

Review Paper

Potential Use of High Rate Algae Ponds for Resource Recovery in the Water-Food-Energy Nexus for Tanzania: A Review

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ABSTRACT

*The use of High Rate Algal Ponds (HRAPs) for the treatment of wastewater and resource recovery has raised interest in recent years. Treatment of wastewater through this technology has proved to have high efficiency in reducing the level of pollution, nutrients, dissolved solids as well as pathogens. HRAPs are more efficient than conventional Wastewater Stabilization Ponds (WSPs) due to their design approach that provides room for high rate bio-chemical processes, which increase the mechanisms of nutrients and pathogens removal as well as the rate of micro-algae production for purposes of resource recovery. This paper reviews the upgrading potential of existing WSPs to HRAPs for resource recovery from products of wastewater for biofuel production, as a plant nutrient or for irrigation purposes and animal feeding. Several results have reported HRAPs to have efficiency in reducing bacterial contamination in excess of 99% while the removal of organic matter of up to 84% for Chemical Oxygen Demand (COD) and 88% for Biochemical Oxygen Demand under normal conditions have been reported. The removal for nitrogen was indicated to vary from 50 to 98% while that of phosphorus varies from 32 to 99% depending on the culture conditions. It was further noted that, the potential for resource recovery from HRAPs is high in terms of energy and nutrients recovered through algae biomass, particularly for biofuel and animal feed production. Whereas among the dominant algal species of the HRAP *Chlorella vulgaris* revealed to have suitability in both treatment of wastewater and achieved a higher effluent quality and having nutrients contents essential for lipid extraction for biofuel and as a protein source for animal feeding which is largely attributed by their ability to grow very rapidly and to tolerate varieties of cultural conditions. To date, limited research attention has been given to studying the re-use potential of wastewater for irrigation purposes in Africa.*

Keywords: *High rate algal ponds, Wastewater Treatment, Micro-algae, Resource Recovery.*

INTRODUCTION

The application of High Rate Algal Ponds (HRAPs) for the treatment of wastewater has raised interest and caught global attention in recent years (Pittman *et al.*, 2011) although numerous studies on use of

high rate ponds for nutrients removal for resource recovery have been carried out for over 60 years (Oswald *et al.*, 1957; Oswald and Golueke, 1960; García *et al.*, 2000; Craggs *et al.*, 2014; Drira *et al.*, 2016). HRAPs were first developed in the United States of America in the middle of

the 20th century for wastewater treatment (Oswald *et al.*, 1957; Oswald and Golueke, 1960). They are now being used in different parts of the world including Israel, South Africa (Azov *et al.*, 1982; Abeliovich, 1986; Buhr and Miller, 1983; Shelef and Azov 1987), Morocco (Bouchaib, 2009), Australia (Young *et al.*, 2016), France (Picot *et al.*, 1991), the United Kingdom (Fallowfield and Garrets, 1985), Spain (García *et al.*, 2008), China, and New Zealand (Craggs *et al.*, 2012; Craggs *et al.*, 2014).

Previous studies have shown that HRAPs are more efficient than conventional Waste Stabilization Ponds (WSPs) for the treatment of wastewater and algae production that can be used for various resource recovery applications (Sayre, 2010; Craggs *et al.*, 2014; Butler *et al.*, 2017). They are considered as effective reactors that reclaim water; nutrients and energy from organic wastewater (Young *et al.*, 2017). Micro-algae possess the potential to produce bio-oils as a source of energy, carbohydrates, proteins, amino acids and other value-added products (Cooney *et al.*, 2011). In accordance with Burlew (1953), the first micro-algae cultivation started in early 19th century with *Chlorella vulgaris*. The mass cultivation of micro-algae began in the 1940s in the United States, Germany, and Japan, while the first commercial large-scale micro-algae culture system using *Chlorella* was developed in the 1960 (Park *et al.*, 2018).

In most of urban centres, management of both faecal sludge and wastewater is posing a lot of challenges (Brandes *et al.*, 2015). Due to high rates of urbanisation, population growth and economic development, the generation of wastewater is increasing rapidly, especially in the Global South (Phuntsho *et al.*, 2017). It has been estimated that 80% of wastewater generated globally, is directly discharged into the environment without being treated

or being re-used, with 90% in developing countries (D'Andrea *et al.*, 2015). Several technologies for the treatment of faecal sludge and wastewater are practiced, but the most common treatment technology used in tropical climate is Wastewater Stabilization Ponds (WSPs) because of the favourable climatic conditions (Mara, 2013; Craggs *et al.*, 2003). This enables most of the biological systems to function effectively without human interference (Mayo, 2013).

The conventional WSPs systems discharge high levels of nutrients in their effluents, which contribute to water eutrophication, which in turn can affect the aquatic life of receiving water bodies (Garcia and Marine, 2000). High levels of nutrients in the effluents also accelerate the growth of algae blooms on the surface of the storage ponds (Mbwele, 2006). Some scholars have reported that excessive nitrogen in water in form of nitrate can cause *methaemoglobinemia* in infants and susceptible populations, and in the form of ammonia it is toxic to fish and exerts oxygen demand in receiving water by nitrifiers (Mayo, 2013; Picot *et al.*, 2009). In accordance with Liu *et al.* (2017) several pathogens, such as bacteria and helminths, responsible for causing communicable diseases are found in effluents of conventional ponds, and their values generally exceed the permissible limits that pose risks to public health. Unfortunately, many of the pond systems are not designed to optimise the recovery of resources from wastewater.

HRAPs are closed-loop, paddlewheel-mixed ponds, which can take a few metres of an area in which a typical design consist of a series of parallel meandering channels (Figure 1). Among the operational features of HRAPs, depth has been taken as a crucial input for the pond performance (García and Marine, 2000; Craggs *et al.*, 2012; Sutherland *et al.*, 2014). Basing on various recommendations from literature,

HRAPs should maintain a shallow depth as much as possible since shallow depth allows much light to penetrate throughout the pond system whereby micro-algae cells are exposed to optimal light. The optimal range of depth reported in literature ranges from 0.2 to 0.5 m. The technology essentially consists of shallow race track reactors with mechanical mixing which recirculate the contents of the pond in which the interchange of CO₂ and O₂ is promoted between algae and aerobic organisms (Buhr and Miller, 1983).

Studies show that high rate pond systems have incorporated many improvements of

conventional ponds (Young *et al.*, 2017). Their designs provide room for high rate bio-chemical processes, which speeds up the removal of sludge disposal, minimizes bad odours and increases the mechanism of nutrients and pathogens removal (Craggs *et al.*, 2014). They are more economic than conventional ponds and they provide micro-algae for biofuel production, food and animal feed (Paulo *et al.*, 2009; Sayre, 2010; Rupiper, 2016). The purpose of this paper is to review the potential uses of HRAPs with focus on opportunities of re-using the resources recovered from wastewater.



Figure 1: A laboratory-scale High Rate Algal Pond at New Mexico State University (source: <https://www.google.com/search?q=Photos+of+high+rate+algal+pond>)

PERFORMANCE OF HRAPs IN WASTEWATER TREATMENT

Operational factors that manage the performance of HRAPs include the pond depth and its influence on light penetration which account for the light regime to which the photosynthetic organism has to be exposed, Hydraulic Retention Time (HRT) of the effluent in the pond as well as effect of turbulence on nutrients availability and exposure to light intensity.

The operating characteristics of HRAPs are largely determined by the various interactions between the several chemicals and the biological processes within the system as well as the environmental factors.

Removal of Chemical and Biochemical Oxygen Demand

The organic compounds of wastewater comprise of a large number of compounds

with all having at least one carbon atom. The carbon atom in these compounds can be oxidized biologically by bacteria to yield CO₂. Some of the algae species have been reported to have high efficiency of removing organic matter. In accordance with literature (Choi and Lee, 2012) the removal efficiency of organic matter

increased with an increased amount of *Chlorella vulgaris*. By increasing *Chlorella vulgaris* concentration from 1 to 10 g/L, the removal efficiency of organic matter increased from 80.4% to 82.9% for BOD₅ and 78.3% to 82.3% for COD (Table 1).

Table 1: Chemical and Bio-chemical Oxygen Demand Percentage Removal Efficiency in High Rate Ponds

Algal Specie	COD		BOD ₅		Reference
	% Removal	Condition/addition	% Removal	Condition/addition	
<i>Micractinium</i>	77.4%	Normal	N/A	N/A	Nurdogan and Oswald (1995)
	84%	80mg/l CaO	N/A	N/A	
Not Specified	35%	HRT = 4 days	N/A	N/A	García <i>et al.</i> (2006)
	38%	HRT = 7 days	N/A	N/A	
<i>Chlorella vulgaris</i> Phormidium Sp Scenedesmus Sp	10%	HRT = 4 days	N/A	N/A	Cromar and Fallowfield (1997)
	30%	HRT = 7 days	N/A	N/A	
<i>Chlorella vulgaris</i>	67.2%	Normal	68.4%	Normal	Colak and Kaya. (1988)
<i>Fragilaria,</i> <i>Euglena,</i> <i>Chlorella,</i> <i>Micratinium,</i> <i>Cyclotella,</i> <i>Navicula</i>	50%	Normal	N/A	N/A	Chen <i>et al.</i> (2003)
Not Specified	N/A	N/A	~ 50%	BOD ₅	Craggs <i>et al.</i> (2012)
	N/A	N/A	~ 87%	fBOD ₅	
<i>Scenedesmes obliquus</i> and <i>Micracitinium pusillum</i>	31%	Normal	32%	Normal	Doma <i>et al.</i> (2015)
<i>Chlorella vulgaris</i>	66%	Normal	70%	Normal	Sahu (2014)
<i>Chlorella vulgaris</i>	78.3%	1 g/L <i>C. vulgaris</i>	80.4%	1 g/l <i>C. vulgaris</i>	Choi and Lee (2012)
	82.3%	10g/L <i>C. vulgaris</i>	82.9%	10g/l <i>C. vulgaris</i>	
Not Specified	N/A	N/A	88%	Normal	Hamouri <i>et al.</i> (1994)

HRT= Hydraulic retention time, BOD₅ = Biochemical oxygen demand after five days, fBOD₅ = Filtered Biochemical oxygen demand after five days COD = Chemical oxygen demand and N/A = Not applied

Nutrients Removal

Nutrients like nitrogen and phosphorous can be removed in HRAPs through biological treatment of wastewater. In sewage effluent, nitrogen primarily arises from metabolic inter-conversions of several compounds, while 50% or more of the phosphorus arises from synthetic detergents. Principally, nitrogen in wastewater occurs in form of NH₄⁺ (Ammonia), NO₂⁻ (nitrite), NO₃⁻ (nitrate) and phosphorus in most cases is in the

form of PO₄³⁻ (orthophosphate). The removal of these two elements is by nutrients stripping, uptake by micro-organisms and precipitation. When these nutrients are in excess in receiving water bodies, they cause eutrophication which in turn leads to excessive growth of harmful microalgal blooms (Abdel-Raouf *et al.*, 2012). However, several studies have shown that a HRAP fed with clarified domestic wastewater with CO₂ supplement, can remove nutrients to levels of concentration better than those achieved

in conventional ponds (Woertz *et al.*, 2009; Craggs, 2012; Batter, 2013).

Nitrogen transformation and removal in High Rate Algal Ponds

Organic nitrogen and NH_3 enter into the system of HRAPs with the influence of wastewater. Organic nitrogen in faecal matter and other organic materials undergoes conversion to NH_3 and ammonium ion (NH_4^+) by microbial activity. When HRAPs efficiently operate in treating wastewater, nitrogen is effectively removed (Jones *et al.*, 2016). The concentration of nitrogen is lowered in the effluent by bacteria, denitrification, algal assimilation and NH_3 volatilization when pH is very alkaline. The major mechanisms for removal of nitrogen are uptake of ammonia by algae and nitrification-denitrification process and to some extent stripping of ammonia (NH_3) (Mayo, 2013; Mayo and Mutamba, 2004). Nutrient removal is highly influenced by massive growth of micro-algae and chlorophyll concentration whereby pH and temperature are the main contributing factors (Mayo *et al.*, 2018). For example, in summer, higher temperatures favour algal productivity and performance of HRAP in reducing $\text{NH}_4\text{-N}$ in the effluent and with high pH, a large portion of the nitrogen is removed through ammonia volatilization. High daytime pH generated in the ponds due to algal uptake of bicarbonates, shifts the equilibrium in favour of NH_3 , which may volatilize into the atmosphere when pH exceeds 9.0.

According to Chen *et al.* (2003), in a study, ammonia in the influent was by 71% while in the effluent concentration reduced to less than 12% and also the oxidized forms, the nitrite and nitrate appeared to be 19.3% with the mass balance showing a loss of nitrogen by 44.6%.

Micro-algae normally lower the concentration of nitrogen in the effluent through algal assimilation when the algae harvesting is incorporated and in turn contributes to the conditions that are favourable for the massive growth of algae whereby algal biomass and chlorophyll increase (Abdel-Raouf *et al.*, 2012). On the other hand, bacteria nitrification also plays a role of oxidation of $\text{NH}_4\text{-N}$ into $\text{NO}_2\text{-N}$ and $\text{NO}_3\text{-N}$ in the pond system. Ammonium is nitrified to nitrite (NO_2^-) by Nitrosomonas bacteria then to nitrate (NO_3^-) by Nitrobacter (USEPA, 2011). In anoxic conditions nitrate may be bacteria as an electron acceptor of electrons released by organic matter, thus reducing it to nitrogen gas.

Mechanism of phosphorous removal

In wastewater, phosphorus can be found in three forms; Organic phosphorus compounds, polyphosphates or condensed phosphates and orthophosphates, which carries 80% of the total phosphate in wastewater. Organic phosphorus compounds are mainly insoluble phosphor-proteins, nucleic acids and polysaccharides. Polyphosphates are in form of polymers of phosphoric acid while orthophosphates in HRAPs is as a result of complete hydrolysis of polyphosphates and total decomposition of organic phosphorus compounds through biological treatment of sewage (Nurdogan and Oswald, 1995). There are several forms of orthophosphates which is as a result of function values of pH. At the neutral pH of the domestic wastewater; the predominant form of pH is HPO_4^{2-} . At high rate of photosynthesis, the pH of wastewater in HRAPs may raise up to 11 in the afternoons of the summer days and around 9 during winter seasons. However, pH can increase in the pond due to photosynthetic depletion of dissolved CO_2 under inorganic – carbon limited growth of algae (Woertz *et al.*, 2009).

Orthophosphates are essential for growth of algae and other macrophytes. To avoid eutrophication in receiving water bodies, they have to be removed during wastewater treatment process. In algal cells, phosphates typically fall within the range of 0.35 to 1% (Craggs *et al.*, 2012) and may also reach 3.16% when there is luxury uptake. Phosphorus may be removed from wastewater by precipitation resulting from chemical addition or elevated pH levels (Chen *et al.*, 2003;

Rodrigues, 2013) and sometimes with longer Hydraulic Retention Time (HRT) (Table 2). Polyphosphates and organic phosphorus are known to be removed by adsorption on CaCO₃ crystals, which are formed in significant amounts in the pH range of HRAP operation. Precipitation is the main cause of phosphate removal in calcium – rich ponds. Therefore, calcium must be added in ponds with low concentration of calcium (Picot *et al.*, 1991).

Table 2: Nutrient Percentage Removal Efficiency in High Rate Ponds

Algal Specie	Nitrogen		Phosphorus		Reference
	% Removal	Condition/addition	% Removal	Condition/addition	
<i>Chlorella vulgaris</i>	86%	Normal	78%	Normal	Lau <i>et al.</i> (1996)
Not Specified	50%	Normal	85.7%	Normal	Colak and Kaya. (1988)
<i>Micractinium</i>	85%	Normal	45-55%	20-40mg/l CaO	Nurdogan and Oswald (1995)
	90%	60-80mg/l CaO	99%	60mg/l CaO	
Not Specified	73%	HRT = 7 days	43%	HRT = 7 days	García <i>et al.</i> (2006)
	57%	HRT = 4 days	32%	HRT = 4 days	
Multiple species	69%	HRT = 4 days	17% Con P	HRT = 4 days	Cromar and Fallowfield (1997)
<i>Chlorella vulgaris</i>	69% ~	HRT = 4 days	45% Ex P	HRT = 4 days	
<i>Phormidium Sp</i>	78% ~	HRT = 7 days	69% Con P	HRT = 7 days	
<i>Scenedesmus Sp</i>	78% ~	HRT = 7 days	93% Ex P	HRT = 7 days	
Not Specified	94%	Normal	71%	Normal	Picot <i>et al.</i> (1991)
<i>Fragilaria, Euglena, Chlorella, Micractinium, Cyclotella, Navicula</i>	87%	Normal	40%	Normal	Chen <i>et al.</i> (2003)
Mixed cultures	>98%	CO ₂	>96%	CO ₂	Woertz <i>et al.</i> (2009)
Not Specified	~65%	CO ₂	~19%	CO ₂	Craggs <i>et al.</i> (2012)
Not Specified	~60%	CO ₂	N/A	N/A	Park and Craggs (2010)
<i>Scenedesmes obliquus and Micracitinium pusillum</i>	56%	Normal	N/A	N/A	Doma <i>et al.</i> (2016)
<i>Chlorella vulgaris</i>	71%	Normal	67%	Normal	Sahu (2014)
<i>Chlorella vulgaris</i>	81.04%	1g/L <i>C. vulgaris</i>	32.26%	1g/L <i>C. vulgaris</i>	Choi and Lee (2012)
	84.81%	10g/L <i>C. vulgaris</i>	36.12%	10g/L <i>C. vulgaris</i>	
Not Specified	69%	Normal	52%	Normal	Hamouri <i>et al.</i> (1994)

HRT= Hydraulic retention time, Con p = Control pond, Ex p Experimental pond and N/A = Not applied

Mortality of Faecal Bacteria

There are several pathogenic organisms in wastewater including bacteria such as *Salmonella* and *Shigella*, protozoa, viruses and helminth eggs (Abdel – Raouf *et al.*, 2012). Several results have reported that

HRAPs are efficient in reducing bacterial contamination and the number of nematodes eggs. Apart from HRAPs being used for resource recovery attributed by massive growth of algae, it is also very important in treatment of wastewater (Oswald and Goueke, 1960; Oswald,

1995). Algae supply the oxygen demanded for bacteria degradation of organic matter, and bacteria excrete mineral compounds that provide the algae with nutrition which in turn accelerate the rate of photosynthesis. High rate of photosynthesis increases the level of pH which increase the mortality rate of pathogens. Among the pathogenic organisms, bacteria provide a large number of microbial communities in all biological wastewater treatment processes and several studies have reported the number in the range of 10^6 and above (Hamouri *et al.*, 1994; Bahlaoui, and Troussellier, 1997; Abdel – Raouf *et al.*, 2012; Doma *et al.*, 2015). However, considerable pathogen removal of more than 99% can be achieved in HRAPs (Bahlaoui and Troussellier, 1997).

At rapid growth of algae, the pH can rise up to and above 9, which is favourable for bacterial removal (Parhad and Rao, 1974; Young *et al.*, 2017). When algal activity is at its peak, carbonate and bicarbonate ions react to provide more carbon dioxide for algae, leaving an excess of hydroxyl ions. A pH above 9 for 24 hours ensures nearly 100% killing of *E. coli* and presumably most pathogenic bacteria (Young *et al.*, 2017). Other factors for faecal bacteria die-off include high temperature with increased time (Marais, 1974; Mancini, 1978; Mills *et al.*, 1992), starvation (Gann *et al.*, 1968), microbial antagonism (Polprasert *et al.*, 1983), production of toxic substances by algae (Merz *et al.*, 1962) as well as high light intensity due to shallow depth (Mayo, 1989; Mayo, 1995). Light of wavelength 425-700 nm can damage faecal bacteria. Ultraviolet radiation is known to disinfect bacterial cells, even those resistant to antibiotics. Meckes (1982) reported that total coliform isolates resistant to streptomycin, tetracycline, and chloramphenicol were disinfected by ultraviolet radiation. Fujioka *et al.* (1981) and Kapuscinski and

Mitchell (1983) have reported that visible light can also disinfect coliforms.

MICRO-ALGAE BIOMASS PRODUCTION

Among other plants, algae have been mentioned to have more efficiency to utilize energy from visible light. Micro-algae have the ability to grow very fast and yield high biomass, using non-fresh water streams as substrate (Park *et al.*, 2013). They do not interfere with food security if produced for biofuels, and can be harvested daily. The generated fuel has less emission of CO₂ compared to petroleum-based fuels, and therefore might reduce greenhouse gas emissions (Park *et al.*, 2018). In high rate algal ponds, up to 30 tons/ha/year of algae can be produced and their yield may increase up to 60 tons/ha/year if CO₂ is artificially applied for extra carbon supply. In conventional WSPs, the algae production is much lower, and can only go up to 10 tons/ha/year (Craggs *et al.*, 2011; Craggs *et al.*, 2014; Montemezzani *et al.*, 2015).

The key input for the algae growth include wastewater, sunlight and high solar radiation, sustainable source of CO₂ and nutrients (Batten, 2013). Production of algae is more reliable compared to other traditional plants as algae have the ability to operate in two distinct environments. This is aerobic and anaerobic alteration of photosynthesis-respiration relationship, which in turn leads to continuous production of massive microalgae (Bala-Amutha and Murugesan, 2011). Several studies have reported that the mutual interaction between algae and bacteria has a significant impact on algal growth (Buhr and Miller, 1983; Medina and Neis, 2007; Fuentes *et al.*, 2016), since the presence of symbiotic relationship between bacteria and algae is beneficial to the massive production of micro-algae and algal products. Both algae and bacteria alter their metabolism to meet each other's

needs, micronutrients like vitamins and macronutrients like nitrogen and carbon are usually exchanged between algae and bacteria (Medina and Neis, 2007), and plant hormones excreted from bacteria also promote algal growth. A typical example showing the mutual relationship is when bacterial species supply vitamin B12 to an algae as an exchange for fixed carbon. When some algae are grown with an artificial consortium of mutualistic bacteria, they supply fixed organic carbon to the consortium and in return, they show enhanced growth (Wrede *et al.*, 2014; Fuentes *et al.*, 2016).

Efficiency of algal biomass production also depends on harvesting and dewatering mechanisms (Golueke and Oswald, 1965). The mechanisms involved include centrifugation, flocculation, filtration, screening, gravity sedimentation, floatation and electrophoresis techniques. Harvesting techniques depend on the properties of the microalgae such as size, shape, density and also uses of the targeted outputs. Dewatering process is equally important for the biomass production according to the study done by Batten *et al.* (2013).

Several methods for drying can be deployed for the achievement of concentration of 99% to 100% suspended solids before the biomass being used for targeted purposes (Christi, 2008; Cooney *et al.*, 2009). Upon drying, extraction can be followed whereby the internal triglycerides and free fatty acids can be extracted from the algal biomass into biofuel such as biodiesel or jet fuel (Cooney *et al.*, 2011). To some of algae species such as *Nannochloropsis spp.*, *Chlorella spp.*, and *Scenedesmus spp.*, 100% extraction has to be taken into consideration as most of the oleaginous microalgae possess hard cell walls that coupled with small cell sizes hinder the total oil extraction in strains. (Cooney *et al.*, 2009).

In a single use energy stream, the fuel would be a final valuable product and the nutrients and energy would be lost while in a closed energy loop system, the products feed back into the production e.g. burning biofuels result in production of carbon dioxide, which can be recycled for algal growth. Therefore, since carbon and nutrients are cycled with the use of energy from the sun, the system is renewable and carbon neutral (Rupiper, 2016).

MICRO-ALGAE BIOMASS UTILIZATION

Little attention has been paid in utilization of micro-algae that can be generated in wastewater ponds (Craggs *et al.*, 2014; Young *et al.*, 2017). The harvested algal biomass can be potentially used as fertilizer, protein-rich animal feed, or can be converted into biofuel; like biogas via anaerobic digestion (Heubeck *et al.*, 2007), bio-ethanol via carbohydrate fermentation (Hwang *et al.*, 2016), bio-crude oil via high temperature liquefaction (Jegathese and Farid, 2014), or biodiesel via lipid trans-esterification (Craggs *et al.*, 2011; Montemezzani *et al.*, 2015; Driver *et al.*, 2014).

Micro-algae for Bio-fuels

The consumption of fossil fuels is increasing at an alarming rate globally. Petroleum reserves are shrinking at a fast pace, in turn creating demand for alternative sources of fuel (Mutanda *et al.*, 2011). The current systems for production of alternative energy do not account for the water and energy crisis, neither for the food security since the production of traditional crops for biofuel and feed e.g. terrestrial oil seed plants, soybeans, corn, sunflower, palm, *Jatropha*, cassava, coconut, rice straws, witch grass, need arable land for their cultivation and hence conflicting with agricultural land used for food crops (Park *et al.*, 2018). All these

challenges ought to have alternative source of nutrients and energy, which can be obtained from the utilization of microalgae.

Due to the current consumption trend, fossil fuels may run out within several decades (Khan *et al.*, 2017; Park *et al.*, 2018). Over 80% of energy consumption comes from fossil fuels, which is not only non-renewable energy, but it is also one of the main contributors of global climate change (Pittman, 2011; Jegathese and Farid, 2014; Mehrabadi *et al.*, 2016; Young *et al.*, 2017). It is factual that, in life cycle while algal biodiesel reduces carbon emissions and 50% loss life cycle production of greenhouse gases, petrol based fuel release it (Slade and Bauen, 2013). Algae can sequester CO₂ about 10 to 50 times more efficiently than land crops (Maity *et al.*, 2014; Rupiper, 2016). It is prospected that algae biodiesel will almost completely replace conventional biodiesel by 2040, to reduce global warming and achieve CO₂ emission (Khan *et al.*, 2017). One of the objectives of the Copenhagen Accord which took place in the past few years, was to promote the use of renewable energy to replace the fossil fuels since they are environmentally friendly and carbon neutral (Lee, 2011; Lau *et al.*, 2012). It is important to understand that, micro-algae for bio-fuel production are advantageous because apart from contributing to alternative energy, they avoid using food crops for fuel production, hence enhances food security. In accordance with Khan *et al.* (2017), algae species such as *tribonema*, *ulothrix* and *euglena* have good potential of biodiesel production and it is estimated that the use of HRAPs for wastewater treatment could save up to 50% of energy that typical mechanical systems use (Rupiper, 2016).

One of the strongest facts in comparison between cost-benefit analysis of using algal biofuel to petrol-diesel is that, biofuel

is a renewable fuel while petrol-diesel have a limited and diminishing supply. Therefore, as time goes on, the cost implications of petrol-diesel will be increase because of limited supply whereas algal biofuel production from wastewater will not face that shortfall (Maity *et al.*, 2014; Rupiper, 2016). Therefore, the use of biodiesel from wastewater algae is promising and potentially cost effective compared to petro-diesel (Craggs *et al.*, 2011; Pittman, 2011). The petrol-diesel market price is still more expensive, even at the average cost it is almost four times more expensive than biodiesel by as much as US\$ 2.67 per gallon (Slade and Bauen, 2013; Maity *et al.*, 2014; Rupiper, 2016). A study done by Rupiper (2016) found that biodiesel is more cost-effective than petroleum-diesel based fuel.

Several studies have indicated that algae have oil content with different composition depending on the species (Greenwell *et al.*, 2010; Park *et al.*, 2013). Some have good fatty acid value, hence highlighting the potentials of their utilization (Khan *et al.*, 2017). A study done by Drira (2016) found out that *Chlorella sp* have high fatty acid content, almost 70% of lipids extracted from the harvested biomass with more palmitic and stearic acids. In accordance with several researchers the algae harvested from full-scale HRAP treating domestic wastewater through increase of pH, performs the recovery of more than 96% of biomass *chlorella vulgaris*, *Dunaliella tertiolecta*, *Tibonoma minus*, *Nannochloropsis* and *Tetraselmis* (Greenwell *et al.*, 2010; Mutanda *et al.*, 2011; Jegathese and Farid, 2014; Milledge *et al.*, 2014; Wrede *et al.*, 2014; Hwang *et al.*, 2016; Mehrabadi, 2016).

The high oil content and rapid production of algal biodiesel cycle can ensure stable supply (Dermibas, 2010). Many studies using micro-algae conducted to produce biofuels most especial biodiesel and this is because generally micro-algae contain

relatively low carbohydrate contents but high lipid contents in their cells (Mata *et al.*, 2011). Micro-algae contain glucose - based carbohydrates, which is suitable sugars for bioethanol production. From the

study done by Mehrabadi *et al.* (2016), *Chlorella vulgaris* is a prominent algae specie that is appropriate for all bio-fuel production types (Table 3).

Table 3: Recently Published Results of Biofuel Production from Algal Biomass

Biofuel Type	Microalgae species	Algae composition (%)			Reaction Temperature (°C)	Time	Yield
		L	P	C			
Biodiesel						Minutes	g biodiesel/ g biomass
	<i>Dunaliella tertiolecta</i>	19	NG	NG	340	0.5	NG
	<i>Chlorella vulgaris</i>	38.9	NG	NG	60	120	0.3
	<i>Chlorella sp</i>	12	NG	NG	60	1140	NG
	<i>Nannochloropsis oceanic</i>	24.8	NG	NG	60	2880	0.2963
Biogas						Days	CH ₄ in biogas %
	<i>Scenedesmus spp</i> & <i>Chlorella ssp</i> (50%) and 50% of waste paper.	NG	NG	NG	35	10	68-72
	<i>Scenedesmus</i> (30%) and 70% <i>Chlorella</i>	NG	NG	NG	37	23	56-60
	<i>Scenedesmus</i> (40%) and 40% <i>Chlamydomonas</i>	NG	NG	NG	35	NG	40-60
	<i>Scenedesmus obliquus</i>	NG	NG	NG	33	30	0.61
Bio-ethanol						Days	g bio-ethanol / g biomass
	<i>Chlorococum sp</i>	NG	NG		30-40	60	0.23-0.37
	<i>Chlorococum humicola</i>	NG	NG	32.52	30	50	0.027-0.52
	<i>Scenedesmus obliquus</i>	NG	NG	29	30		0.023
	<i>Spirulina platensis</i>	NG	NG	58	30	24	0.13-0.16
	<i>Chlorella vulgaris</i>	NG	NG	55	33	26	0.167
Bio-crude oil						Minutes	g bio-oil/ g biomass
	<i>Chlorella vulgaris</i>	25	55	9	350	60	0.28-038
	<i>Chlorella pyronoidosa</i>	0.1	71.3	NG	280	120	0.359
	<i>Scenedesmus obliquus</i>	16.8	28		250-375	5	0.176-0.505
	<i>Nannochloropsis oceanica</i>	24.8	19.1	22.7	300	30	0.40
	<i>Dunaliella tertiolecta</i>	23.4	50.8	NG	250-275	5	0.553

Modified from Mehrabadi *et al.* (2016)

NG = not given, L= lipids, C= Carbohydrates, P= Proteins

Micro-Algae for Food and Feed

Organisms require food to supply the energy that they need for movement and other activities in which they engage, as well as building blocks for their growth. The rise in global population has led a concern of exploring alternative sources of food. Corn and soybean remain to be the main staple food crops in so many human societies, as a dominant source of energy and protein (Lum *et al.*, 2013). However,

food processing for animals directly competes with the human consumptions. From several studies (Benemann, 2013; Lum *et al.*, 2013; Norambuena *et al.*, 2015), it is seen that the micro-algae biomass has been generated for potential biofuel production may be a viable replacement of food crops due to their high level of protein, relatively well balanced amino acids and rich contents of minerals and vitamins together with bioactive compounds (Lum *et al.*, 2013).

Microalgae and cyanobacteria are a promising source of protein for food and feed purposes, (Craggs *et al.*, 2014; Smetana *et al.*, 2017). Several researchers have reported that paddle-wheel mixed algal growth ponds are not only cost-effective for wastewater treatment, but are also very efficient for reclaiming nutrients in algal biomass, which in turn can be used for animal feed (Oswald 1995; Batten, 2013; Hwang *et al.*, 2016). Considerable efforts have been directed towards removal of algae from the effluent polishing pond for the purposes of upgrading the quality of effluent, and recovering a valuable source of food for animals (Golueke and Oswald, 1965, García *et al.*, 2000).

Micro-algae are a main source of omega-3 (n-3) polyunsaturated fatty acids, including docohexaenoic acid (DHA) and eicosapentaenoic acid (EPA), which can also be obtained in eggs, meat and milk. They also contain α -linoleic acid (Austic *et al.*, 2013; Benemann, 2013) and have high iodine content (He *et al.*, 2002). The biomass has high crude protein content and could be used as animal feed with proper processing, and could thus be considered as an attractive alternative for animal feed-stocks (Norambuena *et al.*, 2015). However, recent studies have shown that sewage grown algae such as *chlorella* and *scenedesmus sp.*, have drawn attention as potential nutrients sources due to their high crude protein and carotenoid contents, and the recent estimates indicate that 30% of the global algae production is used by the animal feed industry (Becker, 2004). Since protein is considered to be the most expensive nutrient in animal feeding (Rezael *et al.*, 2013), proper utilization of algae can be of great benefits.

The advantage of harvesting micro-algae is that the same harvested product can be used for two purposes. Firstly, for the extraction of lipids and secondly, the remaining product serve as animal feed

(Lum *et al.*, 2013). The remaining micro-algae skeletons after lipid extraction, the so-called de-fatted micro-algae biomass can be used as animal feed and adjusting the current competition with human food crops supply.

Micro-algae Value for Animal Nutrition

Several studies have reported different potentials of cultivated algae as effective in maintaining animal growth, performance and sometimes improve daily body weight gain. It is reported that 10% supplement of *chlorella sp.* into a diet deficient in riboflavin and vitamin A improve feed efficiency and growth of chicks (Combs, 1952). Blue-green algae (e.g. *spirulina sp.*) seem to have positive impacts on overall growth performance, organ health, and reproductive characteristics of animals. Some of the blue-green algae can supplement diets for broiler chickens with up to 20% as to that of conventional crops. However, over the past few decades, pond-grown algae were found to sustain fish growth in aquaculture while today; algae from ponds are used for feeding various animal species (Shields, 2012). De-fatted biomass of micro-algae species derived from the biofuel production has of recently shown feasibility in replacement of corn and soybean meal in diets for poultry, swine, cattle, and sheep (Austic *et al.*, 2013; Lum *et al.*, 2013). Although some studies have reported supplementing the de-fatted biomass from *strauropsiro sp.* to replace 7.5% corn and soya bean meal in diet for weanling pig cannot affect their overall grown (or growth?) performance, Supplementation of micro-algae to the diets of ruminants, increases the concentration of n-3 PUFA in milk. In lambs and horses, dietary microalgae increased the n-3 fatty acid content in a meat and blood, respectively, while in pigs, dietary microalgae increased DHA concentration in the ion and subcutaneous fat (Sard *et al.*, 2006; He and Rambeck

2002; Hess *et al.*, 2012) and they are greatly used in dairy cows as source of n-3 fatty acid.

Nutritional profiles of micro-algae vary with different algal species although the majority are characterized by proteins, carbohydrates, and lipids contents, which similar; they are in other ways closely related to the conventional feed (Norambuena, 2015). This diversity of nutritional contents makes certain algae species have potential for cultivation of diet—needs for humans and animals (Table 4). For example, a commonly cultivated algae species for human consumption is *Spirulina maxima*, which are rich in vitamin B1, B2 and β -carotene and crude protein of up to 71% which is more compared to the dietary soybean which contains 48% crude protein (Lum *et al.*, 2013).

Focusing on nutritional value, micro-algae contain large amounts of the most limited amino acids, lysine and methionine, hence become potential for all dietary amino acids although they are somehow deficient in the sulphur-containing amino acids like cysteine. Therefore, in order to balance and maximize amino acid utilization by animals, diets can be typically generated by mixing different feedstuffs to balance amino acids to meet their nutrients requirement. For example, it is reported by Austic *et al.* (2013) that the decreased growth performance of broilers fed by the de-fatted *staurospira sp* biomass in the first three weeks was prevented by the supplementation of essential amino acids (Lum *et al.*, 2013).

Another study on laying hens reported that inclusion of 10% *Polphyridium sp* Red algal biomass did not affect their body weight, egg production rate, or egg weight, but lowered egg yolk cholesterol level by 24% (Ginzberg *et al.*, 2000). Fish from ponds, or fish product from reared fish ponds represent the major source of n-3

fatty acid while marine fish species are incapable of synthesizing n-3 fatty acids by themselves; they may obtain n-3 fatty acids by consuming micro-algae or other algae consuming fish. Micro-algae biomass or oil may be supplied in the feed of ruminant to manipulate their milk fatty acid composition. From the study that was conducted to compare algae and co-supplementation with sunflower oil in sheep diet, nutrition profile of milk showed the milk DHA concentration was increased as dietary algae concentration rose (Lum *et al.*, 2013).

REUSE OF WASTEWATER FOR IRRIGATION

Wastewater treatment has no alternative option since it can have several impacts on human health and the environment. Treated wastewater can be potentially useful for agricultural purposes (Michunaka *et al.*, 2017). The use of high rate algal ponds becomes of paramount importance as their aim is to maximize wastewater treatment conditions for massive growth of algae and sufficient oxygen which are the key factors for the removal of organic matter, nutrients and pathogens (Young *et al.*, 2017). Since recovered wastewater nutrients can be used as fertilizer, the treated wastewater can be used for irrigation purposes (Paulo *et al.*, 2009). However, reclaimed water from the HRAP, present two options; one reduces consumption of the processed water for domestic purposes and the second is to reduce the cost of nutrients as well as avoid environmental impacts that arises with the discharge of large volumes into the receiving environment (Young *et al.*, 2017; Cooney *et al.*, 2011).

Basing on problem of water scarcity, urban wastewater use in agriculture is now considered as an important practice (Rivera, 2016). Water shortage, threats to food security among urban dwellers, has limited farmers from practicing urban

agriculture (Zhang and Shen, 2017). This raises concerns against using wastewater and it is obvious that urban utilities generate a lot of wastewater from their treatment plants, which in most cases they face many operational problems (Keraita and Akatse, 2012). It is been estimated that more than 20 million hectares are currently being irrigated with wastewater worldwide by about 200 million farmers and the large part of it is practiced in Latin America (D'Andrea *et al.*, 2015).

Based on various literature, the situation shows the HRAPs are now merely applied for biofuels production, whereas the focus on the final effluent being used for irrigation purposes is still not very promising. Although they can also be utilized as food supplements for humans and animal dietary, there is little exploration in this area, which indicates there is still little attention on re-use of reclaimed wastewater for agricultural purposes.

Table 4: Nutritional Values for Microalgae close Related to Conventional Crop based

Algae species	Nutrients contents			Conventional feed staffs	Nutrients contents		
	P	C	L		P	C	L
<i>Synechococcus sp</i>	73	15	11	Egg	49.8	2.7	47.4
<i>Spirulina maxima</i>	71	NG	18	Soybean	48	NG	2
<i>Arthospira maxima</i>	60-70	13-16	6-7	Meat	43	1	34
<i>Spirulina plantesis</i>	61-64	15-16	7-8	Soybean	37	30	20
<i>Apharizomenon flos-aquae</i>	62	23	3	Peas (green)	28.8	47.1	3.7
<i>Chlorella vulgaris</i>	51-58	12-17	14-22	Milk	26	38	28
<i>D. salina</i>	57	32	6	Tomato	14.5	58.3	3.5
<i>Chlorella pyrenoidosa</i>	57	26	2	Wheat	14	84	2
<i>Scenedesmus obliquus</i>	50-56	10-17	12-14	Carrot	11.0	63.3	1.8
<i>Anabaena cylindrical</i>	43-56	25-30	4-7	Rice	10	77	2
<i>Chlamydomonas</i>	48	17	21	Corn	9	85	4

Modified from Mehrabadi *et al.* (2016); Lum *et al.* (2013).

NG = Not Given, P = Protein, C = Carbohydrates, L = Lipids

POTENTIAL OF APPLICATION OF HRAPs IN TANZANIA

Application of this type of technology in Tanzania is significant since it is going to solve problems in several areas. Proper wastewater management contributes to improved public health, environmental protection as well as economic benefits.

i. Environmental benefits

Due to HRAPs efficiency in treating wastewater through reduction of nutrients, receiving water bodies are protected against pollution, which can lead to eutrophication (Garcia and Marine, 2000). Removal of organic matter, pathogens and nutrients from wastewater produces cleaner water and has several indirect

environmental benefits including safer and more stable aquatic ecosystems. Wastewater being released into the environment without any treatment can pollute drinking and recreational waters and become less potential for multiple uses and increase costs of treatment of drinking water (Slovak Republic, 2018).

ii. Health benefits

Treatment of wastewater and re-use will reduce the health risks of diseases causing organisms responsible for water borne diseases like cholera, diarrhea, typhoid fever, dysentery and hepatitis that are normally found in effluents of conventional ponds (Liu *et al.*, 2017). Reduction of pathogens and pollutants in the water cycle decreases the morbidity

and mortality among the population using water for domestic use. In accordance with the World Health Organization (2017), contaminated drinking water is estimated to cause 502,000 diarrhea deaths each year. Since it has been reported by United Nations Environmental Program, (2010) that 88% of all diarrhea incidents globally are connected to poor hygiene and drinking of unsafe water, the country has no option in adopting a proven technology that will work appropriately to protect public health as well as minimize costs that are associated with healthcare for water-borne disease, hospitalization as well as preventing the productivity losses due to sickness.

iii. Social Economic benefits

HRAPs offers opportunities through resources recovery, and thus the reclaimed wastewater will substitute the use of potable waters particularly in urban farming through irrigation of horticulture crops and gardening which in turn reduces Government costs for treating water for potable uses and reduces the water stress.

Currently, wastewater from waste stabilization ponds is being used for irrigation of horticultures by small scale farmers in some part of the country such as Arusha and Moshi municipalities. To some communities, wastewater from this type of the source is used to grow food products like maize, beans and banana (Paulo *et al.*, 2009) which has helped in generating self-employment and increase income for low earning communities. Moreover, since the treated water is rich in nutrients, using it for irrigation reduces the need for chemical fertilizers subject to quality checks. This results in a reliable source of water and an improved food security. As reported by World Health Organization (2017), wastewater might be a key to solve the global water crisis and by 2025, half of the world's population will be living in water-stressed countries (Michinika *et al.*, 2017). Application of HRAP has a potential of

reducing water shortages in water-stressed areas through its re-use in aquaculture, agriculture and other uses.

Utilizing algae for biofuel production will improve the energy sector through minimizing the costs of fossil fuel. Energy challenges in Tanzania affect seriously the performance of the country's social and economic sector (Felix and Gheewala, 2011). Poor income, poor health, and education indicators can greatly be improved with adaptation of clean and modern energy (Mkiramweni, 2012). It is estimated that 80% of Tanzanians depend on biomass as a source of energy by burning firewood and charcoal. Application of algae as a source of biofuel will potentially reduce the burden on the forest resources as well as consequences of air pollution which can lead to complications of breathing, chronic respiratory diseases and stinging eyes due to indoor air pollution that comes from burning charcoal and firewood inside homes (Mkiramweni, 2012). Nutrients from wastewater through utilization of microalgae for animal feed will improve individual's incomes since algal products will supplement the conventional source of food which is always expensive. Since microalgae and cyanobacteria have proved to have good source of proteins, omega-3 which can also be obtained in eggs, meat and milk, adopting HRAP will improve animal nutrition (Craggs *et al.*, 2014; Smetana *et al.*, 2017).

CONCLUSIONS & IMPLICATIONS

The high-rate algal pond is a low-cost wastewater treatment system designed to achieve two goals. Secondary wastewater treatment and algal biomass production that can be used for resource recovery like, energy as biofuel, microalgae nutrients as protein-rich animal or fish feed in aquaculture and human food, and reclaimed water for irrigation purposes. Use of HRAP has economic impact through

generating self employment, increase income to low earning communities as well as minimizing the issues of food, energy and water crisis and reduce the emission of green house gases. However, since there is partial utilization of microalgae for food and feed, it is necessary to determine limiting factors of the microalgae biomass that hinder its digestion and utilization by animal. The tremendous potential of using the reclaimed wastewater should be fully explored.

AREAS FOR FURTHER RESEARCH

For the upcoming research the focus should also include the removal of pathogens in large scale; protozoa, nematode eggs and all prominent pathogens that pose threat to public health rather than just *E. coli* and faecal indicator bacteria (Young *et al.*, 2017). Reclaimed wastewater for resource recovery should be given much attention. However, since there is partial utilization of microalgae for food and feed, It is necessary to determine limiting factors of the microalgae biomass that hinder its digestion and utilization by animals.

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REFERENCES

Abdel-Raouf N., Al-Homaidan A.A. and Ibraheem I.B.M. (2012). Microalgae and wastewater treatment. Saudi

Journal of Biological Sciences, 19(3): 257–275.

<https://doi.org/10.1016/j.sjbs.2012.04.005>.

Abeliovich A. (1986). Algae in wastewater oxidation ponds. In Handbook of microalgal mass culture, 331-338. Amon, Richmond, California.

Austic R.E., Mustafa A., Juny B., Gatreu S. and Lei X.G: (2013). Potential and limitation of new defatted diatom microalgal biomass in replacing soybean meal and con diets for broiler chickens, J. Agric. Food Chem., 61: 7341-7348. DOI: 10.1021/jf401957z

Azov Y., Shelef G. and Moraine R. (1982). Carbon limitation of biomass production in high rate oxidation ponds. Biotechnology and Bioengineering, XXIV: 579-594. <https://doi.org/10.1002/bit.260240305>

Bahlaoui M.A., Baleux B. and Troussellier M. (1997). Dynamics of Pollution-Indicator and Pathogenic Bacteria in High-Rate Oxidation Wastewater Treatment Ponds. Water Research, 31(3): 630–638. [https://doi.org/10.1016/S0043-1354\(96\)00299-0](https://doi.org/10.1016/S0043-1354(96)00299-0)

Bala Amutha K. and Murugesan A.G. (2011). Biological hydrogen production by the algal biomass *Chlorella vulgaris* MSU 01 strain isolated from pond sediment. Bioresource Technology, 102(1): 194–199. <https://doi.org/10.1016/j.biortech.2010.06.008>

Batten D., Beer T., Freischmidt G., Grant T., Liffman K., Paterson D. and Threlfall G. (2013). Using wastewater and high-rate algal ponds for nutrient removal and the production of bioenergy and biofuels. Water Science and Technology, 67(4): 915–924. <https://doi.org/10.2166/wst.2012.618>

Becker W. (2004). Microalgae in human and animal nutrition. In Handbook of microalgal culture. Edited by Richmond A. Oxford, UK: Blackwell. Publishing Ltd, 312-335.

- Benemann J. (2013). Microalgae for biofuels and animal feeds. *Energies*, 6(11): 5869–5886. <https://doi.org/10.3390/en6115869>.
- Bouchaib E.H. (2009). Rethinking natural, extensive systems for tertiary treatment purposes: the high-rate algae pond as an example. *Desalin. Water Treat.*, 4: 128–134. DOI: 10.5004/dwt.2009.367
- Brandes K., Schoebitz L., Kimwaga R. and Strande L. (2015). Sheet Flow Diagram Promotion Initiative report, SFD Promotion Initiative, Dar es Salaam, Tanzania.
- Buhr H.O. and Miller S.B. (1983). A Dynamic model of the high rate algal-bacterial wastewater treatment pond. *Water Research*, 17: 29-37. [https://doi.org/10.1016/0043-1354\(83\)90283-X](https://doi.org/10.1016/0043-1354(83)90283-X)
- Burlew J.S. (1953). Current status of the large-scale culture of algae. *Algal culture, From laboratory to pilot plant*. Carnegie Institute of Washington, Washington DC.
- Butler E., Hung Y.T., Suleiman A.M., Yeh R.Y.L., Liu R.L.H. and Fu Y.P. (2017). Oxidation pond for municipal wastewater treatment. *Applied Water Science*, 7(1): 31–51. <https://doi.org/10.1007/s13201-015-0285-z>
- Chen P., Zhou Q., Paing J., Le H. and Picot B. (2003). Nutrient removal by the integrated use of high rate algal ponds and macrophyte systems in China. *Water Science and Technology*, 48(2): 251-257. DOI: 10.2166/wst.2003.0128
- Choi H. and Lee S. (2012). Effects of Microalgae on the Removal of Nutrients from Wastewater: Various Concentrations of *Chlorella vulgaris*, *Environmental Engineering Research*, 17(S1): 53–58. <http://dx.doi.org/10.4491/eer.2012.17.S1.S3>
- Christi Y. (2008). Biodiesel from microalgae beats ethanol. *Trends in biotechnology*, 26: 125-131. doi: 10.1016/j.tibtech.2007.12.002
- Colak O., and Kaya Z. (1988). A study on the possibilities of biological wastewater treatment using algae. *Doga Biyolji serisi*, 12(1): 18-29.
- Combs G.F. (1952). Algae (*Chlorella*) as a source of nutrients for chicks. *Science*, 116: 453-454. DOI: 10.1126/science.116.3017.453
- Cooney M.J., Young G. and Pate R. (2011). Bio-oil from photosynthetic microalgae: Case study. *Bioresource Technology*, 102(1): 166–177. <https://doi.org/10.1016/j.biortech.2010.06.134>
- Cooney M.J., Young G., and Nagle N. (2009). Extraction of bio-oils from microalgae, a review. *Journal of separation and purification Review* 38, 291-325. <https://doi.org/10.1080/15422110903327919>
- Craggs R., Park J., Heubeck S. and Sutherland D. (2014). High rate algal pond systems for low-energy wastewater treatment, nutrient recovery and energy production. *New Zealand Journal of Botany*, 52(1): 60–73. <https://doi.org/10.1080/0028825X.2013.861855>.
- Craggs R., Sutherland D. and Campbell H. (2012). Hectare-scale demonstration of high rate algal ponds for enhanced wastewater treatment and biofuel production. *Journal of Applied Phycology*, 24(3): 329–337. <https://doi.org/10.1007/s10811-012-9810-8>
- Craggs R.J., Davis –Colley R.J., Tanner C.C. and Sukias J.P.S. (2003). Advanced pond system; Performance with high rate ponds of different depth and areas. *Water Science and Technology*, 48: 259-267. PMID: 14510219
- Craggs R.J., Heubeck S., Lundquist T.J. and Benemann J.R. (2011). Algal biofuels from wastewater treatment high rate algal ponds. *Water Science*

- and Technology, 63(4): 660–665.
<https://doi.org/10.2166/wst.2011.100>
- Cromar N.J. and Fallowfield H.J. (1997). Effect of nutrient loading and retention time on performance of high rate algal ponds, *Journal of Applied Phycology*, 9(4): 301–309.
- D’Andrea L.G.D., Barboza A.G.J.S., Garcés V., Alvarez M.S.R., Iribarnegaray M.A., Liberal V.I., Fasciolo G.E., van Lier J.B. and Seghezzi L. (2015). The Use of (Treated) Domestic Wastewater for Irrigation : Current Situation and Future Challenges *Int J Water and Wastewater Treatment*, (2): 1-10,
<https://doi.org/10.16966/ijwwwt.107>
- Demirbas A. (2010). Biodiesel from Oilgae, *Biofixation of Carbon Dioxide by Microalgae: A Solution to Pollution Problems*. *Applied Energy*, 88: 3541-3547.
<http://dx.doi.org/10.1016/j.apenergy.2010.12.050>.
- Doma S.H., El-Liethy, A.M., Abdo S.M. and Ali G.H. (2016). Potential of Using High Rate Algal Pond for Algal Biofuel Production and Wastewater treatment. *Asian Journal of Chemistry*, 28(2): 399-404.
DOI: 10.14233/ajchem.2016.19378
- Dirra N., Piras A., Rosa A., Porcedda S. and Dhaouadi H. (2016). Microalgae from domestic wastewater facility’s high rate algal pond: Lipids extraction, characterization and biodiesel production. *Bioresource Technology*, 206: 239–244.
<https://doi.org/10.1016/j.biortech.2016.01.082>
- Driver T., Bajhaiya A. and Pittman J.K. (2014). Potential of Bioenergy Production from Microalgae. *Current Sustainable/Renewable Energy Reports*, 1(3): 94–103.
<https://doi.org/10.1007/s40518-014-0011-8>
- Fallowfield H. and Garrett M. (1985). The treatment of wastes by algal culture. *J. Appl. Bacteriol.*, 59: 187S-205S.
<https://doi.org/10.1111/j.1365-2672.1985.tb04900.x>
- Felix, M., & Gheewala, S. H. (2011). A Review of Biomass Energy Dependency in Tanzania. *Energy Procedia*, 9(2): 338–343.
<https://doi.org/10.1016/j.egypro.2011.09.036>
- Fuentes J.L., Garbayo I., Cuaresma M., Montero Z., González-Del-Valle M. and Vilchez, C. (2016). Impact of microalgae-bacteria interactions on the production of algal biomass and associated compounds. *Marine Drugs*, 14(5): 100,
<https://doi.org/10.3390/md14050100>
- Fujioka R.S., Hashimoto H.H., Siwak E.B. and Young R.H.F. (1981). Effect of sunlight on survival of indicator bacteria in seawater. *Applied Environmental Microbiology*, 41(3): 690-696. PMC243761
- Gann J.D., Collier R.E. and Lawrence C.H. (1968). Aerobic bacteriology of waste stabilization ponds. *Journal of Water Pollution Control Federation*, 40(2): 185-191.
<https://www.jstor.org/stable/25036006>
- García J., Green B.F., Lundquist T., Mujeriego R., Hernández-Mariné M. and Oswald W.J. (2006). Long term diurnal variations in contaminant removal in high rate ponds treating urban wastewater, *Bioresource Technology*, 97: 1709–1715.
<https://doi.org/10.1016/j.biortech.2005.07.019>
- García J., Mujeriego R. and Marine H. (2000). High rate algal pond operating strategies for urban wastewater nitrogen removal. *Journal of Applied Phycology*, 12: 331-339.
<https://doi.org/10.1023/A:1008146421368>
- García M., Soto F., González J.M. and Bécares E. (2008). A comparison of bacterial removal efficiencies in constructed wetlands and algae-based systems. *Ecol. Engrg*, 32: 238–243.
DOI: 10.1016/j.ecoleng.2007.11.012

- Ginzberg A., Cohen M., Sod-Moriah U., Shany S., Rosenshtrauch A. and Arad S. (2000). Chicken fed with biomass of the red microalgae *porphyridinn* sp have reduced blood cholesterol level and modified fatty acid composition in egg yolk. *Phycol.*, 12: 325-330.
- Golueke C.G. and Oswald W.J. (1965). Harvesting and processing sewage-grown planktonic algae. *Journal of Water Pollution Control Federation*, 37(4): 471-498. <https://www.jstor.org/stable/25035278>
- Greenwell H.C., Laurens L.M.L., Shields R.J., Lovitt R.W. and Flynn K.J. (2010). Placing microalgae on the biofuels priority list: A review of the technological challenges. *Journal of the Royal Society Interface*, 7(46): 703–726. <https://doi.org/10.1098/rsif.2009.0322>
- Hamouri B.E.L., Khallayoune K., Bouzoubaa K. and Rhallabi N. (1994). High-rate algal pond performances in faecal coliforms and helminth egg removals. *Water Research*, 28(1): 171–174. [https://doi.org/10.1016/0043-1354\(94\)90131-7](https://doi.org/10.1016/0043-1354(94)90131-7)
- He M.L., Hollwich W. and Rambeck W.A. (2002). Supplementation of algae to the diet of pigs: a new possibility to improve the iodine content in the meat. *J. Anim. Physiol. Anim Nutr.*, (Berl), 86(3-4): 97-104. PMID: 11972678
- Hess T.M., Rexforg J.K., Hansen D.K., Harris M., Schaurmann N., Ross T., Engle T.E., Allen K.G. and Mulligan C.M. (2012). Effects of two different dietary sources of long chain Omega-3, Highly unsaturated fatty acids on incorporation into the plasma, red blood cell, and skeletal muscle in horses. *J Amin. Sci.*, 90: 3023-3031. doi: 10.2527/jas.2011-4412.
- Heubeck S., Craggs R.J. and Shilton A. (2007). Influence of CO₂ scrubbing from biogas on the treatment performance of a high rate algal pond. *Water Science and Technology*, 55(11): 193–200. <https://doi.org/10.2166/wst.2007.358>
- Hwang J.-H., Church J., Lee S.-J., Park J. and Lee W.H. (2016). Use of Microalgae for Advanced Wastewater Treatment and Sustainable Bioenergy Generation. *Environmental Engineering Science*, 33(11): 882–897. <https://doi.org/10.1089/ees.2016.0132>
- Jegathese S.J.P. and Farid M. (2014). Microalgae as a Renewable Source of Energy: A Niche Opportunity. *Journal of Renewable Energy*, Article ID 430203, 1–10, <https://doi.org/10.1155/2014/430203>
- Jones C.L.W., Taylor R., Mogane M., Mayo M. and Power S. (2016). The underlying mechanisms for nitrogen and phosphorus removal in high rate algal ponds used to treat brewery effluent, harvesting algae using filter feeding fish, and the use of brewery effluent in agricultural crop production and Duckweed as wastewater treatment solution; Report to the Water Research Commission, Department of Ichthyology and Fisheries Science, Rhodes University (WRC Report No. 2284/1/16.
- Kapuscinski R.B. and Mitchell R. (1983). Sunlight-induced mortality of viruses and *Escherichia coli* in coastal seawater. *Environmental Science and Technology*, 17(1): 1–6. <https://doi.org/10.1021/es00107a003>
- Keraita B. and Akatse J. (2012). On-Farm practices for the safe use of wastewater in urban and peri-urban horticulture. On- Farm Practices for the Safe Use of Wastewater in Urban and Peri-urban Horticulture. A training handbook for farmer field schools. Food and Agriculture Organization.
- Khan S., Siddique R., Sajjad W., Nabi G., Hayat K.M., Duan P. and Yao L. (2017). Biodiesel Production from Algae to Overcome the Energy Crisis. *HAYATI Journal of Biosciences*, 24(4): 163–167.

- <https://doi.org/10.1016/j.hjb.2017.10.003>
- Lau P.S., Tam N.F.Y., Wong Y.S., (1996). Wastewater nutrients removal by *Chlorella Vulgaris*; optimization through acclimation. *Environ. Technol.* 17(2): 183-189. <https://doi.org/10.1080/09593331708616375>
- Lau L.C., Lee K.T. and Mohamed A.R. (2012). Global warming mitigation and renewable energy policy development from the Kyoto Protocol to the Copenhagen Accord - A comment. *Renewable and Sustainable Energy Reviews*, 16(7): 5280–5284. <https://doi.org/10.1016/j.rser.2012.04.006>
- Lee D.H. (2011). Algal biodiesel economy and competition among bio-fuels. *Bioresource Technology*, 102(1): 43–49. <https://doi.org/10.1016/j.biortech.2010.06.034>
- Liu L., Macdougall A., Hall G. and Champagne P. (2017). Disinfection Performance in Wastewater Stabilization Ponds in Cold Climate Conditions: A Case Study in Nunavut, Canada. *Environments*, 4(4): 93. doi:10.3390/environments4040093.
- Lum K.K., Kim J. and Lei X.G. (2013). Dual potential of microalgae as a sustainable biofuel feedstock and animal feed. *Journal of Animal Science and Biotechnology*, 4(1): 1–7. <https://doi.org/10.1186/2049-1891-4-53>
- Maity J.P., Bundschah J., Chen C-Y. and Bhattacharga P. (2014). Microalgae for third generation biofuel production, Mitigation of greenhouse gas emissions and wastewater treatment: Present and future perspectives –A mini review, *Energy*, 78: 104-113. <http://doi.org/10.1016/j.energy.2014.04.003>
- Mancini J.L. (1978). Numerical estimates of coliform mortality rates under various conditions. *Journal of Water Pollution Control Federation*, 50(11): 2477~2484. DOI: 10.2307/25040179
- Mara D.D. (2013). *Domestic Wastewater treatment in Developing Countries*. Imprint Routledge, Earthscan, London, UK.
- Marais G.v.R. (1974). Faecal bacteria kinetics in stabilization ponds. *Journal of Environmental Engineering Division, American Society of Civil Engineers*, 100(1): 119~139.
- Mata T.M., Martins A.A., and Caetano N.S. (2010). Microalgae for biodiesel production and other applications: A Review *Renewable and Sustainable Energy Reviews*, 14(1): 217-232.
- Mayo A.W. (1989). Effect of pond depth on bacterial mortality rate. *Journal of the Environmental Engineering Division, American Society of Civil Engineers*, 115(5): 964-977. [https://doi.org/10.1061/\(ASCE\)0733-9372\(1989\)115:5\(964\)](https://doi.org/10.1061/(ASCE)0733-9372(1989)115:5(964))
- Mayo, A.W. (1995). Modeling Coliform Mortality in Waste Stabilization Ponds. *Journal Environmental Engineering Division, American Society of Civil Engineers*, 121(2): 140~152. [https://doi.org/10.1061/\(ASCE\)0733-9372\(1995\)121:2\(140\)](https://doi.org/10.1061/(ASCE)0733-9372(1995)121:2(140))
- Mayo A.W. (2013). Nitrogen mass balance in waste stabilization ponds at the University of Dar es Salaam, Tanzania. *African Journal of Environmental Science and Technology*, 7(8): 836–845. DOI: 10.5897/AJEST2013.1495.
- Mayo A.W. and Mutamba J. (2004). Effect of HRT on nitrogen removal in coupled HRP and Unplanted subsurface flow gravel bed constructed wetland. *Journal Phy. Chem. Earth*, 29: 1253-1257. doi:10.1016/j.pce.2004.09.005
- Mayo A.W., Muraza M. and Nobert. (2018). Modelling nitrogen transformation and removal in Mara river basin wetlands upstream of Lake Victoria. *Journal Phy. Chem. Earth*, 105: 136-146. <https://doi.org/10.1016/j.pce.2018.03.005>

- Mbwele L.A. (2006). Microbial Phosphorus Removal in Waste Stabilisation Pond Wastewater Treatment Systems. A Licentiate thesis, School of Biotechnology, Royal Institute of Technology, Stockholm, Sweden.
- Meckes M.C. (1982). Effect of UV light disinfection on antibiotic-resistant coliforms in wastewater effluents. *Applied Environmental Microbiology*, 43(2): 371~377.
- Medina M. and Neis U. (2007). Symbiotic algal bacterial wastewater treatment: Effect of food to microorganism ratio and hydraulic retention time on the process performance. *Water Science and Technology*, 55(11): 165–171. <https://doi.org/10.2166/wst.2007.351>
- Mehrabadi A., Craggs R. and Farid M.M. (2016). Biodiesel production potential of wastewater treatment high rate algal pond biomass. *Bioresource Technology*, 221: 222–233. <https://doi.org/10.1016/j.biortech.2016.09.028>
- Merz R.C., Zehnippfennig R.G. and Klima J.R. (1962). Chromatographic assay of extracellular products of algal metabolism. *Journal of Water Pollution Control Federation*, 34(2): 103-115. <https://www.jstor.org/stable/25034574>
- Michinaka A., Hijikata N., Shigemura H. and Kawasumi R. (2017). Potential of Treated Wastewater Reuse for Agricultural Irrigation in Ouagadougou, Burkina Faso. *Sanitation Value Chain*, 1(1): 27–34. Available online at http://www.chikyu.ac.jp/sanitation_value_chain/journal/img/001/03.pdf
- Milledge J.J., Smith B., Dyer P.W. and Harvey P. (2014). Macroalgae-derived biofuel: A review of methods of energy extraction from seaweed biomass. *Energies*, 7(11): 7194–7222. <https://doi.org/10.3390/en7117194>
- Mills S.W., Alabaster G.P., Mara D.D., Pearson H.W. and Thitai W.N. (1992). Efficiency of faecal bacteria removal in waste stabilization ponds in Kenya. *Water Science and Technology*, 26(7-8): 1939-1748. <https://doi.org/10.2166/wst.1992.0617>
- Mkiramweni, L. L. N. (2012). The Impact of Biogas Conversion Technology for Economic Development: A Case Study in Kilimanjaro Region, 2012. <https://doi.org/10.5402/2012/424105>
- Montemezzani V., Duggan I.C., Hogg I.D. and Craggs R.J. (2015). A review of potential methods for zooplankton control in wastewater treatment High Rate Algal Ponds and algal production raceways. *Algal Research*, 11: 211–226. <https://doi.org/10.1016/j.algal.2015.06.024>
- Mutanda T., Ramesh D., Karthikeyan S., Kumari S., Anandraj A. and Bux F. (2011). Bioprospecting for hyper-lipid producing microalgal strains for sustainable biofuel production. *Bioresource Technology*, 102(1): 57–70. <https://doi.org/10.1016/j.biortech.2010.06.077>
- Norambuena F., Hermon K., Skrzypczyk V., Emery J.A., Sharon Y., Beard A. and Turchini G.M. (2015). Algae in fish feed: Performances and fatty acid metabolism in juvenile Atlantic Salmon. *PLoS ONE*, 10(4): 1–17. <https://doi.org/10.1371/journal.pone.0124042>
- Nurdogan Y. and Oswald W.J. (1995). Enhanced nutrient removal in high-rate ponds Enhanced nutrient removal in high-rate ponds, *Water Science and Technology*, 31(12): 33–43. [https://doi.org/10.1016/0273-1223\(95\)00490-E](https://doi.org/10.1016/0273-1223(95)00490-E)
- Oswald W.J. (1995). Ponds in the twenty First Century. *Water Science and Technology*, 31: 1-8. [https://doi.org/10.1016/0273-1223\(95\)00487-8](https://doi.org/10.1016/0273-1223(95)00487-8)
- Oswald W.J. and Golueke C.G. (1960). Biological transformation of solar energy. *Adv Appl. Microbiol.*, 2: 223-262.
- Oswald W.J., Gotaas H., Golueke C.,

- Kellen W., Gloyna E. and Hermann E. (1957). Algae in waste treatment [with discussion]. *Sew Ind. Wastes*, 29: 437-457.
- Parhad N.M. and Rao N.U. (1974). Effect of pH on survival of *Escherichia coli*. *Journal of Water Pollution Control Federation*, 56(5): 980-986.
- Park J., Han T., Yarish C. and Kim J.K. (2018). Chapter 11: Microalgae and Alcohol. In *Microalgae in Health and Disease Prevention*, Levine I.A. and Fleurence J. (Editors). 1st Edition, Elsevier Inc., ISBN 978-0-12-811405-6.
- Park J.B.K. and Craggs R.J. (2010). Wastewater treatment and algal production in high rate algal ponds with carbon dioxide addition. *Water Science and Technology*, 61: 633–639. doi: 10.2166/wst.2010.951.
- Park J.B.K., Craggs R.J. and Shilton A.N. (2013). Enhancing biomass energy yield from pilot-scale high rate algal ponds with recycling. *Water Research*, 47(13): 4422–4432. <https://doi.org/10.1016/j.watres.2013.04.001>
- Paulo J., Senzia M., Mohamed J., Kimaro T. and Tendwa O. (2009). Integration of resource-oriented sanitation in informal settlements: The case of Arusha, 34th WEDC International Conference, Addis Ababa, Ethiopia, *Water, Sanitation and Hygiene: Sustainable Development and Multisectoral Approaches*, 599–603.
- Phuntsho S., Shon H.K., Vigneswaran S. and Kandasamy J. (2017). Wastewater Stabilisation Ponds (WSP) for Wastewater Treatment. *Water and Wastewater Treatment Technologies, II*. Available online at <http://www.eolss.net/sample-chapters/c07/e6-144-12.pdf>. Retrieved on 20th June 2018.
- Picot B., Andrianarison T., Olijnyk D.P., Wang X., Qiu J.P. and Brissaud F. (2009). Nitrogen removal in wastewater stabilisation ponds. *Desalination and Water Treatment*, 4(1–3): 103–110. <https://doi.org/10.5004/dwt.2009.363>
- Picot B., El Halouani H., Casellas C., Moersidik S. and Bontoux J. (1991). Nutrient removal by stabilization ponds. *J. Wat. Pollut. Control Fed.*, 55(3): 285-296.
- Pittman, J. K., Dean, A. P., & Osundeko, O. (2011). The potential of sustainable algal biofuel production using wastewater resources. *Bioresource Technology*, 102(1), 17–25. <https://doi.org/10.1016/j.biortech.2010.06.035>
- Republic S. (2018). Estimating environmental benefits of wastewater treatment in Slovakia, Institute for environmental policy, Ministry of environment of Slovak Republic. *Economic Analysis No 3*.
- Rezaei R., Wang W., Wu Z., Dai Z., Wang J. and Wu G. (2013). Biochemical and physiological bases for utilization of dietary amino acids by young pigs. *J Anim. Sci. Biotechnol.*, 4(1):7 doi: 10.1186/2049-1891-4-7.
- Rivera A.E. (2016). A Review of Reclaimed Water for Irrigation Use in an Urban Watershed. Graduate thesis and dissertations. Scholar Commons, University of South Florida, Tampa bay, Florida, USA. Available online at <http://scholarcommons.usf.edu/etd/6576>.
- Rupiper A. (2016). Potential for biofuel production from algae-based wastewater treatment in California: Can algal biofuels be cost-competitive with traditional petroleum-based diesel? Master's project and capstones. The University of San Francisco, USA. Retrieved from <https://Repository.usfca.edu/capstone%0Ahttps://repository.usfca.edu/capstone>.
- Sahu O. (2014). Reduction of Organic and Inorganic Pollutant from Wastewater by Algae, *International Letters of Natural Sciences*, 13: 1–8. Available online at <https://doi.org/10.18052/www.scipress.com/ILNS.13.1>

- Sardi L., Martelli G., Lambertini L., Parisini P. and Modenti A. (2006). Effect of dietary Supplement of DHA-rich marine algae on Italian heavy pig production parameters, *Livestock Science*, 103: 95-103. <https://doi.org/10.1016/j.livsci.2006.01.009>
- Sayre R. (2010). Microalgae: The Potential for Carbon Capture. *BioScience*, 60(9): 722-727. doi: 10.1525/bio.2010.60.9.9.
- Shelef G. and Azov Y. (1987). High rate oxidation ponds. The Israeli experience. *Water Science and Technology*, 19(12): 249-255. <https://doi.org/10.2166/wst.1987.0153>
- Shields R.J. and Lupatsch I. (2012). Algae for aquaculture and animal feeds, *J Anna Sci.*, 21: 23-37.
- Slade R. and Bauen A. (2013). Microalgae cultivation for biofuel: cost, energy balance, environmental impacts and future prospects. *Biomass and Bioenergy*, 53: 29-38. <http://doi.org/10.1016/j.biombioe.2012.12.019>.
- Smetana S., Sandmann M., Rohn S., Pleissner D. and Heinz V. (2017). Autotrophic and heterotrophic microalgae and cyanobacteria cultivation for food and feed: Life cycle Assessment. *Bioresource Technology*, 245: 162-170. Available online at <https://doi.org/10.1016/j.biortech.2017.08.113>
- Sutherland D.L., Turnbull M.H. and Craggs R.J. (2014). Increased pond depth improves algal productivity and nutrient removal in wastewater treatment high rate algal ponds. *Water Research*, 53: 271-281. <https://doi.org/10.1016/j.watres.2014.01.025>
- United Nations World Water Assessment Programme (2017). The United Nations World Water Development Report 2017. Wastewater: The Untapped Resource, UNESCO, Paris.
- Woertz I., Fulton L. and Lundquist T. (2009). Nutrient Removal & Greenhouse Gas Abatement with CO₂ Supplemented Algal High Rate Ponds, Paper written for the WEFTEC annual conference, Water Environment Federation, October 12~14, Orlando, Florida, 7924-7936.
- World Health Organisation (WHO) (2017). Drinking-water. Available online at <http://www.who.int/mediacentre/factsheets/fs391/en/>. Accessed on 20 11 2017.
- Wrede D., Taha M., Miranda A.F., Kadali K., Stevenson T., Ball A.S. and Mouradov A. (2014). Co-Cultivation of Fungal and Microalgal Cells as an Efficient System for Harvesting Microalgal Cells, Lipid Production and Wastewater Treatment. *PLoS ONE* 9(11): e113497. <https://doi.org/10.1371/journal.pone.0113497>
- Young P., Buchanan N. and Fallowfield H.J. (2016). Inactivation of indicator organisms in wastewater treated by a high rate algal pond system. *J. Appl. Microbiol.*, 121: 577-586. doi: 10.1111/jam.13180.
- Young P., Taylor M. and Fallowfield H.J. (2017). Mini-review: High rate algal ponds, flexible systems for sustainable wastewater treatment. *World Journal of Microbiology and Biotechnology*, 33(6): 117. <https://doi.org/10.1007/s11274-017-2282-x>
- Zhang Y. and Shen Y. (2017). Wastewater irrigation: past, present, and future. *Wiley Interdisciplinary Reviews: Water*, e1234. <https://doi.org/10.1002/wat2.1234>