Upotreba otpadne vode od uzgoja riba u RAS-u za ugoj mikroalga

Prečanica, Mario

Master's thesis / Diplomski rad

2020

Degree Grantor / Ustanova koja je dodijelila akademski / stručni stupanj: **University of Dubrovnik / Sveučilište u Dubrovniku**

Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:155:504956

Rights / Prava: In copyright / Zaštićeno autorskim pravom.

Download date / Datum preuzimanja: **2024-12-27**

SVEUČILIŠTE U DUBROVNIKU
UNIVERSITY OF DUBROVNIK

Repository / Repozitorij:

Repository of the University of Dubrovnik

zir.nsk.hr

dabar
DIGITALNI AKADEMSKI ARHIVI I REPOZITORIJI

SVEUČILIŠTE U DUBROVNIKU

ODJEL ZA AKVAKULTURU

DIPLOMSKI STUDIJ MARIKULTURA

Mario Prečanica

Use of wastewater from fish culture in RAS for culture of microalgae

DIPLOMSKI RAD

Dubrovnik, 2020.

SVEUČILIŠTE U DUBROVNIKU

ODJEL ZA AKVAKULTURU

DIPLOMSKI STUDIJ MARIKULTURA

Mario Prečanica

Use of wastewater from fish culture in RAS for culture of microalgae

DIPLOMSKI RAD

Mentor: doc. dr. sc. Kruno Bonačić

Komentor: doc. Ing. PhD. Jan Mraz

Dubrovnik, 2020.

ABSTRACT

This experiment assessed usability of wastewater from recirculating aquaculture system for freshwater species under different disinfection methods for *Chlorella vulgaris* cultivation. Before wastewater was used for culturing algae, it flowed through aerobic mineralization process and disinfection using: active chlorine, UV, UV with chlorine and ozone. The goal was to investigate if disinfection method had an effect on nitrogen and phosphorus levels in wastewater and growth dynamics of microalgae cultivated in wastewater, which in turn was an indicator of disinfection efficiency. Best results were achived with a combination of UV and active chlorine, where highest concentration of microalgae were achieved. Furthermore, UV in combination with active chlorine and UV alone resulted in higher levels of phosphorus. Regarding the nitrates, UV and ozone group showed highest levels. After wastewater was treated with chlorine it showed lowest level of nitrates that can negatively affect microalgae growth. Group where only mechanical filtration was applied showed as the worst group, because cultures of microalgae crashed first due to protozoa contamination. With these findings it was concluded that disinfection methods can be helpfull in reuse of aquaculture wastewater for microalgal cultivation.

Key words: *Chlorella vulgaris*, disinfection methods, nitrogen, phosphorus, wastewater.

SAŽETAK

Ovim je pokusom utvrđena upotrebljivost otpadnih voda iz slatkovodnog recirkulacijskog akvakulturnog sustava koristeći različite metode dezinfekcije za uzgoj vrste *Chlorella vulgaris*. Prije korištenja za uzgoj algi, otpadna voda je podvrgnuta procesu aerobne mineralizacije i dezinfekciji koristeći: kloriranje, UV, UV u kombinaciji s kloriranjem i ozon. Cilj istraživanja bio je istražiti utječe li metoda dezinfekcije na promjenu razine dušika i fosfora u otpadnim vodama i na dinamiku rasta kultura mikroalgi uzgajanih u otpadnim vodama, što je ujedno bio i indikator uspješnosti dezinfekcije. Najbolji rezultati postignuti su korištenjem kombinacije UV-a i kloriranja, gdje je postignuta najveća koncentracija mikroalgi. Nadalje, UV u kombinaciji s kloriranjem ili čak sami UV rezultirao je višim razinama fosfora. Skupine tretirane UV-om ili ozonom pokazale su najviše razine nitrata. Otpadna voda koja je tretirana s aktivnim klorom je imala najnižu razinu nitrata što može negativno utjecati na rast mikroalgi. Skupina u kojoj je primijenjena samo mehanička filtracija pokazala se kao najlošija skupina jer su se kulture mikroalgi prve srušile zbog onečišćenja praživotinjama. Rezultati ovog istraživanja pokazuju da metode dezinfekcije mogu biti korisne u ponovnoj upotrebi otpadnih voda iz akvakulture za uzgoj mikroalgi.

Ključne riječi: *Chlorella vulgaris,* dezinfekcijske metode, dušik, fosfor, otpadne vode.

CONTENTS

1. INTRODUCTION

Aquaculture is the fastest growing food sector in the world with average annual growth of 5,8 % since 2010 and, shows no signs of slowing down. It is often viewed as the only solution that can provide more fish products given that harvesting wild stocks have reached an upper limit (FAO, 2014). The fishing effort might continue to increase in the future but it is unlikely that fisheries will be able to supply more aquatic food products than today, averaging at 90 million tonnes per year. It is more likely that current fishing practices and fishing capacity will have to be significantly reduced worldwide to ensure sustainable harvests and to maintain biodiversity and ecosystem functions. On the other hand, aquaculture is considered to be one of the fastest-growing global primary industries with an average annual growth rate of > 4 % over the period 2010 to 2030 (Brugére & Ridler, 2004) and for the first time is set to produce half of the sea food consumed by human societies worldwide (FAO, 2009).

Fast development of intensive fed aquaculture (e.g. finfish and shrimp) throughout the whole world is causing many concerns related to negative environmental impacts, often associated with such monospecific practices. One of the main environmental issues is the direct discharge of wastewater into the environment. The aquaculture industry is realizing the problem of wastewater and it is starting to develop new, innovative, and responsible practices to optimize solutions for continued intensive aquaculture production.

1.1. Filtration in recirculating aquaculture systems

Recirculating Aquaculture Systems (RAS) are generally housed in land-based facilities and consist of tanks for the culture of aquatic animals and water processing, as well as equipment for water treatment and recirculation. It is important that water in the tanks is renewed frequently enough for specific cultured species. In general, water in a tank should be exchanged every few hours because of accumulated levels of ammonia that is highly toxic for most species and because of deficiency of dissolved oxygen in the water. Water flow in the tank is usually from the top and clockwise so that a current is created. That helps water exchange and concentrates waste at the bottom of the tank by creating a vortex so it can be flushed by opening a valve. In an efficient RAS most of the water (around 95 %) is recycled.This is enabled by use of a solids removal treatment (mechanical filter) and biofilter that contains nitrifying bacteria that convert harmful nitrogen compounds (ammonia, nitrites) into the less toxic nitrates. If a RAS is controlled and managed well, it results in efficient feed utilization, which

in the end results in lower waste production and higher profit. Thus, a well managed RAS results in an efficient reduction of effluent wastewater. Typically, all solid waste produced by water organisms is removed into a separate tank that can then be treated before final discharge or before use for other needs. Treatments like that generally involve sludge thickening or nutrient mineralization of that waste (Van Rijn, 2013).

1.1.1. Biofiltration

One of the main challenges of RAS is the fact that the final product of protein metabolism of fish is excreted as ammonia, which is highly toxic for fish. Thus, one of the most important components of a RAS is the biofilter. It contains several species of nitrifiying bacteria with varying functions: *Nitrosomonas* bacteria species that transfer NH_4^+ (ammonium ion) to NO_2^- (nitrite), *Nitrospira* and *Nitrobacter* bacteria species transfer NO_2^- (nitrite) to NO_3^- (nitrate). These bacteria species are aerobic autotrophs, which means they use O_2 as oxidizing agent and CO_2 or HCO_3^- as a carbon source for growth. Because of this, it is important to assure strong aeration that will provide enough oxygen for the bacteria to function. An artificial surface is ususally used for the growth of the bacterial biofilm. This surface should be maximized within the biofilter by having large specific surface areas, usually in the form of ball or wheel-like plastic structures with numerous holes. This way, contact between water flowing through the biofilter and the surface of the bacterial film is maximized (Lekang, 2007).

1.1.2. Solids removal

In aquaculture, removal of particles from the water column is needed for several purposes. First is to filter water entering a fish farm for the first time (ambient water from the environment). In this way, water of good quality is ensured for the cultured species and the negative effect that high concentration of particles can have a on the aquaculture equipment (e.g. water pumps) is reduced. Furthermore, it is important to filter effluent water when it is released from fish farms to the environment, in order to prevent pollution. If the water is re-used, like in a RAS, high concentrations of suspended particles can have a negative effect on growth and survival of the cultured organisms (Chen *et al.*, 1994). Except for particles in water that can have a negative effect on the fish, filtration can prevent parasites from entering aquaculture facilites (Liltvedt & Hansen, 1990).

1.2. Aquaculture wastewater treatment

Wastewater treatment is usually a combination of physical, biological and chemical methods in order to remove solids, organic and sometimes inorganic components that can be found in wastewater. It is usually performed in four stages: preliminary treatment, primary treatment, secondary treatment and tertiary treatment.

The main goal of preliminary treatment is removal of bigger particles from wastewater. This is done so wastewater can be treated additionally in next steps if wanted (FAO, 1992).

In primary treatment step, the goal is to remove smaller particles from the water, usually with methods of sedimentation and skimming from the surface. In this step, most of suspended solids and oils are removed from the wastewater. Furthermore, biological oxygen demand (BOD) is reduced by 25 – 50 %.

The secondary treatment is a continuation of the primary treatment, and the goal is to remove suspended solids and organic matter that are left in wastewater. This is achieved with an aerobic biological treatment, similar to a biofilter in a RAS. Aerobic biological treatment is a method that uses aeration in wastewater, providing good life conditions for microorganisms that metabolize most of the organic matter. This process is also called mineralization of wastewater (FAO, 1992).

The tertiary treatment is used when some parts of wastewater have not been removed by previous treatments, such as nitrogen, phosphorus, suspened solids etc. Most often methods that are used in the tertiary treatment are, depending on the goal and volume of wastewater, membrane based filtrations, deep-bed filtration etc. (FAO, 1992).

1.3. Nutrient mineralization of wastewater

1.3.1. Mineralization of fish wastewater

Fish sludge solids are mainly composed of degradable organic matter. The more complex organic molecules (e.g. proteins, lipids, carbohydrates, etc.) are principally built of carbon and they can be reduced to lower molecular weight compounds. During this degradation process, the macronutrients (nitrogen, phosphorus, potassium, calcium, magnesium and sulphur) and micronutrients (iron, manganese, zinc, copper, boron, and molybdenum) are released into water from organic molecules, and they are released in ion form. This process is called nutrient mineralization. If high organic reduction is achieved it will result in high nutrient mineralization. Sludge contains a percentage of undissolved minerals, but it also contains some

macronutrients and micronutrients that are released during the mineralization process of organic matter. Thus, it is important that microorganisms that are in charge of the degradation of organic compounds have good conditions to grow. Optimal pH should be 6 – 8. Also, an oxygen supply is very important, which is often achieved with vigorous aeration (Lennard, 2017).

1.3.2. Batch aerobic mineralization

In this method,solid waste is placed into the mineralization tank at one discrete time and in one discrete load. That means that wastewater is added once, it goes through the mineralization process and the end product has released nutrients in that can be removed and utilized (Lennard, 2017).

1.3.3. Continuous aerobic mineralization

This method involves adding wastewater to the mineralization tank on a daily basis and removing clarified liquid daily. The volume of newly added wastewater each day has to equal the volume of processed wastewater removed each day (Lennard, 2017).

1.4. Wastewater disinfection

Mineralized wastewater often still contains numerous microorganisms that may or may not be harmful to cultured organisms and wildlife. For this reason, mineralized wastewater should be disinfected before further use.

1.4.1. Disinfection with ozone

Ozone gas (O_3) is unstable and will quickly be broken down to oxygen (O_2); the half-life of O_3 is around 15 min. It is therefore necessary to produce the ozone on site. Ozone is produced by the corona method; air or pure oxygen gas is passed through a high voltage electric field. Most pathogens are killed by an ozone dose of 0,1 – 1 mg/L and contact time of 1 – 10 min, but this varies with the organism (Lekang, 2007).

One way to measure ozone dosage for removing organic compounds in water is by measuring chemical oxygen demand (COD). This test essentially determines the amount of oxygen to convert all of the organic carbon in the sample to CO_2. This is the most direct way to determine the amount of ozone needed. For organic compounds that are treatable with ozone,

a rule of thumb can be applied for an initial estimate of ozone demand. The recommendation is to use 2,5 mg ozone per mg of COD (https://www.spartanwatertreatment.com/how-much-ozone-do-i-need-to-treat-water/). The most common test method is the colorimetric analysis after oxidizing the COD with acid and using indicator compounds, such as hexavalent dichromate. In some instances, however, there are compounds that will interfere with the colorimetric analysis, and the titration is required to determine COD levels.

1.4.2. Disinfection with UV

In commercial plants, a normal UV dose is in the range 30 – 35 mWs/cm^2, and this is adequate for disinfection of three of the most common aquaculture bacteria (Lekang, 2007).

1.4.3. Disinfection with active chlorine

Active chlorine process is widely accepted and used method for disinfection of water. It is effective in killing microorganisms like bacteria, microalgaes, protozoans, etc. Chlorine as a strong oxidiating agent, reacts with organic molecules and with a process of oxidation, kills them (Calderon, 2000).

1.5. Treatment of wastewater with microalgae

Mineralized wastewater retains high levels of micro and macronutrients which act as fertilizer for plants and microalgae. Thus, microalgae can remove most common nutrients from the water column, such as nitrogen, phosphorus, carbon dioxide, but also heavy metals and even pathogens that are present in wastewaters, providing an efficient management of wastewater before it is released to natural bodies of water (Ansari *et al*., 2017). Many studies have demonstrated the big potential of *Chlorella vulgaris* for treating wastewater by fixating up to 74 % carbon dioxide, absorbing 45 – 97 % nitrogen, 28 – 96 % phosphorus and in reducing the chemical oxygen demand (COD) by 61 – 86 % from numerous types of wastewaters such as textile, sewage, municipal and agricultural (Aslan & Kapdan, 2006). For this reason, *C. vulgaris* is considered as one of the best microalgae species for preventing environmental pollution with wastewaters (González *et al*., 1997).

1.6. Wastewater from aquaculture for cultivation of microalgae

There are several common wastes in the effluents of aquacutlure wastewater: sludge, organic waste, inorganic waste, nutrients, toxins, pathogens, etc. For microalgal growth, nutrients in wastewater have a high potential because of the possibility to use those nutrients, and with that, make wastewater from RAS potentially useful. The requirements for culturing microalgae consist of three main macronutrients: carbon, nitrogen, and phosphorus. Micronutrients required in the trace are silica, calcium, magnesium, potassium, iron, manganese, sulphur, zinc, copper, and cobalt. These essential micronutrients rarely limit algal growth when wastewater is used (Knud-Hansen *et al*., 1998). nitrogen can be present in wastewater in the form of ammonia (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-). Phosphorus is primarily in the form of phosphates (PO_4^{3-}). Inorganic nitrogen and phosphorus cause big problems to remove from wastewater. Microalgae have the ability to use wastewater pollution for growth and have an especially good ability to reduce concentrations of inorganic nitrogen and phosphorus in wastewater (Ahluwalia & Goyal, 2007). Many species of microalgae show good adaptability and resistance to grow successfully in wastewater because they can use those high concentrations of inorganic nitrogen in form of NH_4^+ and in the wastewater (Martin *et al*., 1985). The only limiting factor of culturing microalgae in wastewater can be carbon deficiency, but luckily the atmosphere provides infinite quantities of carbon that microalgae can use.

Competition among the microbial community in wastewater can be really intense, sometimes making growth of microalgae in wastewater very difficult. This is why one cannot expect optimal or high concentrations of microalgae using untreated wastewater. Usually, species that contain high lipid concentrations are more likely to be outcompeted by some more rigid, tougher and faster-growing microalgae or other microorganisms, meaning it is almost impossible to have a monoculture (Vasudevan & Briggs, 2008). When wastewater is used, there are usually a few species of microalgae that just naturally dominate such environments. In monocultures grown for nutritional supplements or other bioproducts, microalgae cultures are susceptible to contamination by less desirable strains of microalgae and microorganisms. However, it is important to note that available disinfection methods (ozone, UV, chlorine) have the potential to alter the nutrient composition of mineralized wastewater, but very little research has been done in this regard (Lehtola *et al*., 2001; Kim *et al*., 2003; Singer & Zilli, 1975).

1.7. Biology and culture of *Chlorella vulgaris*

C. vulgaris is is a green eukaryotic species of microalgae, which belongs to the following scientific classification: Domain: Eukaryota, Kingdom: Protista, Divison: Chlorophyta, Class: Trebouxiophyceae, Order: Chlorellales, Family: Chlorellaceae, Genus: *Chlorella*, Species: *Chlorella vulgaris*.

1.7.1.Morphology of C. vulgaris

C. vulgaris is a spherical microscopic cell that is 2 – 10 μm in diameter (Yamamoto *et al*., 2004). During its early formation in its autosporangia, the newly formed cell wall is fragile, forming a 2 nm thin electron-dense unilaminar layer. The cell wall of the daughter cell gradually increases in thickness until it reaches 17 – 21 nm after maturation. Microfibrillar layer is formed representing a chitosan-like layer composed of glucosamine. Which creates a more rigid cell. In the mature stage, cell wall thickness and composition are not constant because they can change according to different growth and environmental conditions which make them suitable species to grow in harsh conditions that are often occurring in wastewater (Němcová & Kalina, 2000).

Cytoplasm is the gel-like substance that is positioned in the cell and it is composed of water, soluble proteins, and minerals, and it hosts all internal organelles of the *C. vulgaris* (Solomon *et al*., 1999).

Every mitochondrion contains some genetic material and the respiratory apparatus has a membrane that is double layered. Outer membrane wraps the whole organelle and it is composed of an equal ratio of proteins and phospholipids, but the inner membrane is composed of three-time more proteins than phospholipids, and it surrounds the internal space called the matrix, which contains the majority of mitochondrial proteins (Solomon *et al*.,1999).

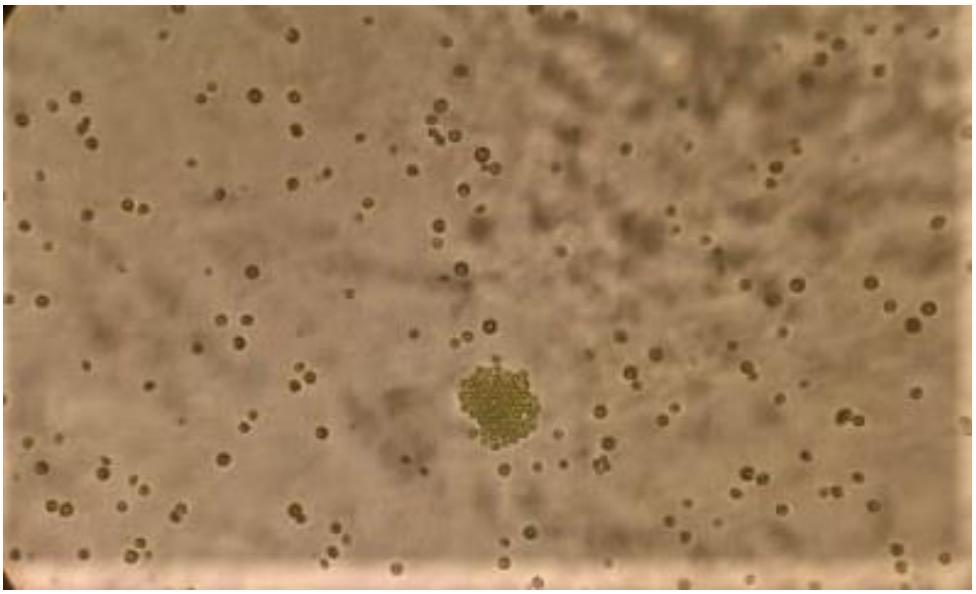

Figure 1. Microscope image of *C. vulgaris* (source: M. Prečanica).

1.7.2. Reproduction of C. vulgaris

*C. vulgaris*is a non-motile reproductive cell (autospore); it reproduces asexually. If conditions are good, this species reproduces by autosporulation which is a common way for asexual reproduction in microalgae; the mother cell has four daughter cells that are formed inside of it (Yamamoto *et al*., 2004). The new formed cells make a hole and are released from the mother cell. During this process the mother cell is destroyed and becomes food for the daughter cells (Yamamoto *et al*.,2004).

1.7.3. Culture of C. vulgaris

Open water bodies are the most common and the cheapest method for large-scale algal biomass production. These systems are categorized into natural waters like lakes, lagoons, and ponds or even artificial water bodies like containers and artificial hand made ponds. They are usually built next to an industry that produces high quantities of waste that is rich in carbon dioxide then the biomass is used for absorption of nutrients that can be found in that waste. Those water bodies should be shallow, not deeper than 50 cm to allow good exposure of all the cells to sunlight (Brennan & Owende, 2010). Because one cannot fully control all parameters, open pond systems have some disadvantages. Things that can go wrong are water evaporation, contaminants, contamination withun desirable species of microalgae and bacteria that can cause

the collapse of the target species. Furthermore, temperature, CO_2 concentration and sunlight exposure are almost impossible to control so one cannot predict with high accuracy the biomass production. It is recommended to at least add aeration to supply biomass with enough oxygen and to mix all the layers of the water.

1.7.4. Microalgae growth phases

There are several stages of growth in algal culture (Figure 2). The first stage is lag or induction phase. At this initial stage there is a slow increase in cell density. Cultures inoculated with exponentially propagating algae have a rapid lag phase, which can greatly reduce the time required for upscaling. Deviation from growth is attributed to the physiological adaptation of cell metabolism to growth, such as an increase in the levels of enzymes and metabolites involved in cell division and carbon fixation (FAO, 1996).

The second phase is the phase of exponential growth. During this phase, cell density increases. The specific growth rate mainly depends on the species of algae, light intensity and temperature.

The third stage is the phase of declining growth. Cell division slows down when nutrients, light, pH, carbon dioxide or other physical and chemical factors begin to limit growth.

The fourth phase is the stationary phase that occurs when the limiting factor and the growth rate come into equilibrium, resulting in a relatively constant cell density.

The declining phase is in the last phase, when water quality deteriorates and nutrients are already depleted to levels unsuitable for growth maintenance. Cell density decreases rapidly, and algae culture finally collapses. In practice, culture failures can have a variety of causes, including nutrient depletion, oxygen deficiency, overheating, inadequate pH or contamination by other organisms.

It is important to emphasize that microalgae are of the highest quality for use when they are in the exponential phase (FAO, 1996).

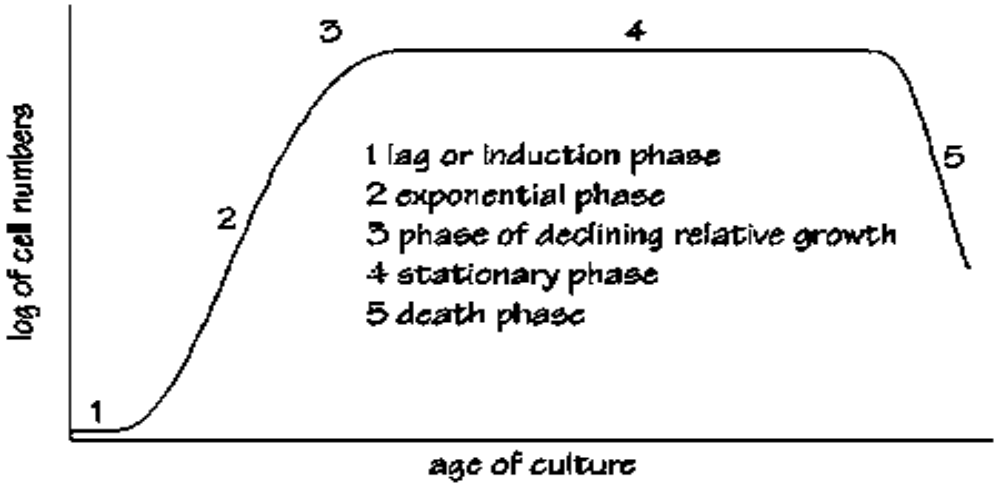

Figure 2. Growth phases of microalgae (taken from FAO 1996).

1.8. Industrial uses for microalgae

Microalgae are one of the best organisms capable of closing the gap between nutrient removal of wastewater from various industries that are causing environmental pollution and the production of biomass that can be used as a renewable energy - biofuels. With growing concerns about the disappearance of fossil fuels, renewable biofuels have received a large amount of attention in recent times. One of the most important characteristics that *C. vulgaris* has is the accumulation of lipids. If *C. vulgaris* goes into nitrogen deficiency it creates even better fatty acid profile that can be then used for biodiesel production (Zheng *et al*., 2011). Today, biofuels are mostly produced with oil crops and waste oils, but the demand for biofuels highly exceeds the quantity of current possibilities of the same. Because of that, microalgae appear to be a more promising source for the future production of biofuels (Chisti, 2007; Chisti, 2008).

1.9. Objectives

The goal of this experiment is the sustainable and successful management of RAS wastewater for microalgae cultivation and to try to culture monospecific microalgae that can be later used as a feed for zooplankton (e.g. rotifers and *Artemia* spp.) or herbivorous juvenile fish. The idea is to use different disinfection methods for treating wastewater from a fish farmand investigate how different disinfection methods affect nutrient composition of the wastewater and the growth dynamics of *C. vulgaris*. The results of the experiment would contribute to the development of modern sustainable aquaculture, by mitigating and re-utilizing waste from an intensive RAS production system.

2. MATERIALS AND METHODS

This experiment was done at Institute of Fisheries and Protection of Waters, Czech republic. Different wastewater disinfection methods were compared based on their ability to eliminate less desirable microorganisms and provide clean water with nutrients for the monocultivation of microalgae. Wastewater obtained from a RAS mechanical filter was mineralized, mechanically filtered and disinfected by UV, ozone or chlorine, using a combination of the latter two, or left without disinfection. Thus, the study had six experimental groups depending on the disinfection treatment used for processing of the mineralized wastewater: chemical, chemical+UV, UV, ozone and mechanical (no disinfetion), but also a control group where microalgae were grown in a standard culture medium. Each group had three replicas in volume of 1 L that were tested in paralel at same time and place.

Figure 3. RAS where african catfish were farmed (source: M. Prečanica).

2.1. Source of wastewater

Wastewater was taken from RAS (Figure 3) with African catfish (*Clarias gariepinus* Burchell, 1822). The RAS contained approximately 200 kg of African catfish, with an average size of 2,1 ± 0,29 kg per fish. The fish were fed with Merval skretting feed in the quantity of 1

% of body weight. Water parameters were measured with a multimeter (Hanna HI98194) every day. pH was regulated with $CaCO_3$ (calcium carbonate) when needed.

2.2. Mineralization of wastewater

Water containing feces, food waste, and other undesirable particles was filtered by a mechanical filter (DRUM FILTER PP22 ECO) into cylindroconical 100 L vortex tanks (Figure 4). Wastewater from the vortex tanks was flushed once per day into 500 L black mineralization containers with strong aeration in order to start the process of mineralization (Figure 5). Water was left in the mineralization containers until desirable conditions of water were obtained in terms of a good ratio of ammonia, nitrites, and nitrates. Wastewater from these mineralization units were taken when NH_3 started dropping down and, NO_2^- and NO_3^- started rising. The concentrations of NH_3, NO_2^-,NO_3^-, but also phosphates (PO_4^-) were measured every second day over a three week period with a photometer (Hanna instrument, HI 83203 Aquaculture Photometer). The mineralization chamber was aerated via compressed air to promote the respiration of heterotrophic bacteria and to keep anaerobic denitrification processes as minimal as possible.

Figure 4. Mechanical filter and vortex where most of the waste is collected from the RAS (source: M. Prečanica).

Figure 5. Mineralization of wastewater from RAS (source: M. Prečanica).

2.3. Mechanical filtration of mineralized wastewater

After mineralization, the wastewater underwent mechanical filtration and was divided into several canisters that were later used for different disinfection treatments. Mechanical filtration was performed in two stages. In the first stage, a sterile gause was placed in a funnel through which water was poured into a second funnel filled with cellulose pulp. The filtered water flowed into 10 L black canisters (Figure 6) to reduce any possible effect from light. The canisters were previously disinfected for 24 hours with water and 0,2 mL bleach (5 % active chlorine). After 24 h, the chlorine was neutralized with 12 mg/L of sodium thiosulfate pentahydrate and the canisters were emptied. Following disinfection, the canisters were filled with the filtered wastewater.

Figure 6. Black canisters used for mechanical filtration of wastewater used in the experiment (source: M. Prečanica).

2.4.Experimental treatments

The experiment consisted of a control group and five treatment groups: ozone, UV, chlorine, UV + chlorine and mechanical.

2.4.1. Control group

Distilled water was used in the control group, without wastewater. The water was disinfected with 0,2 mL bleach with 5 % active chlorine. After adding bleach, it was mixed and covered with foil so chlorine would not evaporate. Water with active chlorine was left for a period of 24 h for chlorine to disinfect the water. After 24 h, the chlorine was neutralized with

12 mg/L of sodium thiosulfate pentahydrate. Water was poured into three Erlenmeyer flasks of 1 L over a Bunsen burner to prevent possible contamination and then covered with tin foil.

Bg11 nutrient medium (Figure 7) was first sterilized in an autoclave (Melag Sterilizator 75) on 121°C and then added into Erlenmeyer flasks over a Bunsen burner.

Table 1. Bg 11 medium (Allen, 1968, Allen & Stainer, 1968)

Component	Stock solution ($g.l^{-1}$ dH_2O)	Quantity used (per 1 L)
Fe-citrate solution	see following recipe	1 mL
$NaNO_3$	-	1,5 g
$K_2HPO_4.3H_3O$	40	1 mL
$MgSO_4.7H_2O$	75	1 mL
$CaCl_2.2H_2O$	35	1 mL
Na_2CO_3	20	1 mL
Na-EDTA	1,0	1 mL
Trace metals solution	see following recipe	1 mL

Component	Stock solution ($g.l^{-1}$ dH_2O)	Quantity used (per 1 L)
Citric acid	6	1 mL
Ferric ammonium citrate	6	1 mL

Component	Stock solution ($g.l^{-1}$ dH_2O)	Quantity used (per 1 L)
H_3BO_3	-	2,860 g
$MnCl_2.4H_2O$	-	1,810 g
$ZnSO_4.7H_2O$	-	0,220 g
$CuSO_4.5H_2O$	79,0	1 mL
$Na_2MoO_4.2H_2O$	-	0,391 g
$Co(NO_3)_2.6H_2O$	49,4	1 mL

Figure 7. Components of Bg 11 medium after autoclaving (source: M. Prečanica).

2.4.2. Treatment with ozone

10 L of wastewater was taken from the mineralization container and first filtered mechanically. Water was then ozonated with an ozone generator (Sander C-25), (Figure 8). in the duration of 24 h in a black bucket. Sander C-25 produced 25 mg ozone / per hour. After the application of ozone, water was poured over a Bunsen burner into three 1 L Erlenmeyer flasks, which were immediately covered with tin foil.

Measured COD was 96 mg/L so the amount of ozone applied was 240 mg/L.

Figure 8. Ozone generator used in the experiment (source: M. Prečanica).

2.4.3. Tretment with UV-light

10 L of wastewater is taken from the mineralization container and first filtered mechanically. Water was poured into black canister of 10 L, and then treated with UV – light with „UV sterilizátor - UV lamp 2GPM“ (Figure 9). After water flowed through UV light, water was poured over Bunsen burner directly into three Erlenmeyer 1 L flasks. Then Erlenmeyer flasks were covered with tin foil.

Figure 9. UV sterilizer used in the experiment (source: M. Prečanica).

2.4.4. Chemical treatment with active clorine

After mechanical filtration, water was poured into black canister of 10 L. Water was then poured into Erlenmeyer flasks and disinfected with 0,2 mL bleach with 5 % active chlorine (Figure 10). After adding bleach, the water was mixed and left overnight for chlorine to disinfect. The canister was covered with tin foil so the chlorine would not evaporate. After 24 h, the chlorine was neutralized with 12 mg/L of sodium thiosulfate pentahydrate.

Figure 10. Bleach with 5 % of active chlorine that is used for disinfection and sodium thiosulfate pentahydrate that is used for neutralization of chlorine (source: M. Prečanica).

2.4.5. UV light with active chlorine

After mechanical filtration, water was treated withr UV-light and then treated with active chlorine for another 24 h.

2.4.6. No treatment

Wastewater that was mechanically filtered, but not disinfected.

2.5. Microalgae culture

2.5.1. Stocking microalgae cultures

Culture of *C. vulgaris* was brought from Microbiological institute from Trebon, Czech republic. Stocking of experimental groups was done over a Bunsen burner with a sterile pippete. The microalgae were stocked in 18 Erlenmeyer flasks of 1 L, three replicas for each of the six treatments (Control, Ozone, UV, Chemical, UV+chemical, Mechanical), by adding 100 mL of microalgae innoculum.

2.5.2. Aeration

For aeration, air pump „JDK-S-100“ was used (Figure 11). At the beginning of the air tube, an air valve was placed to control air flow to every Erlenmeyer flask. Every air tube had an air filter Whatman that had size of 0,2 μm (Figure 12) before being connected to a glass pipette placed into the Erlenmeyer flasks. The glass pipette went through tin foil and a plug made from cotton wool. The plugs were sterilized before use in an autoclave (Melag Sterilizator 75) at 121°C.

Figure 11. Air pump „JDK-S-100“ used for aeration of the microalgae cultures (source: M. Prečanica).

Figure 12. Whatman filter used for air filtration before entering Erlenmeyer flasks (source: M. Prečanica).

2.5.3. Lighting and temperature

Temperature was maintained at 24°C with AC in a closed room. For every experimental group one white LED light (LED light 36W, 120 cm, 3000 LM, IP65) was used. Lights were placed horizontally 10 cm from E. flasks (Figure 13), not above so they can cover more surface and be more effective. LED lights produced an average of 4322 lux at adistance of 10 cm.

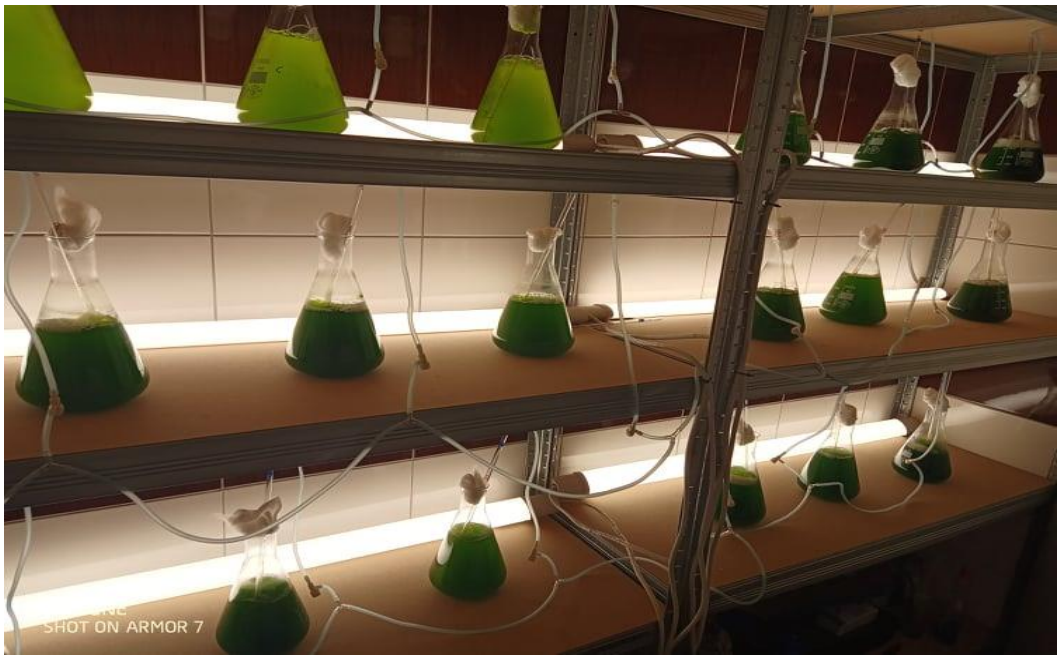

Figure 13. LED light that are used for experiment (source: M. Prečanica).

2.5.4. Counting microalgae cells

Counting was done every day with a cell counting chamber (Neubauer, improved, Blaubrand®, Germany, Figure 14). Samples were taken with a new sterile pipette to avoid contamination. Concentration of microlgae was measured as average number from all three replica in each group. Concentration of microalgae was measured in all three replica, from which average concentration was calculated.

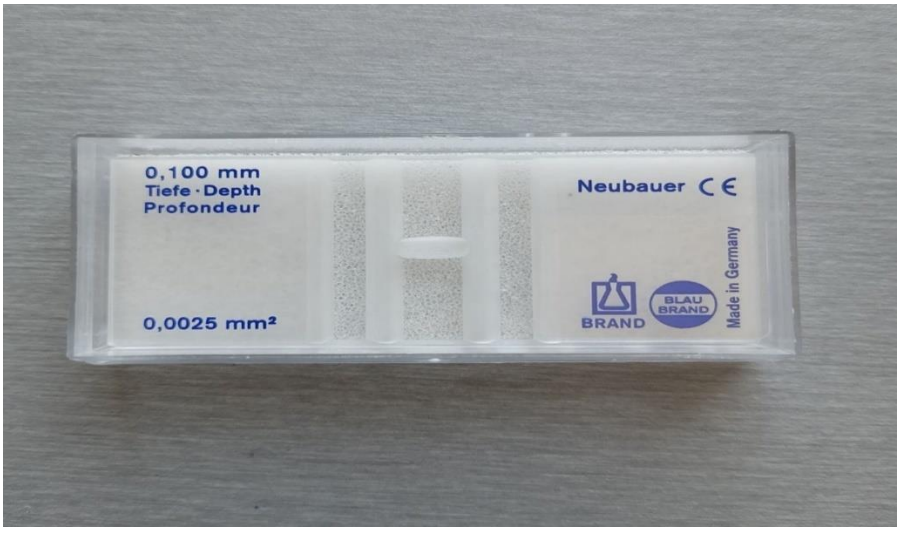

Figure 14. Neubauer chamber for counting algae cells (source: M. Prečanica).

2.6. Measuring concentrations of nitrogen compounds and total phosphorus in microalgae cultures

2.6.1. Nitrogen compounds

Concentrations of nitrogen compounds were measured every third day. The Nessler method (Herbert *et al*., 1971) was used to determine ammonia, nitrite, and nitrate levels in all groups (Figure 15) using a Helion Epsilon spectrophotometer (Thermo Fisher Scientific). Concentration of nitrogen compounds were measured in two replicas (out of the total three), from which final average concentration was calculated.

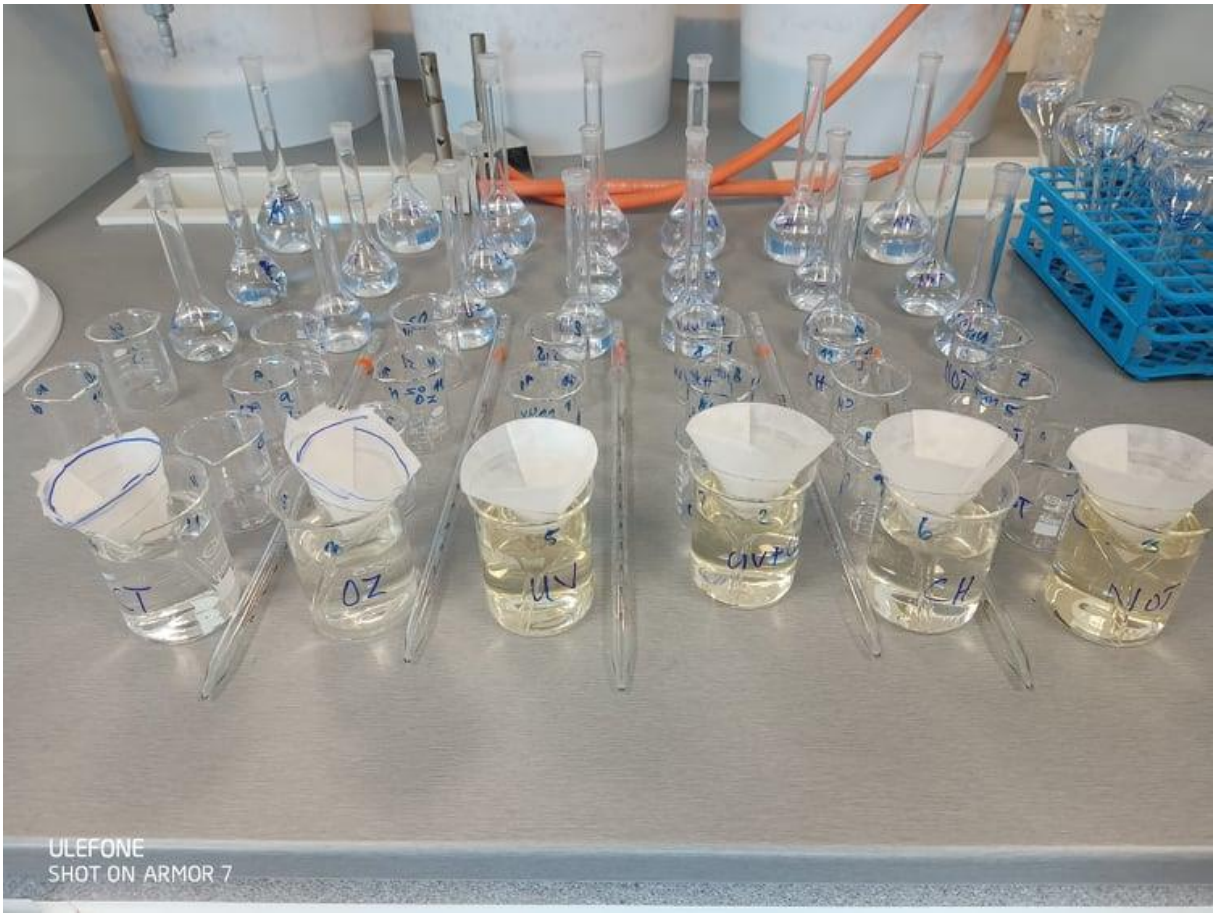

Figure 15. Measuring nitrogen levels using the Nessler method (source: M. Prečanica).

2.6.2 .Total phosphorus

Total phosphorus levels were measured every third day using a Helion Epsilon spectrofotometer (Thermo Fisher Scientific) after reaction with molybdate and reduction with ascorbic acid (APHA, 1995). Concentration of total phosphorus was measured in two replicas (out of the total three), from which final average concentration was calculated.

3. RESULTS

3.1. Nutrient concentrations in the wastewater mineralization container

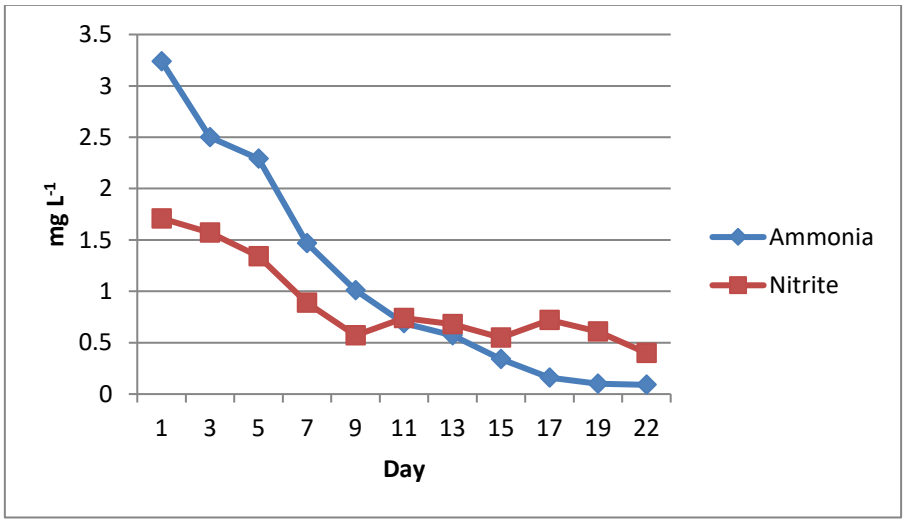

Figure 16. Concentration of ammonia and nitrite of wastewater during mineralization process.

At the beginning of the mineralization of fish wastewater, ammonia concentration significantly decreased from 3,4 to 0,09 mg/L (Figure 16). Nitrites concentration also reduced, from 1,71 mg/L to 0,4 mg/L during the mineralization process.

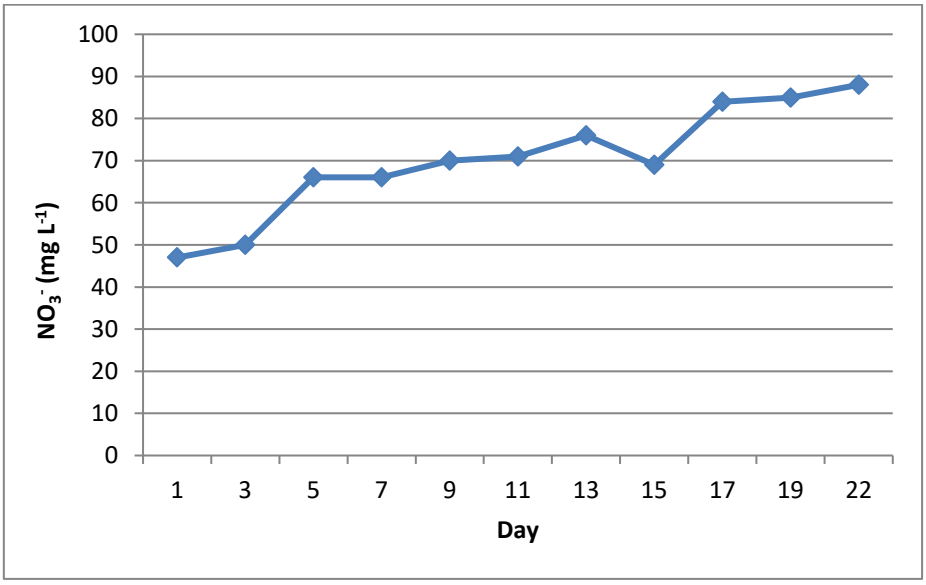

Figure 17. Concentration of nitrates in wastewater during mineralization process.

At the beginning of the mineralization of wastewater, nitrate concentration was 47 mg/L and slowly increased to 88 mg/L during the three week mineralization process (Figure 17).

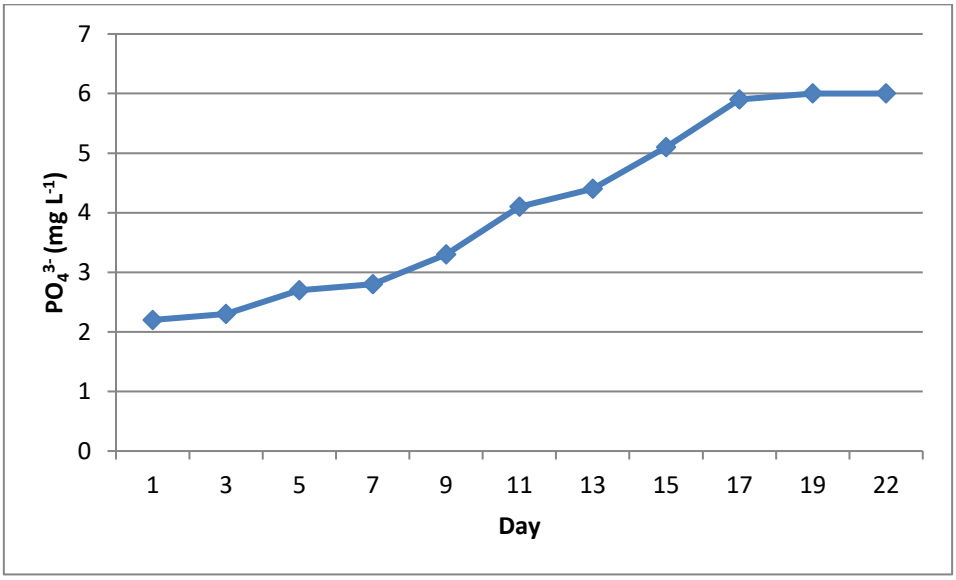

Figure 18. Concentration of phosphates in wastewater during mineralization process.

At the beginning of mineralization, phosphate concentration in wastewater was 2,2 mg/L (Figure 18). After three weeks of the mineralization process, it slowly increased to 6 mg/L and remained at this value for the final six days of mineralization.

3.2. *C. vulgaris* growth

All groups started with an equal concentration of *C. vulgaris* at 640 cells/mL (Figure 19). After seven days, higher growth began to be observed in the Control group when compared to other groups. It reached its peak concentration of 3557 ± 116 cell/mL on the tenth day. All experimental groups followed a similar growth dynamic to the Control group in all stages of growth, but did not exceed 2875 ± 306 cell/mL at peak concentration. The Mechanical group had lowest concentration of microalgae during whole experiment.

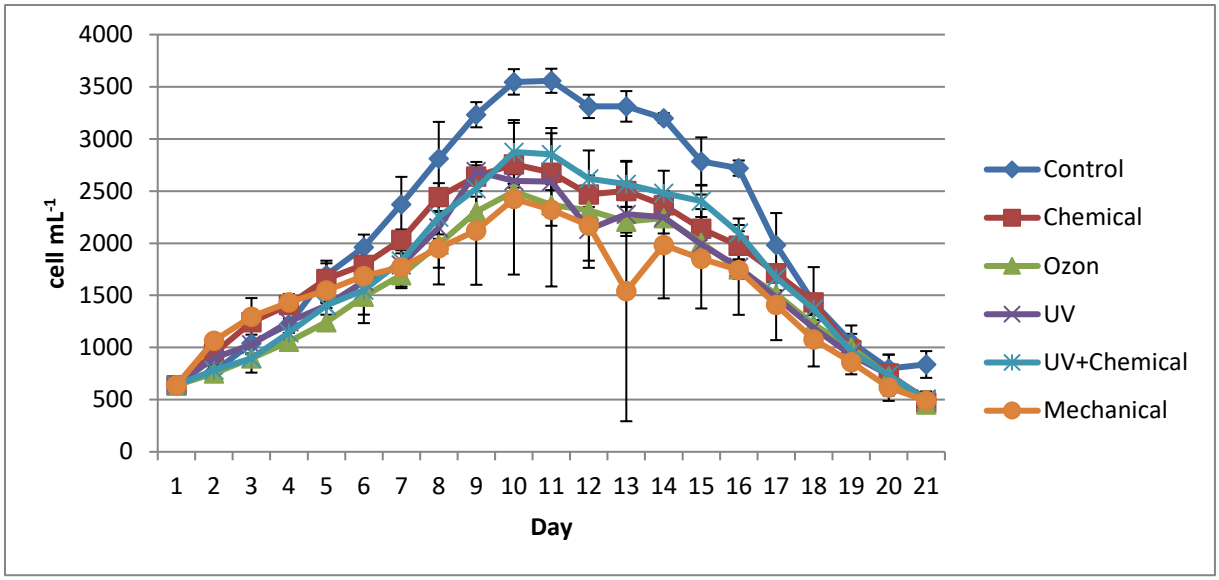

Figure 19. Concentration of *C. vulgaris* cells in all experimental groups during microalgae culture. Values are means ± SD (n = 3).

3.3. Nutrient concentrations in the microalgae cultures

3.3.1. Ammonia concentration

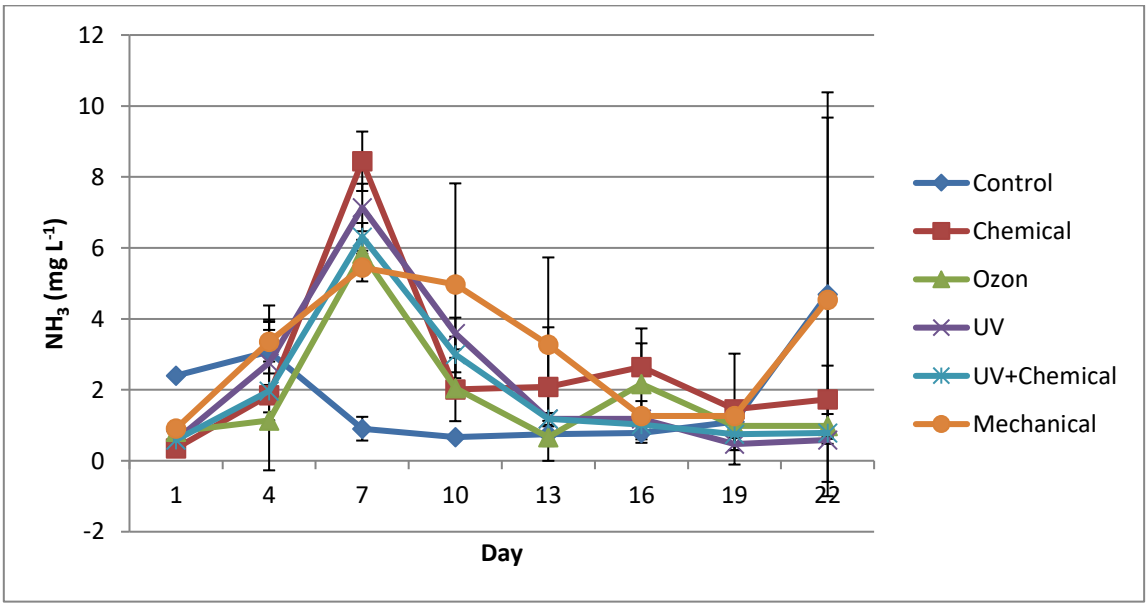

Figure 20. Concentration of ammonia in experimental groups during microalgae culture. Values are means ± SD (n = 2).

Initial concentrations of ammonia after disinfection of the mineralized wastewater did not differ greatly between the experimental treatment groups. The Mechanical group had somewhat higher levels of ammonia (0,91 mg/L) compared to the remaining treatments, while the Chemical group had the lowest values of 0,34 mg/L.

All experimental groups, except the Control group, had an increase of ammonia concentration during the first few days of the algae culture which peaked on the seventh day (Figure 20). However, values never exceeded 8,44 ± 0,84 mg/L. In the Control group, ammonia concentration dropped rapidly after reaching 3,07 ± 0,61 mg/L on the fourth day and remained low until the end of the experiment. The remaining groups had an even more pronounced drop after peaking on the seventh day, with the exception of the Mechanical group where ammonia concentration increased during the final last days, but did not exceed 4,69 mg/L.

3.3.2. Nitrite concentration

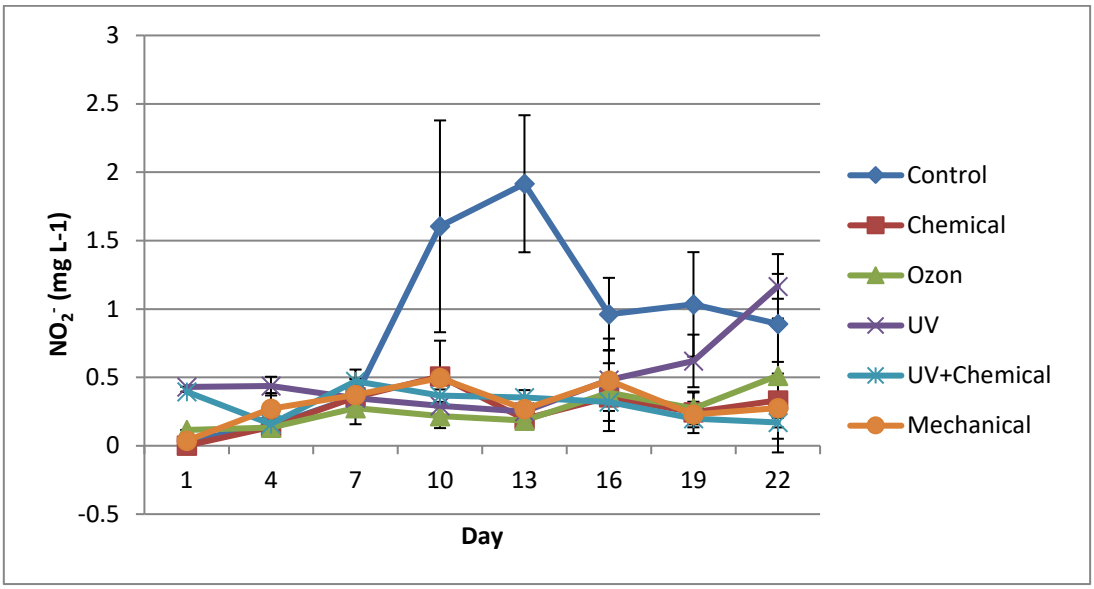

Figure 21. Concentration of nitrites in experimental groups during microalgae culture. Values are means ± SD (n = 2).

Initial concentrations of nitrites were very low, not exceeding 0,43 mg/L, but did differ somewhat between experimental treatment groups (Figure 21). UV and UV+Chemical group had higher levels of nitrites (0,43 mg/L and 0,39 mg/L) compared to the remaining treatments, while the Chemical and Control group had the lowest values of 0,001 mg/L. The Ozone and Mechanical group had similar concentration of nitrites of 0,16 mg/L and 0,04 mg/L,

respectively. Low concentrations of nitrates remained low in all experimental groups untill the end of experiment. The exception was the Control group in which concentrations increased after the seventh day, peaked at 1,92 ± 0,50 mg/L on day 13th and decreased towards the end of the experiment, but never dropped to the level of the remaining groups. Towards the end of the experiment, nitrite concentrations in the UV group started to increase and reached 0,53 ± 0,24 mg/L.

3.3.3. Nitrate concentration

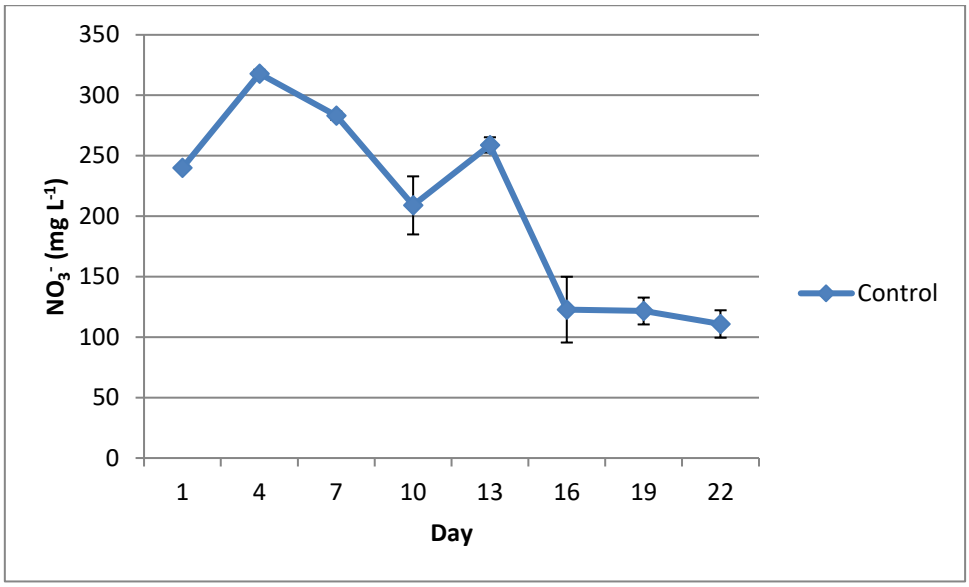

Figure 22. Concentration of nitrates in Control group during microalgae culture. Values are means ± SD (n = 2).

Initial concentrations of nitrates differed between experimental treatment groups after disinfection (Figure 23). The Control group had much higher initial levels of nitrites of 240 mg/L compared to the remaining treatments. Among the experimental groups, the UV group had highest initial concentration of nitrates (122 mg/L), while the Chemical and Mechanical group had the lowest values of 97 mg/L and 99 mg/L, respectively. Ozone and UV+Chemical groups had intermediate concentration of nitrates of 116 mg/L and 109 mg/L, respectively.

The Control group had highest overall levels of nitrates which reached 318 ± 3mg/L on day six, after which they dropped down towards the end of experiment (Figure 22). All other groups followed a similar trend, but having lower starting and overall concentration of nitrates, which did not exceed 122 mg/L on the first day.

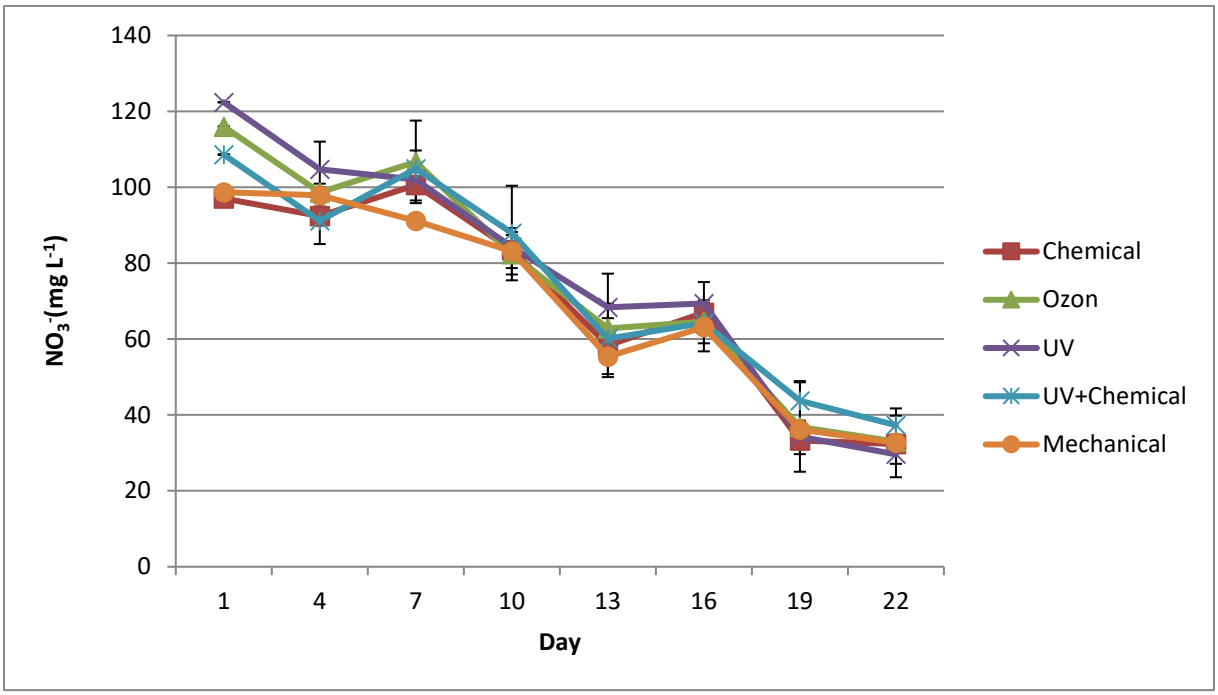

Figure 23. Concentration of nitrates in experimental groups during microalgae culture. Values are means ± SD (n = 2).

3.3.4. Phosphorus concentration

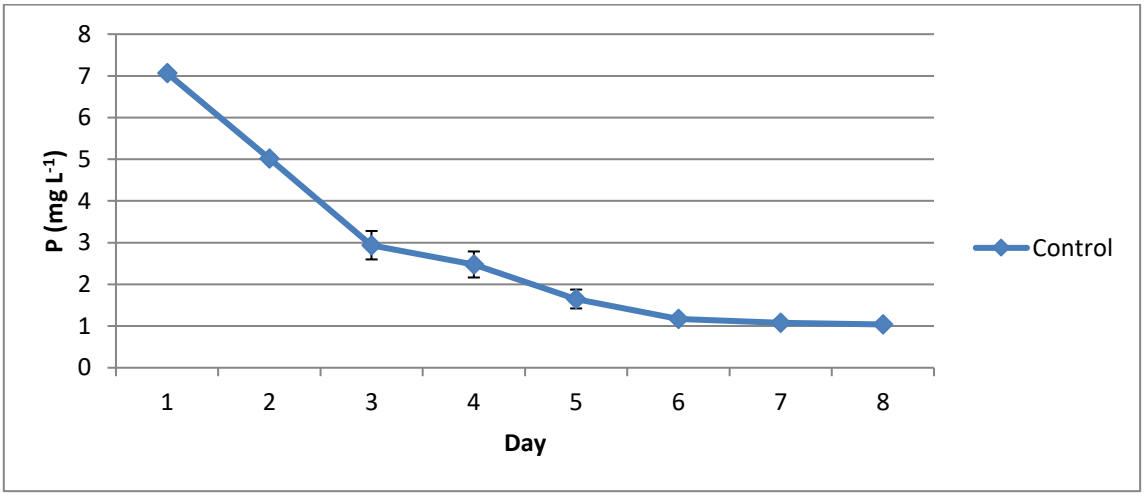

Figure 24. Total phosphorus concentration in Control group during microalgae culture. Values are means ± SD (n = 2).

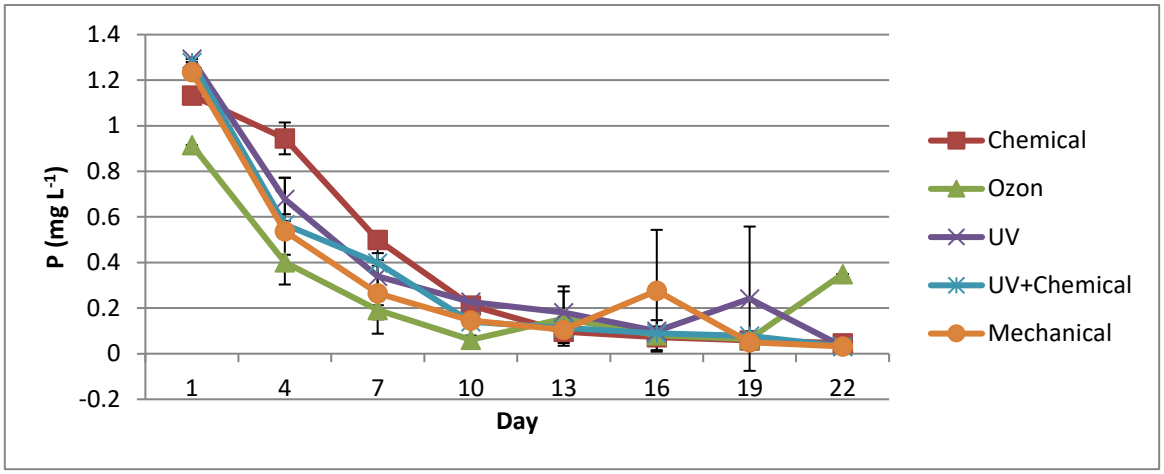

Figure 25. Total phosphorus concentration in experimental groups during microalgae culture. Values are means ± SD (n = 2).

Initial concentrations of phosphorus after disinfection methods were lower in the Ozone group (0,02 mg/L) when compared to the other treatments, while the highest values were observed in the UV+Chemical group (1,28 mg/L) and UV group (1,29 mg/L) (Figure 25).

The Control group had much higher overall phosphorus levels as it used the Bg11 culture medium instead of wastewater, but these decreased from 7,1 mg/L to 1,04 mg/L towards the end of the experiment (Figure 24). The experimental groups started with values similar to those with which the Control group finished (under 1,3 mg/L), but also followed a similar trend during the course of the experiment (Figure 25). In the last days of the experiment, levels of phosphorus oscillated in the Mechanical, UV and Ozone groups, but did not not exceed 0,35 mg/L.

4. DISCUSSION

In this experiment, aerobic mineralization was performed to remove harmful components of wastewater, such as ammonium, and to break down solid waste to molecular and atomic parts with the help of aerobic heterotrophic bacteria.This process brakes down solid waste into nutrients that could be used by microalgae for growth instead of being released into nature where they could potentially be harmful to the environment (Lennard, 2017). During the mineralization process, phosphate levels increased from 2,2 mg/L to 6,0mg/L and nitrates from 47mg/L to 88mg/L, while ammonia and nitrite concentration were almost completely reduced. Ammonia levels were reduced from 3,4 to 0,09 mg/L, and nitrite levels from 1,1mg/L to 0,4 mg/L. These results coincide with those of other studies (Delaide *et al*., 2019) and confirm the successful completion of aerobic mineralization.

High levels of nitrates are important because this form of nitrogen is better used by microalgae and that means there will be better removal and absorption of nitrogen from the water (Markou *et al*., 2014). Phosphates are also important for microalgae growth, however, both total phosphorous and nitrates were still several fold lower after the mineralization process than what they would be in a commercial culture medium, such as what was used in the control group.

In the scope of this study, the efficiency of different methods of wastewater disinfection was analyzed which would ultimately have an effect on the growth of *C. vulgaris*. In addition, the removal of nutrients by microalgae from the culture water was evaluated. The potential effect of these disinfection treatments could have on the availability of nitrogen and phosphorus in the water was also explored. Tejido-Nuñez (2019) have already observed that *C. Vulgaris* is very efficient at removing nitrogen from wastewater, but that the presence of other microorganisms in the culture medium could significantly lower microalgae performance.

4.1. Nutrient levels after different disinfection methods

Results showed different concentrations of ammonia, nitrite, nitrate and phosphorus depending on the disinfection method used, which showed that levels of individual nutrients in mineralized wastewater were indeed affected by the method of disinfection.

It was observed that ammonia concentrations were highest when no disinfection methods were used (0,91 mg/L), although these values did not differ greatly from the other experimental groups. This is surprising as a stronger potential of ozone for ammonia removal

has previously been reported (Singer & Zilli, 1975). However, these differences may have been due to differences in other water parameters between the studies, like alkalinity, which seemed to affect the interaction of ozone and ammonia (Singer & Zilli, 1975). The lowest values of ammonia were observed in treatments where chlorine was used; 0,35 mg/L, and 0,59 mg/L in Chemical and UV+Chemical groups, respectively when in combination with active chlorine. Considering that ammonia levels were already quite low at the end of mineralization, even these slight drops in concentration could be explained by the formation of chloramines as a byproduct of disinfection using chlorine based disinfectants in water sources (Kim *et. al*., 2003). It is important to note that chloramines were not registered by the method of analysis used in this study, but can still pose a threat to living organisms. On the other hand, the success of ammonia removal using UV in combination with active chlorine has also previously been confirmed (Zhang *et al*., 2015).

Similarly to ammonia, nitrite levels were very low at the start of the experiment, due to the activity of nitrifying bacteria during the mineralization process (Lekang, 2007). Active chlorine gave the lowest concentration of nitrites 0,001 mg/L, similar to what was observed by Yang and Cheng (2007), although the Mechanical group and group where ozone were aplied also showed low levels at 0,04 mg/L and 0,16 mg/L, respectively.

Nitrate concentration is probably the most important for microalgae growth when considering nitrogen sources because nitrates are better absorbed and used by microalgae than ammonium and nitrite (Markou, 2014). After mineralization, most of nitrogen is in nitrate form, as was the case in the current study. Nitrates in wastewater were present in much higher quantities than the remaining nitrogen compounds due nitrification processes during mineralization. It is important that the disinfection method itself doesn't change these ratios as microalgae are most effective at utilizing nitrates, and so removing them from wastewater. Chemical and Mechanical groups showed the lowest concentration of nitrates of 97 mg/L and 98 mg/L, respectively. Lekang (2007) stated that if using ozone as disinfection method in aquaculture there is a high possibility that water can get saturated with nitrogen, which could have been the reason for the high (116 mg/L) level of nitrates when ozone was applied to our wastewater, second only to the highest level of nitrates observed after UV treatment. Liu *et al*. (2020) observed that higher levels of nitrites and nitrates are present when water is treated with UV light. Accordingly, in this experiment it was noticed that UV in combination with active chlorine resulted in 109 mg/L and that the UV method resulted in 122 mg/L of nitrates in water, which was higher than in groups where water was treated with chlorine and where there was no disinfection treatment.

Phosphorous levels were technically highest when the UV method was used, alone (1,29 mg/L) and in combination with chlorine (1,28 mg/L), but this did not seem to be much higher than in the Mechanical group (1,24 mg/L) where no disinfection was performed. These results coincide with previous observations that total phosphorus levels do not change significally after treatment with UV (Lehtola *et al*., 2003). On the other hand, use of ozone - one of the most common methods of disinfection of water for aquacutlure purposes – caused phosphorus levels to drop to 0,92 mg/L, which was lowest among all experimental groups. Conversely, Lehtola *et al*. (2001) observed that treatment with ozone resulted in higher levels of phosphorus in water.

4.2. Nutrient levels during culture of *C. vulgaris*

All groups showed similarly low levels of ammonia at the end of the experiment except for the Mechanical group. Two culture replicas from this group started to crash earlier than the third and earlier than the other groups, which may have caused an ammonia increase because of large quantities of dead *C. vulgaris* and accompanying zooplankton that died after consumption of the remaining *C. vulgaris*.

Nitrite concentrations were generally very low throughout the experiment, although some different trends were visible. In the control group the concentration of nitrites increased from very low to highest among all groups, reaching 1,91 ± 0,64 mg/L, possibly because the number of dead microalgae started to increase as the end of the experiment was approaching. However, it seems that this did not cause any negative effects on overall microalgae growth. The UV group also had an increase of nitrite concentration to 1,17 ± 0,37 mg/Ld uring the culture's last days, while the groups had similarly levels of nitrites, below 0,5 mg/L, at the end of the experiment.

At the end of the experiment, all groups showed a significant and constant drop of nitrate and phosphorous concentration from the beginning ofthe experiment to the end of the experiment which showed that *C. vulgaris* was very efficient regarding absorption of these nutrients. An important note is that most of the nitrogen was in nitrate form, which increased the efficiency with which the microalgae could remove it from the wastewater.

4.3. Growth of *C. vulgaris*

The Control group that was grown using the Bg11 culture medium had best results, peaking at 3557 ± 116 cell/mL and also had a higher number of microalgae in all phases of cultivation, except the very start when an equal number of cells was introduced to each flask. The likely reason is that in all groups when compared to the control group, phosphorus and nitrogen levels were much lower in concentration, meaning thecultures could not follow growth of *C. vulgaris* in the control group. Excluding the control group, all experimental groups had similar growth dynamics likely because they had similar phosphorus and nitrogen levels after the mineralization and disinfection treatments. Only one group showed lowest growth, peaking at only 2426 ± 727 cell/mL, and that was the Mechanical group where no treatment for disinfection was used. This resulted in protozoa contamination that likely slowed the growth of *C. vulgaris* towards the end of experiment causing earlier crashes.

Two replicas of the Mechanical group started to crash early on in the experiment, again likely due to contamination with protozoa, but the third replica had great results, almost comparable with the Control group, placing it above the other groups in terms of microalgae growth. Given these results, it might seem that if *C. vulgaris* reaches high enough densities it can out compete remaining microorganisms in the culture, but seeing as two of the three replicas crashed prematurely this would be very risky. Without proper disinfection of the medium it is very hard to assess how the population of microalgae will behave in a given environment, especially when considering wastewater and the large number of microorganisms assosiated with it (Tejido-Nuñez, 2019). Moreover, it has previously been confirmed that *C. vulgaris* is very vulnerable to protozoa (Abou-Shanab *et al*., 2016).

UV, UV+Chemical, Ozone and Chemical groups showed very similar results regarding the growth of *C. vulgaris*, although UV+Chemical treatment showed slightly better and more stable growth, which could be contributed to a combination of two treatment methods and better quality of water in terms of disinfection.

When compared with the Control group, which had optimal conditions for *C. vulgaris* growth, all other groups showed satisfyactory results. The main limiting factor seemed to be the quantity of nutrients available in wastewater that was lower than in the Control group. However, from an economic and environmetal standpoint the use of fish wastewater could potentially be more sustainable and acceptable for cultivation of large quantities of microalgae because nutrients that are used here are basically considered as waste. When comparing with the high prices of microalgae culture mediums, this almost free source of nutrients, even at

lower performance of microalgae cultures, would probably result in a more economic viable production, while reducing the negative environmental impacts of RAS wastewater.

5. CONCLUSION

Aerobic mineralization helps in increasing nitrogen (especially in nitrate form) and phosphates in aquaculture wastewater, which can be used as nutrients for microalgae growth.

In the Mechanical group, where only mechanical filtration was applied without disinfection, growth of *C. vulgaris* was lowest, due to contamination with protozoans. This suggests a form of disinfection must be used before mineralized wastewater can be used to culture microalgae.

Treatment of mineralized wastewater with ozone and UV showed highest levels of nitrates, when treating with UV in combination with chlorine, the highest growth of *C. vulgaris* was observed, as well as highest levels of phosphates, compared with other disinfection methods. This suggests phosphates could be the limiting nutrient in *C. vulgaris* growth and for best culture performance of microalgae disinfection using UV in combination with chlorine should be used.

Cultivation of microalgae in fish wastewater results in usage of free nutrients that are in wastewater and offers an elegant soluting for wastewater treatment. This combination has big potential in economic profitability.

6. REFERENCES

Abou-Shanab R .A. I., Singh M., Rivera-Cruz A., Power G., Bagby-Moon T., Das K. (2016). Effect of *Brachionus rubens* on the growth characteristics of various species of microalgae. Electron. J. Biotechnol., 22, pp. 68-74.

Ahluwalia, S. S., Goyal, D. (2007). Microbial and plant derived biomass for removal of heavy metals from wastewater. Bioresour. Technol., 98, pp. 2243-2257.

Allen, M.M. (1968). Simple conditions for growth of unicellular blue-green algae. J. Gen. Microbiol. 51: 199 - 202.

Allen, M. M., and Stainer, R.Y. (1968). Studies with *Cyanidium caldarium*, an anomalously pigmented chlorophyte. Arch. Mikrobiol. 32: 270-277.

American Public Health Association (1995). Standard Methods for the Examination of Water and Wastewater, 19th Edition, Water Environment Federation, American Water Works Association.

Ansari, F. A., Singh, P., Guldhe, A., & Bux, F. (2017). Microalgal cultivation using aquaculture wastewater: Integrated biomass generation and nutrient remediation. Algal Research, 21, 169–177.

Aslan, S., & Kapdan, I. K. (2006). Batch kinetics of Nitrogen and Phosphorus removal from synthetic wastewater by algae. Ecological Engineering, 28(1), 64–70.

Brennan, L., Owende, P. (2010). Biofuels from microalgae – a review of technologies for production, processing, and extractions of biofuels and co-products. Renew Sustain Energy Rev;14:557–77.

Brugére, C. and Ridler, N. (2004). Global Aquaculture Outlook in the Next Decades: An Analysis of National Aquaculture Production Forecasts to 2030.

Calderon, R. L. (2000). "The Epidemiology of Chemical Contaminants of Drinking Water". Food and Chemical Toxicology. 38 (1 Suppl): S13–S20.

Chen, S., Stechey, D., Malone, R. F. (1994). Suspended solids control in recirculating aquaculture systems. In: Aquaculture water reuse systems, engineering design and management (eds Timmons, M. B., Losordo, T. M.). Elsevier Science.

Chisti, Y. (2007). Biodiesel from microalgae. Biotechnol Adv, 25, pp. 294-306.

Chisti, Y. (2008). Biodiesel from microalgae beats bioethanol. Trends Biotechnol, 26, pp. 126-131.

Delaide B., Monsees H., Gross A., Goddek S. (2019). Aerobic and Anaerobic Treatments for Aquaponic Sludge Reduction and Mineralisation. In: Goddek S., Joyce A., Kotzen B., Burnell G. (eds) Aquaponics Food Production Systems. Springer, Cham.

FAO. (1996). Manual on the production and use of live food for aquaculture. Rome, str. 295.

FAO. (2014). State of the World Fisheries and Aquaculture 2014. Food and Agriculture Organization, Rome.

FAO. (2009). The state of world fisheries and aquaculture 2008. Rome, FAO: 76 pp.

FAO. (1992). Wastewater treatment and use in agriculture. Food and Agriculture Organization, Rome.

González L. E., Cañizares R. O., Baena S. (1997). Efficiency of ammonia and phosphorus removal from a colombian agroindustrial wastewater by the microalgae *Chlorella vulgaris* and *Scenedesmus dimorphus*. Bioresour Technol;60: 259–62.

Herbert, D., Phipps, P. J., & Strange, R. E. (1971). Chapter III Chemical Analysis of Microbial Cells. Methods in Microbiology, 209–344.

Kim J., Chung Y., Shin D., Kim M., Lee Y., Lim Y., Lee D. (2003). Chlorination by-products in surface water treatment process Desalination, 151 (1), pp. 1-9.

Knud-Hansen C. F., McElwee K., Baker J., (1998). Clair Pond fertilization: ecological approach and practical application Pond Dynamics. Aquaculture Collaborative Research Support Program, Oregon State University.

Lehtola, M. (2001). Microbially available organic carbon, phosphorus, and microbial growth in ozonated drinking water. Water Research, 35(7), 1635–1640.

Lehtola, M. J., Miettinen, I. T., Vartiainen, T., Rantakokko, P., Hirvonen, A., & Martikainen, P. J. (2003). Impact of UV disinfection on microbially available phosphorus, organic carbon, and microbial growth in drinking water. Water Research, 37(5), 1064–1070.

Lekang, O. I. (2007). Aquaculture Engineering, 3rd ed. Blackwell Publishing, Oxford.

Lennard W. (2017). Commercial Aquaponic Systems: Integrating Recirculating Fish Culture with Hydroponic Plant Production.

Liltvedt, H., Hansen, B. R. (1990). Screening as a method for removal of parasites from inlet water to fish farms. Aquacultural Engineering, 9: 209–215.

Liu, Z., Xu, B., Lin, Y. L., Zhang, T. Y., Ye, T., Hu, C. Y., Gao, N. Y. (2020). Mechanistic study on chlorine/nitrogen transformation and disinfection by-product generation in a UV-activated mixed chlorine/chloramines system. Water Research, 184:116116.

Markou G., Vandamme D., Muylaert K. (2014). Microalgal and cyanobacterial cultivation: The supply of nutrients. Water Res., 65, pp. 186-202.

Martin, C., de la Noüe, J., & Picard, G. (1985). Intensive cultivation of freshwater microalgae on aerated pig manure. Biomass, 7(4), 245–259.

Singer, P. C., & Zilli, W. B. (1975). Ozonation of ammonia in wastewater. Water Research, 9(2), 127–134.

Solomon E. P., Berg L. R., Martin D. W. (1999). Biology. 5th ed. Fort Worth: Saunders College Publishing.

Tejido-Nuñez, Y., Aymerich, E., Sancho, L., & Refardt, D. (2019). Treatment of aquaculture effluent with Chlorella vulgaris and Tetradesmus obliquus: The effect of pretreatment on microalgae growth and nutrient removal efficiency. Ecological Engineering, 136, 1–9.

Van Rijn, J. (2013). Waste treatment in recirculating aquaculture systems. Aquacultural Engineering, 53, 49–56.

Vasudevan, P. T., Briggs, M. (2008). Biodiesel production - current state of the art and challenges. J Ind Microbiol Biotechnol 35, 421.

Yang, H., Cheng, H. (2007). Controlling nitrite level in drinking water by chlorination and chloramination. Separation and Purification Technology, 56(3), 392–396.

Yamamoto M, Fujishita M, Hirata A, Kawano S. (2004). Regeneration and maturation of daughter cell walls in the autospore-forming green alga Chlorella vulgaris (Chlorophyta, Trebouxiophyceae). J Plant Res;117:257–64.

Němcová, Y., Kalina, T. (2000). Cell wall development, microfibril and pyrenoid structure in type strains of *Chlorella vulgaris*, *C. kessleri*, *C. sorokiniana* compared with *C. luteoviridis* (*Trebouxiophyceae, Chlorophyta*). Arch Hydrobiol;100:95–105.

Zhang, X., Li, W., Blatchley, E. R., Wang, X., & Ren, P. (2015). UV/chlorine process for ammonia removal and disinfection by-product reduction: Comparison with chlorination. Water Research, 68, 804–811.

Zheng, H., Yin, J., Gao, Z., Huang, H., Ji, X., Dou, C. (2011). Disruption of Chlorella vulgaris cells for the release of biodiesel-producing lipids: a comparison of grinding, ultrasonication, bead milling, enzymatic lysis, and microwaves. Appl Biochem Biotechnol;164:1215–24.

Internet links:

1) https://www.spartanwatertreatment.com/how-much-ozone-do-i-need-to-treat-water/ (13.9.2020.).

IZJAVA

S punom odgovornošću izjavljujem da sam diplomski rad izradio samostalno, služeći se navedenim izvorima podataka i uz stručno vodstvo mentora Krune Bonačića.

Mario Prečanica

Potpis: