~stabilization ponds can be optimized by manipulating the existing natural consortium of algae through mass inoculation of specific algae strains of *Chlorella* spp. The reduction of total phosphorus in the unfiltered water (contain algae) after specific algae treatment was 74.7% and 76.4% for water-stabilization ponds 5 and 6, while total nitrogen removal was 43.1% and 35.1% respectively. *Chlorella protothecoides* was the dominant algal species in ponds 4, 5 and 6 after specific algae treatment. The maximum algae abundance (4.6x10^{6} cells mL^{-1} in pond 4 and 6.1x10^{6} cells mL^{-1} in pond 5) were observed in August 2016, while the maximum chl–*a* concentration of 783µg L^{-1} was measured in pond 5 after two months of specific algae inoculation. ~~One of the main concerns using specific strains of algae in SADC countries are temperature requirements of the selected strains. Temperature variations due to climate change can affect biochemical reactions and subsequently biochemical composition of algae. However, *Chlorella* spp., used in the current study, grew in surface water temperatures ranging from 5 °C to 30 °C.~~ Although, the present study showed that inoculation of specific algal strains can potentially enhance the treatment efficiencies of existing rural domestic sewage pond systems, it was also evident from the algae-treated effluent analysis that the algae biomass in the upper surface water layer must be harvested for maximum treatment results.

Keywords: phycoremediation, temperature, phosphorus harvesting, rural, water~~ste-~~ stabilization ponds

Introduction

~~Domestic sewage water constitutes a major component of wastewater generated daily in developing countries in Southern Africa.~~ Current trends in~~of~~ climate change could lead to an average global temperature increase of 2 – 3 °C according to the IPCC (2007). A 2°C increase can lead to a 20 – 30 % decrease in water availability in some vulnerable regions, such as southern Africa and the Mediterranean. Temperature increases can also accelerate the eutrophication process (Carvalho and Kirika 2003) e.g. phosphorus recycling is more intensive in warmer waters, while processes of phosphorus release from lake sediment and mineralization are highly temperature dependent (Hamilton et al. 2001). Nevertheless, the reduction of nutrients, especially phosphorus, from WWTPs is an important aspect in reducing eutrophication and to improve water quality and reuse (Oberholster et al 2009; 2013). Therefore, the photosynthetic capabilities of specific algae are particularly attractive, converting solar energy into useful biomass while incorporating nutrients including nitrogen and phosphorus. Algal systems have previously been used to treat domestic wastewater and agricultural waste (Abinandan et al., 2015; Larsdotter, 2006). Micro algae have also been studied for the removal of toxic minerals (Abdel-Raouf et al., 2012).

Nevertheless, u~~U~~ntreated or partially treated domestic ~~wastewater find~~wastewater finds its way to water bodies resulting in nutrient enrichment and eutrophication (Oberholster, 2013; 2017). In Southern African Development Community (SADC) countries, which are home to nearly 300 million people, domestic waste~~-~~water are principally treated in urban areas via activated sludge plants, trickling filter plants and rotating biological contactors (RBCs). However, a significant proportion of rural municipalities make use of pond-based systems alone. These water~~ste~~-stabilization pond systems (WSP), referred to as oxidation ponds comprise of a series of ponds, all of which are relatively shallow bodies of wastewater contained in earthen basins (Butler et al., 2015). The latter system is ideal for rural areas since it is low cost, not labour

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intensive and needs significant land requirements (±30-50 ha) (Pham et al., 2014). Moreover, for rural municipalities, the cost of conventional treatment systems is excessive and their complexity so great that they are not financially feasible. The Capital expenditure (CAPEX) and maintenance cost is also high as well as energy consumption, in comparison to WSP systems (Gratziou et al., 2006). According to Varon and Mara (2004) the capital cost of a WSP system is around 5.7 million US$. However, cost of conventional treatment systems in comparison to WSP system will depend on size and geographical location (Gratziou et al., 2006). Traditionally, open raceways or High Rate Algae Ponds (HRAP's) have been investigated as an alternative option for rural areas because of their lower cost and easy scale up. These reactors are operated at water depths ranging from 20 to 40 cm with a hydraulic retention time~~rates below 1 Wm^{-2}~~ between 4 and 10 days (Garcia et al., 2000). However, the replacement or upscaling of current traditional water-~~ste~~ stabilization ponds systems in rural areas of ~~Southern African Development Community (~~SADC~~)~~ countries by constructing algae raceway pond systems will be a costly exercise and not a feasible option under the current economic climate in developing countries. A possible alternative would be to optimize the treatment efficiencies of the existing traditional water-~~ste~~ stabilization pond systems by manipulating the natural consortium of algae through the continued inoculation of specific algae strains to improve nutrient treatment efficiency. However, the selected algae strains must be a) fast-growing, to allow for the generation of high cell densities within a short time period, thereby generating a large cumulative internal volume and a large total cell surface, b) the selected algal strains must be able to grow over a wide range of external environmental conditions, c) the selected algal strains should be able to adjust osmotically, and d) allow for cell separation from the water and other post-treatment procedures.

According to Acien et al. (2016) algae from raceway pond systems ~~in WWTP~~ are generally dominated by a single strain making up 90% of the total microalgae population. However, according to König (2000) and Mara and Pearson (1998) the algae found in stabilization ponds in general belong to Cyanobacteria, Chlorophyta, Euglenophyta and Bacillariophyta phylum. Prevailing strains in water~~ste-~~-stabilization ponds based treatment systems include those that are fast growing and tolerant to irradiance and temperature fluctuations. The residence time in the water-stabilization pond must also be higher than the algae reproduction time of > 1.6 days, otherwise they would be removed with the effluent, preventing them to reproduce in time (König, 2000). Genera~~Strains~~ such as *Oscillatoria*, *Scenedesmus*, *Chlorella* and *Nitzschia* spp. have been ranked as the most pollution-tolerant algae in wastewater treatment systems (Palmer 1969). The objectives of the current study were to determine through a field pilot study, (a~~1~~) if nutrient treatment efficiencies in existing traditional water-~~ste~~ stabilization ponds can be optimized through the manipulation of the existing natural consortium of algae by mass inoculation of specific selected algal strains, and (b) to determine if the inoculated algal strains can survive the variations in~~of~~ environmental conditions within the pond system and become dominant over time.

Materials and methods

Description and characteristics of the Motetema wastewater treatment works (WWTW)

Motetema WWTW (25°6'3.87" South and 29°28'6.78"), as pilot study, is situated in the small town of Elias Motsoaledi, Sekhukhune District of the Limpopo Province, South Africa~~ (Fig. 1)~~. Due to the lack of proper WWTW infrastructure and electricity (no aeration or mechanical mixing), a series of ponds are employed at the Motetema WWTW to treat sewage effluent. The WWTW consists of 12 earth ponds organised in two series of six each, parallel to one another, without any algae treatment or mechanical aeration. Of the 12 ponds, only 6 ponds are operated at a time, while the other 6 ponds are dried for sludge removal (Table 1; Fig. 1~~3~~). The WSP system is based on natural overflow from one pond to another, with an average retention time of 28 days. The average total effluent that needs to be treated (for a population of 11 400) by the Motetema WWTW is ~ 2.5 ML day^{-1}. However, Motetema WWTW is over capacitated and treat ~ 4.1 ML day^{-1}. The total average raw sewerage water inflow in pond one is 1.318 m s^{-1}. Wastewater from the Motetema WWTW flows into the phosphate sensitive Olifants River catchment.

Algal culture preparation

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 (Starr, 1964; Coder and Starr, 1978). Both microalgae species occur naturally in South African water bodies. Cultures of *Chlorella vulgaris* and *Chlorella protothecoides* were aseptically transferred from a 0.1 ml aliquot to 5 ml of fresh Sigma Algal Broth Medium (Sigma-Aldrich Chemie GmbH, Switzerland). The algae were cultured under static laboratory conditions at 20°C with a light intensity of 100 µmol m^{-2} s^{-1} (with circadian rhythm of 12 hours day: 12 hours night). To verify if the microalgae cultures were mono-specific population~~axenic~~, a compound microscope at 1250 x magnification was used to examine the different cultures every 3 days; if the cultures were not a mono culture~~axenic~~ the isolation procedure was repeated. Long-term laboratory mono~~axenic~~ cultures of *Chlorella* spp. were maintained by routine serial sub-culturing over 3 months. Each *Chlorella* sp. culture was cultivated for 14 days in liquid algal culture broth (Sigma-aldrich, Germany) before it was sub-cultured. A scale-up approach was followed for the mass culturing of the *Chlorella* spp. : 250 ml flask (starting culture ± 10 000 cells mL^{-1})

→25 L glass tanks→5 000 L algae bioreactor. Total cell counts were recorded using a Countess Automated cell counter (Invitrogen Life technologies, US).

Construction of algae bioreactors and algae mass culturing

Five semi-transparent photo-bioreactor tanks [~~(~~capacity of 5 000 L each, diameter (1800 mm) and height (2040 mm))] were installed at the Motetema WWTW (Fig 2.). In each of these tanks a volume of 25 L of the two algal species (50: 50%) was added to 1000 litre dechlorinated tap water and 10 g synthetic fertiliser (10:20:10). This culture was upscaled to 5 000 L in each photo-bioreactor and released in the selected different oxidation ponds on a 3 to 4 weekly basis, depending on the season. The photo-bioreactors' algae dry weight during the different culturing phases were the following: lag phase [average dry wt. 1.12 (± 0.13) mg L^{-1}]; exponential phase [average dry wt. 1.69 (± 0.15) mg L^{-1}] and in the stationary phase [1.186 (± 0.13) mg L^{-1}].

Algal mass inoculation to ponds

Only maturation ponds 4 and 5 were inoculated with algae (7.500 L per pond) on a 3 to 4 week basis (depending on the growth of the algae - in mid-winter it took 4 weeks and during autumn and spring 3 weeks) with the selected consortium of algae (± 1.2~~0~~ x 10^6~~000~~ cells mL~~l~~$^{-1}$). Due to the fact that the pond system is based on natural overflow from one pond to another, it was assumed that the inoculated algae will move from one pond to another by natural flow (Fig 1~~3~~).

Physicochemical analysis of ~~sewage~~ wastewater from the Motetema WWTW before and after algae treatment

All samples for physical, chemical and biological analyses were collected at the outlet of each pond, in the morning between 9h00 -11h00 a.m. Samples were taken once a month, before algae inoculation, for a period of 6 months during continuous inoculation. Sampling was conducted during the autumn, winter and spring months (March 2016 to August 2016). Dissolved oxygen (DO), temperature (°C), pH and electrical conductivity (EC) were measured *in situ* in the water column using a Hach HQ 40d multiparameter (USA, Hach, Loveland, Colorado). Surface water column samples (top 5 cm) were collected using a grab sampler and kept in polyethylene bottles (1 L) that had been pre-rinsed with diluted sulfuric acid (to pH 2.0) for the analysis of dissolved nutrients. The samples were kept cool whilst transported to the laboratory in a dark container. All water analyses were carried out according to standard methods (APHA, 1992). The following methods were used namely APHA 4500-N: Total Nitrogen, APHA 5310-B: Total Organic Carbon, APHA 5220-D/HACH Method 8000: Total Chemical Oxygen Demand, APHA 4500-P: Total Phosphorus, APHA 2540-D: Suspended Solids, APHA 4500, Cl C: Chloride, APHA 4500-SO_4 G: Sulphate, APHA 4500-NH_3 H: Ammonia, APHA 4500-PO_4 G: Phosphate. Water samples before and after algae treatment of ponds 5, 6 and 7 were filtered through 0.22 µm pore size Whatman GF/filters to separate the algae from the treated water for the determination of Total Nitrogen (TN) and Total Phosphorous (TP) uptake by the algae. Both filtered and unfiltered data was used in the analyses.

Phytoplankton identification

One sample (1 litre bottle), at the outlet of each pond, was taken on a monthly basis over a period of 6 months and divided in three sub-samples for (a) soft algae identification, (b) diatom identification and (c) suspended chl-*a* analyses. For the determination of suspended chl-*a* (µg L^{-1}) in the water column, the protocol of Porra et al. (1989) was followed. The samples for algae identification were preserved in the field by adding 2.5% (v/v) calcium carbonate-buffered glutaraldehyde. The diatom sub-sample from each pond was cleared of organic matter by heating it in a potassium dichromate and sulphuric acid solution and the cleared material was rinsed, diluted and mounted in Pleurax medium for microscopic examination. Algae were identified up to species level using a compound microscope at 1250 times magnification (Van Vuuren et al., 2006; Taylor et al., 2007b; Truter, 1987, Wehr and Sheath, 2003). The samples were sedimented in a Sedgewick-Rafter sedimentation chamber and analysed using the strip-count method (APHA, AWWA and WPCF, 1992). The numerical numbers for general grouping of abundance of algae taxa at each sampling site was categorised according to Oberholster et al. (2013): 1 = ≤ 250, 2 = 251-1000, 3 = 1001-5000, 4 = 5001-25 000 cells mL^{-1}. The Berger-Parker dominance index (Berger and Parker, 1970) was used to measure the evenness or dominance of each algal species at each sampling site using actual algae cell numbers:

$$D = N_{\max}/N \qquad \text{Equation 1}$$

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 site.

Results

The results of the quantitative changes in the algal community pre- and post algae treatment are presented in Table 2. In the pre- algae treated sewage water of pond 4, a total of 15 algal species were recorded with *Micractinium pusillum* as the dominant species. However, after 6 months of inoculation of *Chlorella* spp. only 9 species co-existed in low numbers with the *Chlorella* spp. The average pH of the pre- algae treated water (ponds 4, 5 and 6) was 8.0 and changed to pH 8.7 after treatment with the specific algae (Table 2). ~~The higher pH values were possibly attributed to higher photosynthetic rates of the inoculated algae biomass drawing more CO_2 from the water column (Madhab et al. 2013).~~ Total nitrogen and phosphorus varied from one pond to another before treatment, but decreased along the treatment system after the algae treatment.

The reduction of total phosphorus in the unfiltered water (contain algae) after algae treatment was 74.7% and 76.4% for ponds 5 and 6 respectively. ~~There are two possible explanations for the reduction of phosphorus concentrations during the algae treatment. The first is the incorporation of phosphorus into the algae biomass as shown in Table 3, while the second is the precipitation of phosphorus at high pH values in calcium rich water. The latter is known to occur at pH values greater than 8.5 (Mesple et al., 1995; Moutin et al., 1992).~~ The reduction of total nitrogen was much less than total phosphorus after the algae treatment. The total nitrogen removal (unfiltered) in ponds 5 and 6 were 43.1% and 35.1% respectively. The suspended solids increased from before to after algae treatment. ~~The latter can possibly be related to the dense suspended algal solids after treatment of ponds 5 and 6. The increase of suspended solids after algae treatment was also reported by Madhab et al. (2013).~~

Although the system displayed a reduction of COD after treatment, it was unable to reduce COD levels in the unfiltered samples to meet the South African effluent discharge standard of 75 mg L^{-1}. However, a large portion of this residual COD was possibly related to the algae biomass that increased the chemical demand of dissolved oxygen levels. This phenomenon was evident from the filtered water samples where algal cells were separated from the treated pond water (Table 4).

Chlorophytes were the dominant algal group throughout the pre-algae treatment sampling period in ponds 4, 5 and 6. The chlorophyte *Micractinium pusillum* was the dominant algae (Berger & Parker Index, 0.311; 0.332; 0.289) at ponds 4, 5 and 6 before treatment. These results are similar to those observed by Madhab et al. (2013) who reported dominance of chlorophytes in maturation ponds. The maximum algae abundance was $2.7x10^4$ cells mL~~l~~$^{-1}$ in pond 4 before treatment, while the maximum chl–*a* concentration of 242 µg L^{-1} coincided with the maximum algae biomass in this pond. Algae abundance of $3.1x10^4$ cells mL~~l~~$^{-1}$ was measured in pond 5 before treatment, with a maximum chl–*a* concentration of 341µg L^{-1}.

Diatoms were present in low numbers before and after treatment throughout the study. ~~and can possibly be related to our sampling technique, since Bartel et al. (2008) reported that diatoms preferred the bottom layer of ponds.~~ After treatment with ± 1.2 x 10^6 cells mL^{-1} the dominant algae (Berger & Parker Index, 0.431; 0.462; 0.451) was *Chlorella protothecoides* in ponds 4, 5 and 6 (Table 3). The maximum algae abundance ($4.6x10^6$ cells mL~~l~~$^{-1}$ in pond 4 and $6.1x10^6$ cells mL~~l~~$^{-1}$ in pond 5) were observed in August, while the maximum chl–*a* concentration of 783µg L^{-1} was measured in pond 5 after six months of algae inoculation. The average chl–*a* in ponds 4 and 5 before algae inoculation was 176 µg L^{-1}, which changed to an average of 611µg L^{-1} after four months of algae treatment. ~~Table 5 summerize~~ T~~t~~he behaviour of the system at pond 6 (last pond before releasing treated wastewater in the Olifants River) during autumn, winter and spring of 2016 is summarized in Table 5. From the data it was evident that the algae abundance and chl-*a* ~~did~~ increased from autumn to winter, even with a reduction in irradiance and surface water temperature.

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Discussion

~~Eutrophication and climate change are two key pressures impacting aquatic ecosystems' structure and function. According to the IPCC (2007), current trends of climate change could lead to an average global temperature increase of 2 – 3°C. A 2°C increase can lead to a 20 – 30% decrease in water availability in some vulnerable regions, such as southern Africa and the Mediterranean. Temperature increases can also accelerate the eutrophication process (Carvalho and Kirika 2003) e.g. phosphorus recycling is more intensive in warmer waters, while processes of phosphorus release from lake sediment and mineralization are highly temperature dependent (Hamilton et al. 2001). Nevertheless, the reduction of nutrients especially phosphorus from WWTPs is an important aspect in reducing eutrophication and to improve water quality and reuse (Oberholster et al 2009; 2013). Therefore, the photosynthetic capabilities of specific algae are particularly attractive, converting solar energy into useful biomass while incorporating nutrients including nitrogen and phosphorus. Algal systems has previously been used to treat domestic wastewater and agricultural waste (Zaid-Iso, 1990; Ma et al., 1990; Phang, 1990, 1991). Algae have also been studied for the removal of toxic minerals (Hammouda et al., 1995; Cai-XiaoHua et al., 1995).~~

The current findings from the chemical analyses of the field sampling suggest that the natural treatment was not efficient in reduction of phosphorous and nitrogen to levels below the Department of Water Affairs and Forestry (DWAF) standards for special limits, domestic use and field guidelines (DWAF 1996a; 1996b) at the effluent point of the Motetema WWTW. The reduction of total phosphorus after algae treatment was possibly ~~be~~ related to the following two explanations. The first is the incorporation of phosphorus into the algae biomass as shown in Table 3, while the second is the precipitation of phosphorus at high pH values in calcium rich water. The latter is known to occur at pH values greater than 8.5 (Mesple et al., 1995; Moutin et al., 1992). The higher pH values observed after the algae treatment were possibly attributed to higher photosynthetic rates of the inoculated algae biomass drawing more CO_2 from the water column (Madhab et al. 2013). The increase of suspended solids from before to after algae treatment was related to the dense suspended algal solids after treatment of ponds 5 and 6. The increase of suspended solids after algae treatment was also reported by Madhab et al. (2013). The increase in pond 6 of algae abundance and chl-*a* from autumn to winter, even with a reduction in irradiance and surface water temperature, were possibly~~e~~ related to the inoculation proc~~s~~ess in pond 5. The first inoculation of specific algae in ponds 4 and 5 were started at the beginning of March 2016 and the algae in pond 5 may not have reached pond 6 when measurements were taken at the end of March 2016 (Table 5).

Although the system displayed a reduction of COD after specific algae treatment, it was unable to reduce COD levels to meet the South African effluent standard over the six month period. The latter reduction was also observed by Jayangouder et al. (1983), which reported that *Chlorella vulgaris* induced progressive reduction in both BOD and COD due to high algal growth rate and intense photosynthetic activities.

Previous studies have showed that *Chlorella* sp., under aeration conditions, has a high removal efficiency (more than 80%) of nutrients in primary and secondary treated effluents and has also been found to completely remove ammonia nitrogen, nitrate, total nitrogen (TN) and total phosphorous (TP) (Wang et al. 2010). Evidence from the current study showed that *Chlorella* spp. can be used to optimise the treatment of oxidation ponds without using aeration especially in rural areas of Southern Africa without any electricity. Based on the field data (Table 2 and Table 4), it was evident that there was an effective reduction of nutrients using a combination of the selected algae *C. vulgaris* and *C. protothecoides.*

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. Environmental factors like weather and contamination from strains of bacteria or other outside organisms, often result in undesirable algae or blue-green algae species taking over the desired algal growth in the oxidation ponds. Lee (2001) reported that culturing *Chlorella spp.* in open ponds is often not possible due to the harsh culture conditions. Temperature fluctuations of up to 20°C between day and night makes it very difficult to maintain the optimum growth temperature (Borowitzka, 2005). Most field observations indicated more than one to three dominant phytoplankton species existed at any phase of seasonal development, as predicted by the competitive exclusion theory (Hardin, 1960). The reason for this occurrence is related to the different responses of phytoplankton species to the frequency of disturbances or changes in abiotic resource conditions at different scales (Reynolds, 1984). Nevertheless, in the current study the *Chlorella* spp. in open ponds stayed dominant possibly due to the inoculation on a 4 weekly basis. The low numbers of diatoms before and after treatment can possibly be related to our sampling technique, since Bartel et al. (2008) reported that diatoms preferred the bottom layer of ponds. However, certain phytoplankton species that coexisted with the *Chlorella* spp. after inoculation were *Micractinium pusillum, Melosira varians* and *Scenedesmus* spp. A clear phytoplankton succession consists of species that were originally well adapted to certain ponds, but lost their abilities and was consequently substituted by other species after inoculation of the *Chlorella* spp. A shift of the algae population after inoculation of the *Chlorella* spp. can possibly be related to environmental conditions e.g. pH. Klein (1972) reported that pH changes cause a shift in the relative abundance of various genera in the aquatic system, which is in agreement~~conformity~~ with the present investigation, where along with the increase in pH i.e. from 8.1 to 8.9, ~~caused the change from~~the abundance of genera~~species~~ like *Oscillatoria*, *Scenedesmus* and *Euglena* changed. The only species at ponds 4, 5 and 6 that co-existed with the dominant inoculated *Chlorella* spp. was *Micractinium pusillum*. The latter species are classified under Chlorophyceae and considered opportunistic for their small size and rapid growth favouring its presence in any season of the year (Happey-Wood, 1988). According to Mahapatra et al. (2013) *Micractinium* sp. can be used as indicator to forecast changes in loading conditions in pond systems.

One of the main concerns using specific strains of algae in SADC countries are temperature requirements of the selected strains. Temperature variations can affect biochemical reactions and subsequently biochemical composition of algae. According to Tadross and Johnston (2012), the average regional environmental temperature of Southern Africa ranges between 6-32°C. *Chlorella* spp. used in the current study can grow in surface water temperatures between the ranges of 5 °C to 30 °C (Yang et al., 2010). *Chlorella* grown at 27 °C had a doubling time of 8.6±0.6 h, while at 5 °C, the cell growth was 48.5±2.6 h. According to Shi (2006), *C. protothecoides* appears to be a ‘low temperature’ (optimal between 25°C to 30°C) species in terms of growth. The latter study showed that maximum cell concentration occurred at 28°C, while 35°C gave a much lower value. Converti et al. (2009) showed in their study that *C. vulgaris* grew best at temperature ranges of 25°C-30°C. However, surface water temperatures may reduce rates of photosynthesis. Although the current study showed that inoculation of specific algal strains can potentially enhance the treatment efficiencies of existing domestic sewage pond systems, it was also evident from the algae filtration analyses (Table 4), that the algae must be harvested for maximum water treatment results. According to Acien et al., (2016) 1 kg of algae biomass can be produced per cubic metre of wastewater depending on the nutrient content of the waste water treated. Algae biomass from up to a 1000 ton can be produced from a small rural population of 10, 000 people for the production of bio-fertiliser. This microalgae

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fertiliser does not only contain nutrients, but also phyto-hormones and growth promotors (Acien et al., 2016). Removal of the algae biomass could therefore serve a dual purpose in that it would not only improve the treatment efficacy of the WSP, but the harvested biomass could be used for the production of fuel or bio-fertiliser (Coppens et al., 2016).

Conclusion

The sustainable design and operation of a WWTP is inextricably linked to its environmental and economical footprint. High treatment efficiency and low energy consumption are key to sustainability. The current study showed that the proposed algae technology is low cost and environmental friendly and can easily be sustained, while using existing infrastructure. The novelty of the technology is (1) the consortium of specific algae that were selected on the basis of robustness, maximum nutrient absorption, survival in different temperature ranges and effectively inhibiting coliforms. Also (2), other algae and conventional treatment technologies currently on the market requires high cost investment, electricity for mixing of algae, need skilled labours to maintain the system, and does not use a specific consortium of algae for maximum absorption. Furthermore, conventional waste water treatment plants use non-environmentally friendly chemicals and depend on mechanic mixing, which requires constant electricity supply.

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References

Abinandan S, Shanthakumar S (2015) Challenges and opportunities in application of microalgae (Chlorophyta) for wastewater treatment: a review. Renew Sust Energ Rev 52: 123-132

Abdel-Raouf N, Al-Homaidan, Ibraheem IBM (2012) Microalgae and wastewater treatment. Saudi Journal of Biological Sciences 19: 257-275

Acien FG, Gomez-Serrano C, Morales-Amaral M, Fernandez-Sevilla JM, –Molina-Grim E (2016) Wastewater treatment using microalgae: how realistic a contribution might it be to significant urban wastewater treatment? Appl Microbiol Biotechnol 100:9013-9022

APHA (2006) Standard Methods for Examination of Water and Wastewater, 20th ed. American Public Health Association, Washington, DC. Moet delete

APHA (1992) Standard methods for the examination of water and wastewater, 18th ed. Washington, DC: American Public Health Association.

Barthel L, Oliveira PAV, da Costa RHR (2008) Plankton biomass in secondary ponds treating piggery waste. Braz Arch Biol Technol 51:1287-1298

Berger WH, Parker FL (1970) Diversity of planktonic *Foraminifera* in deep sea sediments. Sci 168:1345-1347

Butler E, Hung Y, Al Ahmad M S, Yeh R Y, Liu R L, Fu Y (2015) Oxidation pond for municipal wastewater treatment . Appl Water Sci DOI 10.1007/s 13201-015-0285-z

Carvalho L, Kirika A (2003) Changes in shallow lake functioning to climate changes and nutrient reduction. Hydrobiologia 506:789-796

Coder DM, Starr M (1978) Antagonistic association of the chlorellavorus bacterium ('Bdellovibrio' chlorellavorus) with *Chlorella vulgaris*. Curr Microbiol 1: 59-64

Converti A, Casazza AA, Ortiz EY, Perego P, Delborghi M (2009) Effects of temperature and nitrogen concentration on the growth and lipid content of *Nannochloropsis oculata* and *Chlorella vulgaris* for biodiesel production. Chem Eng Process 48:1146-1151

Coppens J, Grunert O, Van Den Hende S, Vanhoutte I, Boon N, Haesaert G, De Gelder L (2016) The use of microalgae as a high-value organic slow-release fertilizer results in tomatoes with increased carotenoid and sugar levels. J Appl Phycol 28: 2367-2377

Department of Water Affairs and Forestry (DWAF, 1996) South African Water guidelines. First edition. Volume 8: Field guide.

Department of Water Affairs and Forestry (DWAF, 1996) South African Water guidelines. Second edition. Volume 1: Domestic use.

Garcia J, Mujeriego R, Hernández-Mariné M (2000) High rate algal pond operating strategies for urban wastewater nitrogen removal. J Appl Phycol 12: 331-339

Gratziou MK, Tsalkatidou M, Kotsovinos NE (2006) Economic evaluation of small capacity sewage processing units. Global NEST Journal 8: 52-60

Hamilton DP, Spillman C, Prescott K, Kratz TK, Magnuson JJ (2001) Effects of atmospheric nutrient input on trophic status of Crystal Lake, Wisconsin. Verh Internat Verein Limnol 28:467-470

IPCC (2007) Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change.

König A (2000) Biologia de las lagunas de estabilizacion: algas. In: Sistemas de Lagunas de estabilization: como utilizar aguas residuals tratadas en sistemas de regadio. Mendonca, S.R. (Coord.) McGrawHill, 44-67

Larsdotter K (2006) Wastewater treatment with microalgae-a literature review. Vatten 62: 31-38

Madhab D, Mahapatra H, Chanakya N (2013) Treatment efficacy of algae-based sewage treatment plants. Environ Monit Assess 13:1-19

Mesple F, Troussellier M. Casellas C, Bontoux J (1995) Difficulties in modelling phosphate evolution in a high-rate algal pond. Water Sci Technol 31:45-54

Moutin T, Gal JY, El Halouani H, Picot B, Bontoux J (1997) Decrease of phosphate concentration in a high rate pond by precipitation of calcium phosphate: Theoretical and experimental results. Water Res 26:1445-1450

Oberholster PJ, Botha AM, Myburg JG (2009) Linking climate changes and progressive eutrophication to incidents of clustered animal mortalities in different geographical regions of South Africa. Afr J Biotechnol 8:5825-5832

Oberholster PJ, Botha AM, Chamier J, De Klerk A (2013) Longitudinal trends in water chemistry and phytoplankton assemblage downstream of the Riverview WWTP in the Upper Olifants River. Ecohydrol Hydrobiol 13:41-51

Oberholster PJ, Botha AM, Hill L, Strydom WF (2017) River catchment responses to anthropogenic acidification in relationship with sewage effluent: An ecotoxicology screening application. Chemosphere 189:407-417

Palmer CM (1969) A composite rating of algae tolerating organic pollution. J Phycol 5:78-82

Pearson H, Mara D, Arridge H (1995) The influence of pond geometry and configuration on facultative and maturation waste stabilization pond performance and efficiency. Water Sci Technol 31: 129-139 moet verander in stuk van mara wat eerste is

Pham DT, Everaert G, Janssens N, Alvarado A, Nopens I, Goethals PLM (2014) Algal community analysis in a waste stabilisation pond. Ecol Eng 73: 302-306

Porra RJ, Thompson WA, Kriedemann PE (1989) Determination of accurate extinction coefficient 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 975:384-394

Starr RC (1964) The culture collection of algae at Indiana University. Am. J. Bot. 51: 1013-1044

Shi XM, Liu HJ, Zhang XW (1999) Production of biomass and lutein by *Chlorella protothecoides* at various glucose concentrations in heterotrophic cultures. Process Biochem 34:341-347 moet gedelete word

Tadross M, Johnston P (2012) ICLEI-Local Governments for Sustainability- Africa Climate Systems Regional Report: Southern Africa. ISBN: 978-0-9921794-6-5.

Taylor JC, Harding WR, Archibald CGM (2007) An illustrated guide to some common diatom species from South Africa. – WRC Report, No. TT 282/07. Water Research Commission, Pretoria, South Africa, plates, pp 1-178.

Truter, E. 1987 An aid to the identification of the dominant and commonly occurring genera of algae observed in some South African impoundments. Pretoria, South Africa: Department of Water Affairs, 1-97 pp.

Van Vuuren S, Taylor JC, Gerber A, Van Ginkel C (2006) Easy identification of the most common freshwater algae. North-West University and Department of Water Affairs and Forestry, Pretoria, South Africa, pp 1-200

Varon MP, Mara (2004) Water stabilization ponds. IRC International Water and Sanitation Centre, Leeds, UK.

Wang L, Min M, Li Y, Chen P, Chen Y, Liu Y, Wang Y, Ruan R (2010) Cultivation of green algae *Chlorella* sp. in different sewage wastewaters from municipal sewage wastewater treatment plant. Appl Biochem Biotechnol 162:1174-1186

Wang H, Wang T, Zhang B, Li F, Toure B, Omosa IB, Chiramba T, Abdel-Monem M, Pradham M (2014) Water and Wastewater Treatment in Africa-Current Practices and Challenges. Clean: Soil Air Water 42:1029-1035 in artikel

Wehr JD, Sheath RG (2003) *Freshwater habitats of algae.* In : Wehr JD, Sheath RG (eds.), Freshwater Algae of North America: Ecology and Classification. Academic pp 11-57