BIOPROCESSES AND TECHNOLOGIES FOR NUTRIENT RECOVERY FROM WASTEWATER WITH MICROALGAE AND JELLYFISH

Fridah Valenciah Muthoni, María C. Veiga, Christian Kennes

Chemical Engineering Laboratory, Faculty of Sciences and Center for Advanced Scientific Research (CICA), BIOENGIN group, University of La Coruña (UDC), E-15008-La Coruña, Spain

1. ABSTRACT

The introduction of organic and inorganic substances to the environment is a result of human activities such as agriculture, domestic and industrial wastewaters, which leads to pollution. Treatment processes of these wastewaters are being conducted globally to eliminate easily settled materials and recover nutrients in an attempt to release clear and apparently clean effluents into natural waters. Lack of removing inorganic nitrogen and phosphorus nutrients is the major cause of eutrophication in water bodies, which inhibits life of other organisms, pose a threat to human life, and loss of the economical welfare. Different technologies have been applied and are being developed to recover nutrients as well as heavy metals from wastewaters, in order to meet the permissible limits before discharging effluents. Wastewater treatment using microalgae offers an opportunity to provide tertiary bio-treatment and simultaneous production of valuable biomass. Microalgae use the available inorganic nitrogen and phosphorus for their growth, while they can then be harvested for various uses. Additionally, they have the ability to remove heavy metals and some toxic compounds. The main specific microalgae species in this study is *Chlorella sorokiniana* and the *Aequorea victoria* jellyfish. This paper reviews some of the wastewater treatment processes and focusses on the use of microalgae. It addresses some of the shortcomings of the technologies and how they can be improved to achieve maximum nutrient recovery economically with low energy demand.

Keywords: wastewater; algal biomass; nitrogen; phosphorus; jellyfish

2. INTRODUCTION

Nutrient pollution is a great environmental issue and challenge. Nitrogen and phosphorus are natural components of aquatic ecosystems. They both support the growth of algae and aquatic plants, which provides food and habitat for other organisms. However, in excess quantity in the air or in water they become a nuisance. Nutrient pollution has impacted many streams, rivers lakes and coastal waters for the past years, resulting in serious environmental and human health issues, which also impact the economy (US EPA, 2013a). The rapid growth of algae is called algal blooms and it is considered to be more than the ecosystem can handle. It decreases the oxygen availability that fish and other organisms need to survive and therefore leads to declining fisheries. Harmful algal blooms (HAB) are related mainly to blue-green algae. They can be unsightly and smelly. Some algal blooms produce toxins and support bacterial growth, which is harmful to humans if they come in contact with polluted water or consume tainted fish. Excess nitrogen in the atmosphere can produce ammonia and ozone which could inhibit the ability to breath and alter plant growth. The primary sources of excess nitrogen and phosphorus are from human activities such as agriculture, industries, storm water and domestic water as shown in Figure 1.

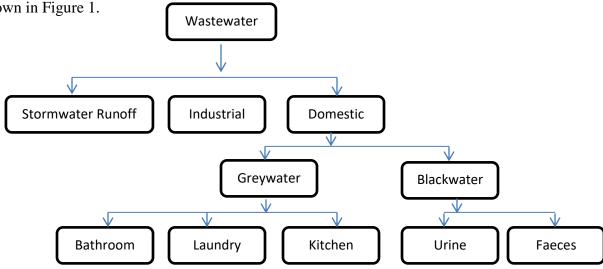


Figure 1 Major sources of wastewaters (Amoatey & Bani, 2011)

In agriculture, one of the main contribution to such pollution is by addition of fertilizers, and when these nutrients are not fully utilized by the plants in farms they end up into water bodies or are leached into groundwater where this can contaminate drinking water wells. Most

homes and businesses direct their wastewaters to water treatment plants for removal of pollutants and nutrients before discharge into the environment. There are still a number of cases of municipal and rural domestic waters discharged directly into water bodies (Abdel-Raouf et al., 2012). Nutrients found in wastewaters are commonly from human waste, food and certain soaps and detergents. Depending on the technologies used and the composition of the wastewater some wastewater treatment plants are able to remove more nitrogen and phosphorus from their discharge than others. Various strategies and technologies are being developed globally to improve N and P removal from wastewater and therewith reducing their load upon discharge to water bodies (US EPA, 2013b). Nutrient enrichment from nitrogen compounds (nitrates, nitrites and ammonia) also create problems even when algal blooms are not involved. Concerns about ammonia toxicity to aquatic organisms have resulted in nitrification strategies being implemented more broadly than even phosphorus removal. Nitrogen in the form of nitrate can infiltrate into groundwater and drain or runoff into surface water. Concentrations greater than 10 mg/L nitrates can cause fatal blood disorder known as blue baby syndrome in children of six months and below. The risk is also detected in pregnant women and individuals with certain gastric disorders.

Eutrophication of water due to the presence of nutrients is a global environmental concern. A substantial fraction of the global nutrients, organic carbon and metals are being dissipated in wastewater treatment plants (Puyol & Batstone, 2017). Besides, the global reserves of important resources such as phosphorus are being rapidly depleted while enriching surface waters worldwide (Fernandes et al., 2017). Wastewaters present an opportunity to recover and re-introduce valuable resources in the market, while achieving a global circular bio-economy. Current wastewater treatment technologies can only recover phosphorus in an insufficient way. However, microalgae can effectively assimilate phosphorus (P) and nitrogen (N) and other macro-nutrients which can then be transformed into valuable products, e.g., fertilizers, bioplastics (Fernandes et al., 2017). Resource recovery from wastewaters is entering a phase of significant technological advancement and scaling-up. Finding efficient solutions to wastewater treatment and their safe discharge entails an integrated process which requires both technical and financial considerations, among others. Appropriate treatment standards are to be selected to meet local conditions and innovative technologies should be considered. There should also be renewed emphasis on removing nutrients from point sources such as municipal wastewater

treatment plants and focus on strategies to reducing nutrients from no point sources such as erosion and agricultural runoff.

3. AVAILABLE TECHNOLOGIES FOR NITROGEN AND PHOSPHORUS REMOVAL

Wastewaters can be treated at primary, secondary or tertiary levels using physical, biological or chemical processes. Table 1 summarizes the main technologies and levels in a wastewater treatment plant. Physical processes include technologies such as sedimentation, filtration and evaporation. Physico-chemical processes comprise operations such as coagulation and flocculation, adsorption, ion exchange, precipitation, membranes and oxidation-reduction. Biological processes can be divided into two categories, i.e., aerobic and anaerobic processes. The first stage involves the removal of heavy solids. Secondary treatment consumes the dissolved organic matter and oxidizes major nutrients and the last stage is the removal of nitrates, phosphates and trace organic compounds (Molazadeh et al., 2019). Wastewaters N:P ratios highly depend on the origin of the wastewater. Municipal wastewaters have a minor contribution compared to industrial wastewaters and most commonly exhibit N:P ratios of approximately 10 (Henze and Comeau, 2008), while wastewaters with animal manure and human excreta can reach N:P ratios of about 40 (Kumar et al., 2010; Tuantet et al., 2013). The average chemical oxygen demand (COD) and biological oxygen demand (BOD) of municipal wastewater is 300-500 mg/l and 200-300mg/l, respectively. Recovery of nutrients from wastewaters will not only prevent eutrophication but it will also provide access to alternative nutrient sources. Conventional water treatment usually involves biological nutrient removal (BNR) to remove phosphorus down to 1-3 mg/L and nitrogen through nitrification-denitrification to typical levels of 8-10 mg/L. Wastewater facilities need to upgrade systems to meet regulatory compliance by improving nutrient removal before effluent discharge (Nutrient Pollution in Wastewater, 2016).

Table 1. Major wastewater treatment levels and technologies (Miah et al., 2012)

Preliminary	Primary	Secondary	Tertiary and Advanced

Purpose	Removal of	Removal of	Biological treatment	Removal of specific
	large solids	suspended	and removal of	pollutants such as
	and grit	solids	common biodegradable	nitrogen,
	particles		organic pollutant.	phosphorus,color, odor
				etc.
Sample	Screening,	Screening,	Percolating/ trickling	Sand filtration,
Technologies	Settling	Sedimentation	filter, activated sludge	membrane bioreactor,
			Anaerobic treatment	reverse osmosis, ozone
			waste stabilization	treatment, chemical
			ponds(oxidation	coagulation, activated
			ponds)	carbon

3.1. NITROGEN REMOVAL IN WASTEWATERS

Depending on the permissible limits, different systems can be operated to optimize the removal or transformation of nitrogen and achieve the desired effluent composition as illustrated in Figure 2 below (Sedlak, 1991).

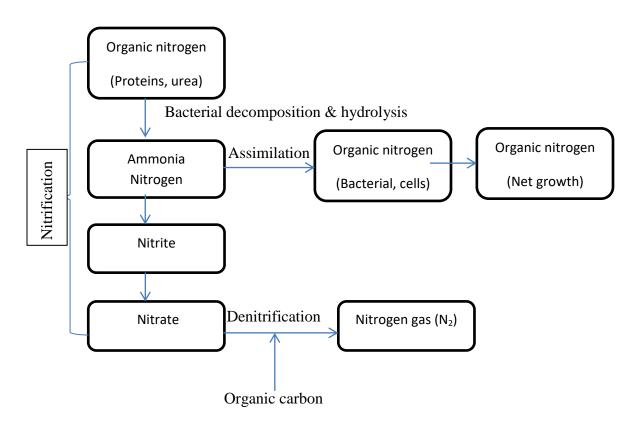


Figure 2 Nitrogen removal in biological treatment processes (Sedlak, 1991)

In any biological treatment system bacterial growth takes place. The organic nitrogen which is present in raw wastewater can be transformed to ammonia through bacterial decomposition as well as hydrolysis of urea. Under appropriate conditions ammonia nitrogen is oxidized in a two-step process to form nitrates. The nitrification process is carried out by microorganisms called nitrifiers in the presence of oxygen. The extent of the nitrification process is dependent on the number and activity of nitrifying organisms present in the wastewater. In the event that there is low alkalinity, this can be added in order to maintain the optimum pH level for nitrification. In the last stage, nitrates can be transformed into nitrogen gas in a process called denitrification. This process is achieved by microorganisms in the absence of oxygen. An organic carbon source is essential for denitrification to occur (Sedlak, 1991). Biological treatment is the most attractive nitrogen removal process from wastewaters but physical and chemical processes may also be technically and economically viable in some situations. They include breakpoint chlorination, selective ion exchange and air stripping.

3.2. PHOSPHORUS REMOVAL IN WASTEWATERS

The major sources of phosphorus in wastewaters are synthetic detergents and household cleaning products as well as commercial, industrial, fecal and waste materials. Phosphorus in wastewater mainly appears as organic phosphorus, polyphosphate and orthophosphate. The main treatment technologies available for phosphorus removal are summarized in Table 2.

Table 2. Main treatment technologies for phosphorus removal (Strom, 2007)

Physical	Filtration for particulate phosphorus	
	Membrane technologies	
Chemical	Precipitation	
	Physical-chemical adsorption	
Biological	Assimilation	
	Enhanced biological phosphorus removal (EBPR)	

Filtration and percolation are largely used in wastewater treatment plants. Phosphorus removal is sometimes carried out in sand filters for suspended and colloidal solids. According to the findings of Bali and Gueddari (2019), the physical process of removing phosphorus by filtration and percolation is not affected by variations in temperatures unless it involves assimilation by microorganisms within the filter. One of the greatest advantages of this technology is the non-production of sludge in comparison with chemical methods. It has been extensively used for the treatment of primary or secondary effluents due to the low energy demands. The wastewater percolates through the high quality sand (or sometimes permeable native soils) and is collected by a drainage system. The overall performance of the filter depends on the type and biodegradability of the wastewater components (Bali and Gueddari, 2019), design and operation of the filter. The organic/mineral phosphorus is physically retained at the surface of the filter bed. The dissolved phosphorus is removed by physical-chemical or biological processes (Kim, 2014). Research conducted by Bali and Gueddari (2019) showed that greater filter depth increases the water residence time, hence retaining phosphorus in the medium. Therefore, the residence time of the wastewater in the filter is a major factor in optimizing phosphorus removal.

Membrane technologies utilize selective barrier/membranes to separate components in a stream either by sieving, diffusion or sorption. The selection is done mainly by particle size and charge. There are basically two types of membrane filtration namely dead-end and cross flow.

The chemical processes used in removal of phosphorus from wastewaters result in the addition of metal salts which react with the soluble phosphate to form solid precipitates. In chemical precipitation the most commonly used chemicals are compounds of calcium, aluminum and iron (Minnesota Pollution Control Agency, 2006). Chemical methods are expensive compared to physical processes and they are accompanied by supplementary pollution. One of the disadvantages of precipitation of P is the production of sludge, especially if the method used in primary treatment involves the application of lime (Strom, 2007). The addition of chemicals is categorized into two scenarios: effluent polishing in the secondary process and two-point chemical addition. The most commonly used technology is the two – point chemical addition shown in Figure 3 because it achieves the most efficient use of chemicals for phosphorus precipitation.

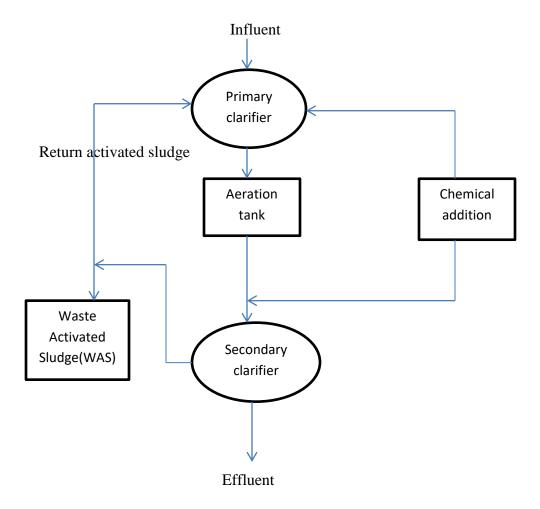


Figure 3 Two-point chemical addition (Minnesota Pollution Control Agency, 2006)

Most progress has been made with the EBPR process which has shown the potential to remove P down to very low levels and at relatively lower costs (Strom, 2007). Biological phosphorus removal is achieved by a selection of bacteria capable of storing polyphosphate (Polyphosphate accumulating organisms, PAO). In anaerobic environments, the bacteria that accumulate phosphorus in excess use the derived energy of the polyphosphate to hydrolyze and fix the organic substratum. The removal of phosphorus is efficient in alternate aerobic and anaerobic environments (Marchetto, 2013), as also illustrated in Figure 4. Research done using synthetic wastewaters to evaluate phosphorus and organic matter removal in a single reactor,

which combined aerobic and anaerobic conditions, managed to reach 92% removal efficiency of phosphorus (Marchetto, 2013).

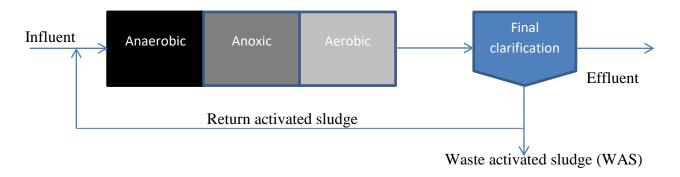


Figure 4 Enhanced Biological Phosphorus Removal (RedCorn & Engelberth, 2018)

4. ALGAE BASED TECHNOLOGIES

The primary and secondary wastewater treatment processes eliminate easily settled materials and oxidize organic materials resulting in a clear apparent effluent which can be discharged into water bodies. However, this effluent can be loaded with inorganic nitrogen and phosphorus which causes eutrophication and additional long term problems. Microalgae offer an interesting solution to the tertiary bio-treatment coupled with production of potentially valuable biomass. Currently, microalgae are being largely considered in the world due to their potential in renewable energy and nutraceutical industries. They are a renewable, sustainable and also economical source of biofuels and food ingredients. Biological methods for the recovery of nutrients from wastewaters are both friendly and environmentally sustainable. During the wastewater treatment process, the algae produce oxygen as a by-product which in turn is used by the bacteria present in the water to produce carbon dioxide for the algae, as illustrated in Figure 5.

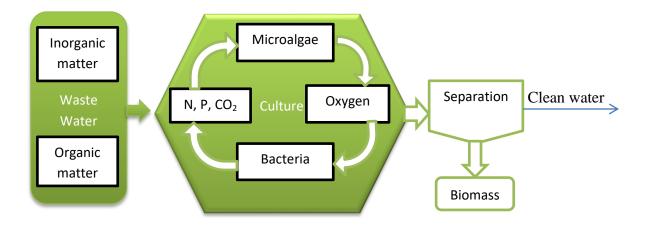


Figure 5 Microalgae- Bacteria consortium (Acién Fernández et al., 2018)

In the past, different species of microalgae have been used to successfully remove nutrients from wastewaters. It has been proven that biological uptake of nutrients can be cheap and ecologically safer than chemical processes. However, this technology is not without its share of challenges. The following scientific-technological overview aims to propose how to optimize the light parameter for nitrogen and phosphorus nutrients recovery by microalgae. The harvested microalgae biomass has numerous uses, such as its use as enriched fertilizer since it contains a wide range of micronutrients (Mg, Fe, Co, among others), which can improve the soil structure and water retention capacity (Metting, 1990; Maurya et al., 2015). Some research proposed the culturing the *Chlorella sorokiniana* green algae species coupled with the *Aequorea victoria* jelly fish in wastewater to remove inorganic N and P. The major biological and technological factors limiting the performance of nutrient recovery with microalgae in the current technologies are the availability of light, nutrient supply, carbon dioxide supply, temperature, pH and solar radiation. Technologies are being improved and upgraded to maximize the biomass production capacity while recovering all major nutrients. Tubular reactors are commonly used for microalgae strains that require strict controlled environment for optimal growth.

Rapidly growing microalgae can take up nitrogen and phosphorus directly in the presence of light and carbon dioxide resulting in valuable algal biomass which can be used as biofuel, carbohydrates, animal feed, polymers or agricultural fertilizer. Some recent research has proven

that Chlorella sorokiniana microalgae can fully remove N and P from black water with 76 mmol NH₄⁺L⁻¹ and 3.4 mmol PO₄⁻³, yielding an N:P ratio of 22. All the P was removed from black water in four days but another eight days were required to recover the entire N (Vasconcelos Fernandez et al., 2015). This may have been due to the high N:P ratio in black water. The rate at which nutrients can be recovered from wastewaters is dependent on the physiological traits of the algae species such as growth rate and nutrient demands. Higher growth rates have previously been associated with higher nutrient uptakes leading to faster removal of nutrients. Algal species with high growth rates may have higher P demands, hence lower N:P ratios (Geider and LaRoche, 2002). The selection of a single species with higher growth rate may enhance the overall nutrient removal rate but it may not support higher N:P removal ratio. On the other hand, optimized N:P recovery can be enhanced by selecting phytoplankton communities with species that have complementary N:P uptake ratios where both resources are depleted with minimum requirements for either species (Tilman, 1982). This overview will establish to what extent the Chlorella sorokiniana green algae and the Aequorea victoria jellyfish can complement each other to facilitate their coexistence and hence optimal removal of both N and P while allowing more effective use of the light spectrum and ease of harvesting the resulting biomass. Past research has shown that green and red cyanobacteria species coexist in white light. Additionally, the green algae would improve the resilience of a system against variations in growth conditions and pathogen infections (Shurin et al., 2013). In waste stabilization ponds (WSPs) raw wastewater is treated by a combination of algal and bacterial processes whereby the bacteria breakdown the organic matter and the algae provide oxygen to support the aerobic process. In some cases, mechanical aerators supply oxygen in the pond. Overall, the application of ecological principles in large scale wastewater treatment using microalgae is still in its infancy.

4.1. GENERAL CHARACTERISTICS OF MICROALGAE

Microalgae are unicellular organisms with chlorophyll which requires carbon dioxide for photosynthesis. Research has proven that these organisms generate about half of the atmospheric oxygen during the photosynthesis process. A very large number of microalgae species exists, which have not been exploited and hence they are an untapped resource since they produce unique products such as antioxidants, enzymes, polymers and sterols, among others (Bonachela et al., 2011). One of the most attractive characteristic of microalgae is their ability to acclimate to changes in environmental conditions such as temperature, pH, CO₂ or nutrient supply (Cardozo

et al., 2007). The green algae possess chlorophyll a and b, in an approximate ratio of 3:1, which gives it its typical green color. Brackish water has been found to support the growth of microalgae due to its nutritional composition.

The three main cultivation methods for wastewater treatment using algae are:

- 1. Open systems
- 2. Closed systems
- 3. Immobilized systems

The open system is preferred due to its simplicity and cheaper operation costs. It can be carried out in large scale natural or artificial ponds. The most common designs for open ponds that have been operated are raceways, unstirred ponds and circular ponds.

In the case of closed photo-bioreactors, they are categorized into two groups, namely covered raceways and tubular reactors. The four types of closed systems are the plate reactor, column reactor, annular reactor and tubular reactor. They have much better light penetrating capabilities than the open ponds hence higher biomass productivity with lower retention time (Harshad Rathod, 2015). However, the greatest disadvantage of this technology is the high operating costs coupled with more energy demands.

The immobilized system consists of trapping the algae cells in a solid medium which prevents them from moving independently. The algae medium is normally shaped as beads. In the several reported laboratory systems this technology has been tested it has been proven that the entrapped algae efficiently removes nitrogen from the wastewater compared to other alternatives (Harshad Rathod, 2015).

For the last 60 years, microalgae have been proposed as an option for wastewater treatment. However, it is yet to be implemented in a cost-effective way on an industrial scale. In most cases open systems such as ponds and deep channels are outdoors while the closed systems are built indoors under artificial light (Molazadeh et al., 2019). Technological and biological aspects are still critical and have to be improved in terms of performance for the conventional systems. There are several reasons as to why the use of microalgae technology is used only in a few facilities despite the great environmental advantages it has to offer with regard to nutrients

recovery. One of the major challenges is the large amount of land required to set up such wastewater treatment systems as well as the hydraulic retention time required, of about 7-11 days, and the high consumption of energy. This can be overcome by improving the system's photosynthetic efficiency. The requirements of land has a significant impact on the economic balance. In spite of the fact that non stirred open ponds are more economical and easier to manage, stirred open ponds facilitate aeration, light and distribution of nutrients present which in turn promotes the growth of microalgae. This overview seeks to explore the trade off and complementarity in nutrient acquisition by microalgae in the presence of light from jelly fish. Removal of carbon, nitrogen and phosphorus is accompanied by high energy consumption. The average depth of non-stirred ponds is half meter or less while the stirred ponds have a depth of between 15 and 25 cm where the water is circulated by a paddle wheel (Molazadeh et al., 2019). Most challenges that are associated with open ponds are reduced in closed pond systems but are coupled with great energy demands and other operation costs.

According to Aqualia, owning one of the largest wastewater treatment plants in Europe which processes up to 500 Mm³/year of wastewater, the average consumption of energy is 0.5 kWh/m³ at a cost of 0.2 euros/m³ which is 50% of the cost corresponding to the energy consumed (Aqualia, n.d.). With this regard it has been reported that using microalgae for wastewater treatment would reduce energy consumption. The capacity to recover nutrients is largely a factor of solar radiation availability. With the available technology it is therefore necessary to reduce land requirement and the hydraulic retention time. Open reactors have very low depth in which water is re-circulated mainly using paddle wheels. Microalgae strains such as *Oscillatoria*, *Chlorella*, *Scenedesmus* and *Nitzchia* have been termed to be the most pollution tolerant in wastewater treatment systems (Acién et al., 2016). With regard to increasing the photosynthetic efficiency microalgae cells perform photosynthesis using solar light, while producing oxygen, which can support bacteria consortia. According to laboratory experiments, the oxygen production rate is much higher than nitrification during the day (Acién et al., 2016).

4.2. EFFECT OF LIGHT ON MICROALGAE GROWTH

According to past research, light intensity, photoperiod and spectral quality are fundamental for proper microalgae photosynthesis. Photo-limitation occurs during low light intensity and photo-inhibition results from high light intensity. The photosynthetic active

radiation (PAR) contains photons between 400 to 700 nm wavelength which accounts for 43% of sunlight and can therefore fully support photosynthesis. The two absorption peaks of chlorophyll a in microalgae are 430 nm which is blue/violet light and 660 nm which is red light. Chlorophyll b has only one absorption peak at 460 nm which is blue light (Pattanaik et al., 2018). The optimum photo-period has been determined to be 12-15 hours of illumination with a mix of red-blue light in the ratio of 3:1.

Traditional High Rate Algae Ponds (HRAPs) and the open race ways are preferred due to their low cost and easy scale up. They are operated at depths varying from 20 cm to about 40 cm with a hydraulic retention time (HRT) ranging from 7 to 10 days. The light availability for the microalgae cells is relatively low, hence with low growth. The use of alternative designs such as photo-bioreactors is coupled with high energy consumption.

Previous research (Kim et al., 2013) to analyze nitrogen and phosphorus removal efficiency by the *Scenedesmus sp.* by mixing wavelength ratios based on light intensities produced the graph shown in Figure 6. Further research obtained results of optimization of wavelength mixing ratios for phosphorus removal as illustrated by figure 7.

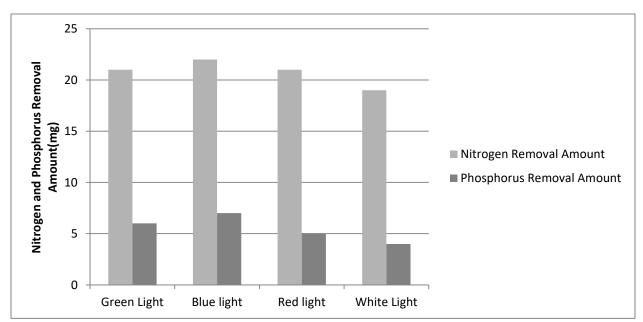


Figure 6 Nitrogen and phosphorus removal amount by *Scenedesmus sp.* under different light wavelengths (Kim et al., 2013)

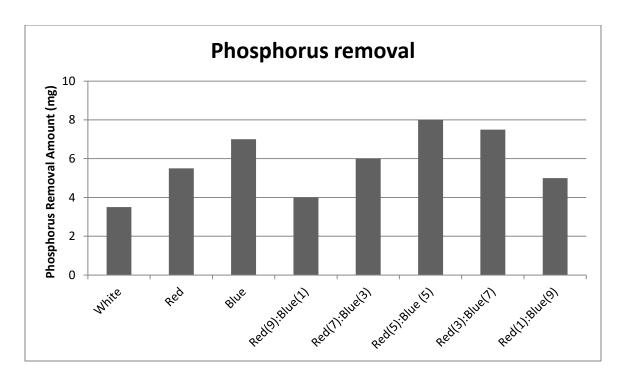


Figure 7 Optimization of wavelength mixing ratios for phosphorus removal (Kim et al., 2013)

5. AEQUOREA VICTORIA JELLYFISH

Many organisms on the planet are bioluminescent. Hundreds of species such as fireflies and corals have different glowing proteins. With this regard it is therefore necessary to indentify the critical wavelength required by the microalgae for optimum photosynthesis and explore if a symbiotic relationship can exist between microalgae and *Aequorea* jellyfish which can fluorescence. This review will explore the light emitting components of the jellyfish to provide light necessary for photosynthesis by the microalgae. The luminescent organs of a jellyfish produce green light. The presence of the green fluorescent protein (GFP) and a chemiluminescent protein called aequorin are the reason for the bioluminescence of the *Aequorea victoria* jellyfish. This enhanced protein has an excitation and emission spectra. The GFP is among the brightest and most photostable (Day and Davidson, 2014). Its ability to glow without requiring help from additional chemicals or enzymes makes it unique (Richard et al, 2014). Research has shown that it has the ability to withstand between mildly acidic pH 5 conditions and extremely basic pH 12 conditions, while resisting temperatures as high as 65 °C. It also has major and minor excitation peaks wavelengths of 395 nm and 475 nm (Ducheyne and Healy, 2011). Previous research has indicated that the Green Fluorescent Protein can be mutated

so as to emit different wavelengths and can fluorescence longer and brighter.

Light intensity and the photoperiod cycle are major influencers in the growth rate of microalgae. The photoautotrophic culture, light regime and photoperiod are critical in determining the biomass production (Wahidin et al., 2013). The light saturation limit required for microalgae to synthesize cell protoplasm is about 600 ft of candles. If microalgae are cultured at higher depth, the light must be increased. Research has been done to find out if the light intensity of the jellyfish is sufficient to affect microalgae growth in wastewater. According to Wahidin et al. (2013), maximum cell growth of the microalgae was obtained with at least six hours of darkness. Generally, cell growth of the microalgae is affected by a combination of environmental parameters including light intensity, photoperiod, temperature and nutrient composition.

According to Parmal et al. (2011), light intensity and photoperiod cycle are the main factors that determine the microalgae growth rate which in turn leads to higher nutrient uptake from wastewater.

All plants require the same core essentials which are light, carbon dioxide, water and nutrients. The ability to control almost every element required for growth is essential to ensure that the plant reaches its maximum potential. In wastewater treatment, photoperiod, alternating light and dark period, has to be controlled to achieve the maximum volume of microalgae growth and eventual nutrient uptake. The sun's light spans a broad spectrum from ultraviolet light to infrared wavelengths, as illustrated also in Figure 8. Plants tend to reflect and transmit the green wavelength more strongly than the blue and red wavelength which are absorbed more effectively within the leaves for photosynthesis (GE Current, n.d.). Different plants have different light needs hence they respond differently to light wavelengths. Recently, scientists have effectively isolated and combined different light wavelengths for plant growth. The properties of light source such as wavelength and intensity are critical for microalgae growth. Traditionally, direct sunlight or external white lights have been used to provide light energy (Figure 9) for different light wavelengths and frequencies. More than 50 % of the light energy from these sources is not utilized by the microalgae for growth. Establishing a symbiotic relationship between Aequorea Jelly fish and microalgae will not only narrow down the emission of unusable wavelength spectrum but also reduce the total consumption of electricity by white lights or LEDs.

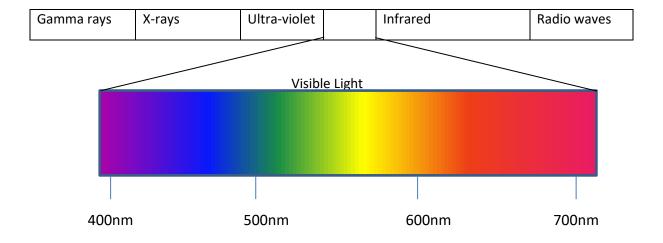


Figure 8 Visible light spectrum (Secades et al., 2014)

	Wavelength	Frequency
Red	625-740nm	480-405THz
Orange	590-625nm	510-480THz
Yellow	565-590nm	530-510THz
Green	520-565nm	580-530THz
Blue	445-520nm	675-580THz
Indigo	425-445nm	700-675THz
Violet	380-425nm	790-700THz

Figure 9 Visible light wavelengths and frequency (University of Bergen, 2013.))

6. SYMBIOTIC RELATIONSHIP

Many living organisms share a relationship for the benefit of at least one of the species, or sometimes both. Symbiosis can be mandatory whereby one organism totally depends on the other to survive or it can be optional whereby the organisms can live independently. In mutual symbiosis, both organisms benefit from each other. Generally, plants make use of the photosynthetic active radiation (PAR) region of the light spectrum ranging between 400 nm and 700 nm. The red and blue light are very essential for the chlorophyll to be converted into

chemical energy (Grow Light Spectrum Explained, 2020). Some studies aimed to use the fluorescence characteristics of the *Aequorea victoria* jellyfish to provide light necessary for growth of microalgae. Microalgae are unicellular photoautotrophic organisms that take in carbon dioxide and give out oxygen during photosynthesis. The gas evolved in photosynthesis is critical for restoring dissolved oxygen content in water. On the other hand, *Aequorea victoria* jellyfish produces green light and survives on very little oxygen and gives out carbon dioxide. It has physiological adaptations that make it possible to take in oxygen and store it for use in low oxygen environments. The wastewater will be responsible for providing the necessary nutrient supply required by the microalgae for growth. Such symbiotic relationship has proven effective.

In wastewater treatment plants photo-bioreactors provide a controlled environment for all parameters helping the efficient growth within a short period. However, they require high power consumption for use light emitting diodes (LEDs) as a source of light. Establishing a symbiotic relationship between *Aequorea victoria* jellyfish and microalgae would largely cut the costs associated with energy demand for lighting. Symbiotic relationships can be very essential to an ecosystem's health as well as economic benefits. The parameters to be measured: and optimized include

- 1. The rate of nitrogen and phosphorus intake by microalgae in the presence of Aequorea jellyfish.
- 2. The greatest depth required to support Aequorea victoria jellyfish life.
- 3. Effect of waste water on Aequorea jellyfish.
- 4. Energy consumption by the system

7. CONCLUSIONS

The *Aequorea* Jellyfish should be able to produce sufficient light for the microalgae to absorb nitrogen and phosphorus from wastewater. Microalgae cultured in the recovery of nutrients from wastewater can be used for energy, agricultural and animal use since it is not allowed for human related applications. Many microalgae species can grow in wastewater and effectively remove N and P, heavy metals and inorganic toxins. However, tolerance to organic pollutants greatly varies from one species to another. A great challenge might lie in the selection

of microalgae species that have great potential to achieve high biomass productivity. It will be necessary to determine the specific growth rate of microalgae as a function of light from the *Aequorea* jellyfish. Identifying the microalgae species suitable for outdoor ponds is fundamental since each species have unique light responses. Although wastewater treatment using microalgae and the luminescence properties of the *Aequorea victoria* jellyfish have been studied over a rather long time, there is no detailed information about combining both processes. Different section in this ovreview reviewed the facts available about both species with regard to light and assumed that they can symbiotically support each other.

8. ACKNOWLEDGMENTS.

The BIOENGIN group, at UDC, thanks Xunta de Galicia for financial support to Competitive Reference Research Groups (ED431C 2021/55).

Institutional Review Board Statement

Not applicable

Informed Consent Statement

Not applicable

Conflict of interest

Declaration no conflict of interest

9. REFERENCES.

Abdel-Raouf, N., Al-Homaidan, A. A., & 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

- Acién, F. G., Gómez-Serrano, C., Morales-Amaral, M. M., Fernández-Sevilla, J. M., & Molina-Grima, E. (2016). Wastewater treatment using microalgae: How realistic a contribution might it be to significant urban wastewater treatment? *Applied Microbiology and Biotechnology*, 100(21), 9013–9022. https://doi.org/10.1007/s00253-016-7835-7
- Acién Fernández, F. G., Gómez-Serrano, C., & Fernández-Sevilla, J. M. (2018). Recovery of
 Nutrients From Wastewaters Using Microalgae. Frontiers in Sustainable Food Systems,
 https://doi.org/10.3389/fsufs.2018.00059
- Amoatey, P., & Bani, R. (2011). Wastewater Management.
- Aqualia. (n.d.). *Eco-efficiency—Aqualia*. Retrieved September 7, 2020, from https://www.aqualia.com/en/web/aqualia-en/eco-efficiency
- Bali, M., & Gueddari, M. (2019). Removal of phosphorus from secondary effluents using infiltration—percolation process. *Applied Water Science*, 9(3), 54. https://doi.org/10.1007/s13201-019-0945-5
- Bonachela, J. A., Raghib, M., & Levin, S. A. (2011). Dynamic model of flexible phytoplankton nutrient uptake. *Proceedings of the National Academy of Sciences of the United States of America*, 108(51), 20633–20638. https://doi.org/10.1073/pnas.1118012108
- Cardozo, K. H. M., Guaratini, T., Barros, M. P., Falcão, V. R., Tonon, A. P., Lopes, N. P.,
 Campos, S., Torres, M. A., Souza, A. O., Colepicolo, P., & Pinto, E. (2007). Metabolites
 from algae with economical impact. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 146(1), 60–78. https://doi.org/10.1016/j.cbpc.2006.05.007
- Ducheyne, P., & Healy, K. E. (2011). *Comprehensive biomaterials. Volume 1, Volume 1,*. Elsevier.

- http://search.ebscohost.com/login.aspx?direct=true&scope=site&db=nlebk&db=nlabk&A N=453974
- Fernandes, T. V., Suárez-Muñoz, M., Trebuch, L. M., Verbraak, P. J., & Van de Waal, D. B. (2017). Toward an Ecologically Optimized N:P Recovery from Wastewater by Microalgae. *Frontiers in Microbiology*, 8. https://doi.org/10.3389/fmicb.2017.01742
- GE Current. (n.d.). *The Science of Indoor Farming*. Retrieved August 14, 2020, from https://www.gecurrent.com/ideas/science-of-indoor-farming
- Grow Light Spectrum Explained: Ideal LED Spectrum for Plants. (2020, April 29). BIOS Lighting. https://bioslighting.com/grow-light-spectrum-led-plants/
- Gupta, S. K., & Bux, F. (2019). Application of Microalgae in Wastewater Treatment: Volume 1:

 Domestic and Industrial Wastewater Treatment. https://doi.org/10.1007/978-3-03013913-1
- Harshad Rathod. (2015). *Algae based waste water treatment*. https://doi.org/10.13140/2.1.1241.8885
- Kim, T.-H., Lee, Y., Han, S.-H., & Hwang, S.-J. (2013). The effects of wavelength and wavelength mixing ratios on microalgae growth and nitrogen, phosphorus removal using Scenedesmus sp. For wastewater treatment. *Bioresource Technology*, 130, 75–80. https://doi.org/10.1016/j.biortech.2012.11.134
- Laboratory, U. P. R. (2012, May 7). Plankton Research Laboratory: Alginate Beads

 (Immobilised algae). *Plankton Research Laboratory*.

 http://usmplanktonlab.blogspot.com/2012/05/alginate-beads-immobilised-algae.html
- Marchetto, M. (2013). Technologies Used in the Wastewater Treatment for Nutrient Removal. 3(2), 4.

- Miah, M., Kazi, R., Uddin, N., & Bhuiyan. (2012). Effluent Treatment Plant: A Tool for Protecting Environment from Pollution. *University Journal of Business*, 33.
- Minnesota Pollution Control Agency retrieved from https://www.pca.state.mn.us/sites/default/files/wq-wwtp9-02.pdf accessed on 20th September, 2020
- Molazadeh, M., Ahmadzadeh, H., Pourianfar, H. R., Lyon, S., & Rampelotto, P. H. (2019). The Use of Microalgae for Coupling Wastewater Treatment With CO2 Biofixation. *Frontiers in Bioengineering and Biotechnology*, 7. https://doi.org/10.3389/fbioe.2019.00042
- Nutrient Pollution in Wastewater: An Emerging Global Problem Analyte Guru Post. (2016, August 26). Analyte Guru. https://analyteguru.com/nutrient-pollution-in-wastewater-an-emerging-global-problem/
- Pattanaik, A., Sukla, L., & Pradhan, D. (2018). Effect of LED Lights on the Growth of Microalgae. 14.
- Plant morphology and spectrum: How plants respond to light quality. (2019, June 6). P.L. Light Systems. https://pllight.com/plant-morphology-and-spectrum-how-plants-respond-to-light-quality/
- Puyol, D., & Batstone, D. J. (2017). Editorial: Resource Recovery from Wastewater by Biological Technologies. *Frontiers in Microbiology*, 8. https://doi.org/10.3389/fmicb.2017.00998
- RedCorn, R., & Engelberth, A. S. (2018). Quantifying Glycogen in Solids at Full-Scale
 Enhanced Biological Phosphorous Removal Wastewater Facilities. *Journal of Environmental Engineering*, 144(9), 04018088. https://doi.org/10.1061/(ASCE)EE.1943-7870.0001438

- Secades, C., O'Connor, B., Brown, C., Walpole, M., UNEP World Conservation Monitoring Centre, & Secretariat of the Convention on Biological Diversity. (2014). *Earth observation for biodiversity monitoring: A review of current approaches and future opportunities for tracking progress towards the Aichi biodiversity targets*. http://www.deslibris.ca/ID/242836
- Sedlak, R. (Ed.). (1991). *Phosphorus and nitrogen removal from municipal wastewater: Principles and practice* (2nd ed). Lewis Publishers.
- Ssblakely. (2008). *Aequorea victoria*. Own work. https://commons.wikimedia.org/wiki/File:Aequorea4.jpg
- Strom, P. (2007). Technologies to Remove Phosphorus from Wastewater. *New Jersey Effluents*, 40, 31–35.
- University of Bergen. (n.d.). *The Electromagnetic spectrum*. University of Bergen. Retrieved September 7, 2020, from https://www.uib.no/en/hms-portalen/75292/electromagnetic-spectrum
- US EPA, O. (2013a, March 12). *The Issue* [Overviews and Factsheets]. US EPA. https://www.epa.gov/nutrientpollution/issue
- US EPA, O. (2013b, March 12). *The Sources and Solutions: Wastewater* [Overviews and Factsheets]. US EPA. https://www.epa.gov/nutrientpollution/sources-and-solutions-wastewater
- Wahidin, S., Idris, A., & Shaleh, S. R. M. (2013). The influence of light intensity and photoperiod on the growth and lipid content of microalgae Nannochloropsis sp. *Bioresource Technology*, 129, 7–11. https://doi.org/10.1016/j.biortech.2012.11.032

Wollmann, F., Dietze, S., Ackermann, J., Bley, T., Walther, T., Steingroewer, J., & Krujatz, F. (2019). Microalgae wastewater treatment: Biological and technological approaches. *Engineering in Life Sciences*, 19(12), 860–871. https://doi.org/10.1002/elsc.201900071