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NUTRIENT REMOVAL FROM WASTEWATER BY MICROALGAE CHLORELLA VULGARIS

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Abstract: In Europe, 2.52 kg nitrogen and 0.51 kg phosphorous / inhabitant / year are discharged in wastewater. Conventional biological and chemical methods for wastewater treatment are costly and not fully efficient in removing nutrients, and the discharge of effluents in rivers and lakes enhances the eutrophication. The use of microalgae species to remove nutrients available in wastewater, as a sustainable and low-cost treatment option, and also the use of different systems for algal cultivation in wastewater, aiming to obtain biomass and bio-products, is a growing field of research. Green microalgae, especially Chlorella species, play an important role in wastewater treatment while producing algal biomass, with numerous studies proving the high potential of Chlorella to consume nutrients from different categories of wastewater: municipal wastewater, wastewater from agriculture, wastewater from zootechnics, industrial wastewater. Cultivated in autotrophic, heterotrophic or mixotrophic conditions, in open or closed systems, microalgae have an important role in reducing greenhouse gases, consuming 1.83 kg CO_2 / kg dry algal biomass. Algal biomass can be capitalized to obtain many value-added products, including biofuels. The aim of this study is to review the advances in the utilization of microalgae Chlorella vulgaris in the treatment of municipal and zootechnical wastewater, emphasizing the removal of nutrients (nitrogen and phosphorus).

Keywords: wastewater, nutrients, biomass, microalgae, Chlorella

INTRODUCTION

Depending on their origin, municipal, agricultural, and industrial wastewaters contain variable amounts of organic matter, nutrients, heavy metals, pharmaceuticals and other emerging contaminants, detergents, greases, oils, microplastics, insecticides, fungicides, herbicides, sulfates, phosphates, fluorides, chlorides and a variety of pathogens.

Nutrients (nitrogen, phosphorus and other minerals) are the main elements for eutrophication of natural water bodies (lakes), which manifests by oxygen depletion, harmful microalgal blooms and disturbances in the balance of the ecosystem. Nutrients discharged from wastewater are getting increased attention and have been strictly regulated worldwide. Even if it has low concentrations, the ammonia in wastewater effluents can be toxic to aquatic organisms and fish. Furthermore, the phosphate and ammonia may cause serious health issues in humans, such as methaemoglobinaemia in new-borns (Yamashita et al., 2014).

The nitrogen in wastewater is primarily due to the metabolic interconversions of extra derived compounds, and over 50 % of phosphorus comes from synthetic detergents (Abdel-Raouf et al., 2012) used in households and different industrial activities. Nutrient concentrations and availability vary across the wide types of wastewater. For example, total nitrogen and phosphorous concentrations can reach values of 10–100 mg/L in municipal wastewater, more than 1000 mg/L in agricultural wastewater and 500–600 mg/L in zootechnical wastewater (Cheunbarn and Peerapornpisal, 2010). In Europe, on average, 2.52 kg nitrogen and 0.51 kg phosphorous / inhabitant / year are discharged in wastewater (EU-EEA, 2015). The European Directive 6498/15/EC establishes a limit of 10 mg/L for total nitrogen and 1 mg/L total phosphorous contained in effluents before discharge in natural waters. However, the effluents from wastewater treatment plants have much higher values: 20–70 mg/L nitrogen and 4–12 mg/L phosphorous (Rinna et al., 2017). Hence, wastewater should not be discharged unless proper treatment. If not recycled, the nitrogen in wastewater is lost as N_2 or as important greenhouse gases such as N_2O, which can contribute up to 78 % of the carbon footprint of a conventional wastewater treatment plant (Vasilaki et al., 2018).

The main forms in which they occur in wastewater are NH_4^+ (ammonia), NH_2^- (nitrite), NO_3^- (nitrate) and PO_4^{3-} (orthophosphate). In the secondary effluent, the concentration of ammonia is between 40-48 mg NH_4^+ - N/L, and the concentration of phosphorous is 9–12 mg PO_4^- - P/L (Martinez et al., 2000).

Nitrogen is conventionally removed through biological nitrification and denitrification using aerobic and anoxic reactors, or by the application of dual sludge treatment, while phosphorus is removed by coupling anaerobic and aerobic reactors. The disadvantages of biological methods are the need of aeration, high energy demand and carbon footprint. Chemical methods like chemical precipitation, ion exchange and adsorbtion, or electrochemical methods like electrooxidation, are also used in wastewater treatment plants to remove the nutrients, but these methods have the disadvantage of generating high amounts of sludge from chemical reactions and precipitates. Conventional treatment of municipal wastewater (primary settling and biological treatment) could remove 40 % of total nitrogen and 12% of total phosphorus (Rinna et al., 2017). Overall, regardless their category, the conventional methods for nutrients removal include many steps, with complicated operation for which are required skilled personnel, are energy intensive, produce large quantities of sludge and have high costs.

With the new construction and upgrade of wastewater treatment plants and stricter disposal policies, alternative and sustainable approaches are needed for treating the ever increasing volumes of wastewater.

MATERIALS AND METHODS

Bio-treatment, bioremediation or phycoremediation of domestic, agricultural and industrial wastewater involves the use of microalgae and has attracted increasing attention in recent years.

Microalgae have the ability to adjust in adverse conditions; they grow naturally in many types of wastewater thriving on nutrients and forming an abundant resource of biomass. Microalgae have a rapid growth-rate (12 days), their doubling time during exponential growth are only 3.5 hours (Ungureanu et al., 2020) and can double their biomass within 24 hours. During their growth, algal cells might accumulate high amount of lipids and carbohydrates, which makes them an important feedstock for biofuel production.

Because of their photosynthetic capabilities, they convert the solar energy into abundant useful biomass. Microalgae also play an important role in CO_2 sequestration, because the CO_2 needed for their photosynthetic metabolism can be provided from industrial flue gases. When CO_2 from flue gases is consumed during microalgae growth, this leads to a decrease of greenhouse gas emission to the atmosphere simultaneously with carbon fixation. It was estimated that 1 kg of dry algal biomass consumes about 1.83 kg of CO_2 (Rosenberg et al., 2011). Microalgae have carbon fixation rates much higher than those of land-based plants by one order of magnitude (Kumar et al., 2011).

In addition to CO_2 mitigation, microalgae produce oxygen as a byproduct of photosynthesis, which can be used by aerobic bacteria to decompose biologically the organic matter in the wastewater. Thus, microalgae can help to reduce the need for mechanical aeration during wastewater treatment (Otondo et al., 2018).

Microalgae cultivation can take place in photoautotrophic, mixotrophic, or heterotrophic conditions (Figure 1). As an emerging wastewater treatment method, microalgae treatment provides an alternative and sustainable pathway for the removal (uptake) of the inorganic nitrogen and phosphorous from wastewater. Sunlight or UV light, pH, temperature, conductivity and water salinity are parameters for controlling and adjusting the process of microalgae cultivation (Nedelcu et al., 2019). Many other factors including nutrients, the concentration of organics and metal ions, or trace elements such as Mn, Fe, Zn and Cu, can influence the microalgal growth in wastewater.

Microalgae either grow naturally or are cultivated in open systems like wastewater lagoons, shallow algal ponds, high rate algal ponds, raceway ponds, constructed wetlands, and these require large areas with considerable light exposition. About 90 % of the global algae production is conducted in the open systems (Placzek et al., 2017).

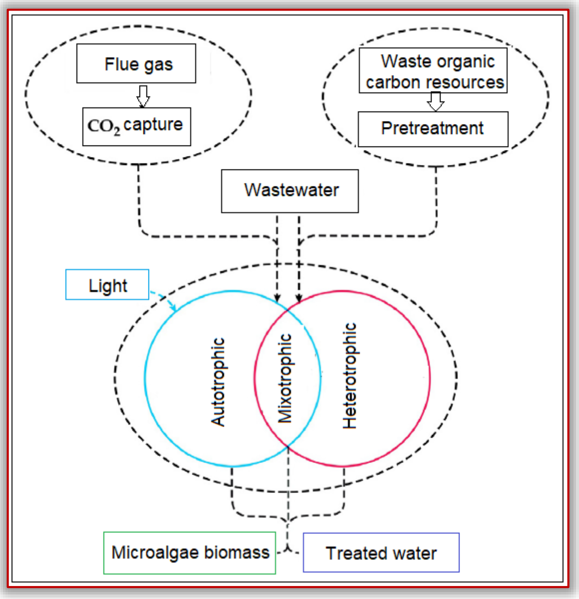

Figure 1 – Microalgae cultivation methods for wastewater treatment (Sundar Rajan et al., 2019)

In recent years, a large variety of closed systems (photobioreactors) were developed. This equipment must be supplied with CO_2 and light and represent a possible economically viable alternative to conventional aerobic biological methods for wastewater treatment due to their potential of resource recovery and recycling (Christenson and Sims, 2011). The cultivation costs in closed systems are high and this limits their commercial applications to high-valued compounds, but the costs can be reduced by efficient design of photobioreactors, which are able to achieve high areal biomass productivities (Placzek et al., 2017).

To some extent, in addition to the uptake by microalgae, some of the nutrient removal might be due to physical and chemical processes like volatilization, precipitation or adsorption (AlMomania and Örmeci, 2016). Algae uptakes the phosphorus as inorganic orthophosphate, preferably as $H_2PO_4^-$, PO_4^- or HPO_4^{2-} (AlMomania and Örmeci, 2016).

Besided wastewater, there are other possible culture medium for microalgae growth. Centrate is a stream generated from dewatering of sludge from primary and secondary settling, and mainly contains phosphorus, ammonia, and nitrogen. According to Wang et al. (2010), centrate is the best among all municipal wastewater streams for algal cultivation with high biomass accumulation and high efficiency wastewater nutrient removal. In addition to different types of wastewater, activated sludge contains 90–95 % organic matter and nutrients and there are some studies that have shown that activated sludge might be an alternative medium that could provide the necessary nutrients for microalgae cultivation. Digestate from anaerobic digesters can also be used as suitable medium for microalgae cultivation, preferably after dilution with synthetic culture medium, secondary/tertiary wastewater or seawater (Dickinson et al., 2015).

Microalgae can improve the quality of the final effluent through natural disinfection because they reduce the pathogenic organisms, viruses, protozoa and coliform bacteria such as Salmonella, Shigella found in municipal and livestock wastewater (Ungureanu et al., 2020).

Wastewater treatment by microalgae can be achieved in the form of suspended free-cells culture and immobilized cells.

The suspended free-cells culture is the condition of microalgae living cells to move independently within the flasks containing a medium under the condition to ensure uniform cells distribution (Katarzyna et al., 2015). The use of suspended free-microalgae cells culture involves the removal of nitrogen and phosphorus from wastewater whilst simultaneously providing oxygen (O_2) for aerobic bacteria coexisting in the culture. The immobilized cells culture is the condition of microalgae living cells to be prevented from flow freely from their original location to all parts of the medium (Emparan et al., 2019). Several residual polymeric materials like alginate and chitosan are currently used for microalgae immobilization. Biofilms also provide a medium for immobilization of microalgae, because they are slimy, green layers consisting of large numbers of microalgae entrapped in a gel-like matrix (Ungureanu et al., 2019).

Research on algae-based wastewater treatment has focused mostly on the conventional microalgae and cyanobacteria, including Arthrospira sp., Botryococcus sp., Chlorella sp., Chlamydomonas sp., Haematococcus sp., Nannochloropsis sp., Scenedesmus sp. and Spirulina sp.

Unicellular green microalgae Chlorella sp. is spherical shaped, flagellate organism with a diameter of about 2-10 μm (Figure 2). Chlorella sp. is mostly used in the tertiary treatment of wastewater, but it has high removal efficiency (> 80%) of nutrients (nitrogen and phosphorus) in primary and second treatment effluents.

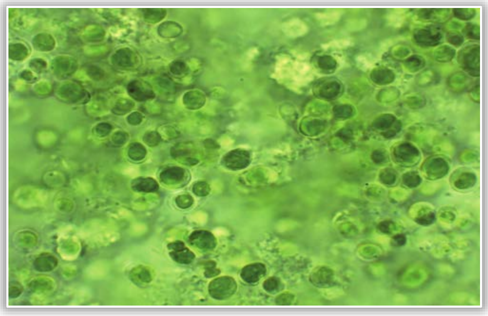

Figure 2 – Microalgae Chlorella vulgaris (Brzychczyk et al., 2016)

Under certain conditions, Chlorella sp. can completely remove ammonia nitrogen, nitrate nitrogen and total phosphorous, hence it has wide application in the treatment of agricultural and fecal wastewater (Wang et al., 2010). Its photoautotrophic growth is usually limited by lack of nutrients (particularly nitrogen), weak light, carbon limitation, changes in wastewater pH, and accumulation of photosynthetic oxygen (Yuvraj and Singh, 2016). Chlorella vulgaris produces more oxygen than all other plants in the world (Malothu R., 2020).

In the context of a circular and bio-based economy and the development of biorefinery concepts, after wastewater treatment, the harvested microalgal biomass can be processed to obtain high-value products, including: cosmetics, pharmaceutical products (with antiviral, antibacterial, anticancer, antihistamine role), steroids, algal toxins, pigments, organic fertilizers rich in N, P and K minerals, protein sources, animal feed, food products (juices, sauce, cheese, noodles, beverages), preservatives in food industry, bioplastics, biosorbents (ion exchangers that can bind toxic heavy metals), bioenergy (biofuels including biogas, biodiesel, bioethanol, biobutanol, biohydrogen, biochar etc), and digester residues (compost and vermicompost).

RESULTS

— Microalgae Chlorella in the treatment of municipal wastewater

As reported by Wang et al. (2010), Chlorella sp. cultivated in wastewater from a local municipal wastewater treatment plant achieved removal rates of NH_4-N, phosphorus and COD of 74.7%, 90.6% respectively 56.5%, before the primary settling stage. It was also reported a high removal efficiency of NH_4-N and phosphorus from secondary wastewater.

Feng et al. (2010) cultivated Chlorella vulgaris in synthetic wastewater containing 78 mg/L N-NH_4^+ and 400 mg/L COD was provided by glucose. Cultivation was done at 30ºC, under 3000 lx light emission and 0.5 vvm of air bubbling. The microalgae grew for 7 days, and the removal efficiency of nitrogen, phosphate and COD were 96%, 97% and 85%, respectively. In another study, Chlorella sp. grown in a highly concentrated municipal wastewater (centrate) achieved approximately 90 % of total nitrogen removal (Li et al., 2011).

Choi and Lee (2012) investigated the effect of optimal concentration of Chlorella vulgaris (FC-16) with cell diameter between 3–8 μm, for the removal of nutrients in real wastewater obtained from preliminary sedimentation of a wastewater treatment plant, using batch reactor operation. They observed that increasing the concentration of Chlorella vulgaris from 1 to 10 g/L produced an increase of removal rates thus: total nitrogen from 81.04% to 84.81%; total phosphorous from 32.26% to 36.12%; NH_3-N from 96.90% to 97.26%, PO_4-P from 44.76% to 48.71%, COD from 78.33% to 82.30%, respectively BOD from 80.41% to 82.92%.

In a study conducted by Ebrahimian et al. (2014), Chlorella vulgaris was cultivated in batch system and mixotrophic conditions supplied with CO_2 using a mixture of primary and secondary wastewater with 25, 50 and 75 volume percent of the primary wastewater. As presented in Figure 3, the removal rates using 25% of the primary wastewater were 100% for ammonium, 82% for nitrate and 100% for organic matter (COD).

AlMomania and Örmeci (2016) tested the efficiency of Chlorella vulgaris, Neochloris oleoabundans and mixed indigenous microalgae collected from a wastewater treatment plant, for the removal of nitrogen, phosphorus and organic carbon from primary effluent, secondary effluent, and centrate. The indigenous microalgae culture has proven to be more effective than Chlorella vulgaris and Neochloris oleoabundans in removing inorganic nitrogen (63.2–80.8%), phosphorus (30.8–70%) and chemical oxygen demand (64.9–70.4%). Zhou et al. (2014) obtained removals of 76.7–92.3% of total nitrogen and 67.5–82.2% of total phosphorus by Chlorella vulgaris, Chlorella pyrenoidosa, Chlamydomonas reinhardtii and Scenedesmus obliquus, during wastewater treatment.

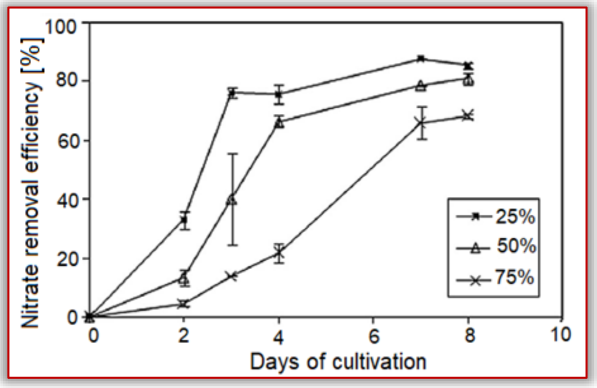

Figure 3 - Removal of nitrate in a mixture of primary and secondary wastewater (Ebrahimian et al., 2014)

Caporgno et al. (2015) tested freshwater microalgae species Chlorella vulgaris and Chlorella kessleri and the marine microalgae species Nannochloropsis oculata cultivated in urban wastewater, in batch mode using a flat-panel airlift photobioreactor. Both freshwater microalgae species achieved reductions of nitrogen concentration around 96% and 95%, and of phosphorous concentration around 99% and 98%. Nannochloropsis oculata was able to uptake nutrients from wastewater to grow but with less efficiency, indicating the need of microalgae acclimation or process optimization to achieve high nutrient removal.

Bacteria are inevitably present in the wastewater and they interact with the microalgae, on the one hand potentially restricting their growth and on the other hand potentially enhancing the efficiency of wastewater treatment due to stimulation of microalgae growth. Some bacteria could kill the algae by releasing enzymes to break down the microalgae cell wall, and extracellular substances produced by bacteria could cause algae lysis (Ma et al., 2014).

In this regard, He et al. (2013) studied the effect of Chlorella vulgaris with or without co-existing bacteria on the removal of nitrogen, phosphorus and organic matter from wastewater. In the algae–bacteria system Chlorella vulgaris had a dominant role in the removal of nitrogen and phosphorus, while bacteria removed most of the organic matter from the wastewater. Using the algae–bacteria consortium resulted in the removal of 97% ammonium, 98% phosphorus and 26% dissolved organic carbon at a total nitrogen level of 29–174 mg/L. A study conducted by de-Bashan et al. (2002) proved that the co-immobilization of microalgae Chlorella vulgaris in alginate beads with bacteria Azospirillum brasilense was superior to a stand-alone microalgae system, obtaining removal rates of up to 100% ammonium, 15% nitrate and 36% phosphorus.

Kube et al. (2019) also proved that algae immobilization in alginate was more efficient and stable for synthetically-made municipal wastewater treatment, achieving a maximum nitrogen removal efficiency of 95% after 84 h of treatment.

Ma et al. (2016) cultivated Chlorella vulgaris in wastewater with waste glycerol generated from biodiesel production, to improve nutrients removal and enhance lipid production. The tested concentrations of glycerol were 0, 1, 5 and 10 g/L. The optimal concentration of the pretreated glycerol for Chlorella vulgaris was 10 g/L with biomass concentration of 2.92 g/L, lipid productivity of 163 mg/L·day. Under these conditions, it was obtained the removal of 100% ammonia and 95% of total nitrogen (Figure 4).

The highest COD removal efficiency of the culture with the pretreated glycerol was 98%, possibly because some organic compounds in the crude glycerol, which were not removed by pretreatment, were not fully absorbed by Chlorella vulgaris.

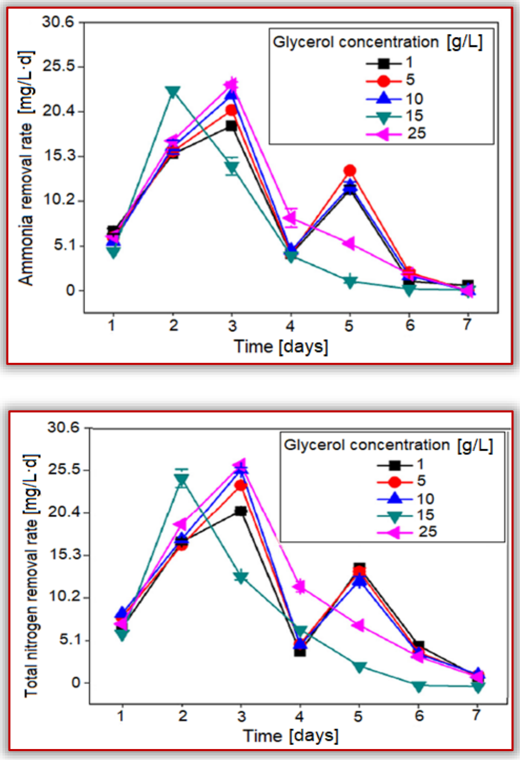

Figure 4 - Effect of concentration of pretreated glycerol on nutrients removal by C. vulgaris (Ma et al., 2016)

In a recent study, iron-magnetic nanocomposite particles (Fe_3O_4@EPS) synthesized by co-precipitation of iron (III) chloride and iron (II) sulfate (Fe_3O_4 nanoparticles) with exopolysaccharides (EPS) derived from the microalga Chlorella vulgaris were tested for the removal of nutrients from wastewater. Under optimum conditions (3.5 g/L of Fe_3O_4@EPS, pH 7.0 and 13 hours of incubation) 91 % PO_4^{3-} and 85 % of NH_4^+ were effectively eliminated, showing the potential of Fe_3O_4@EPS application in removing nutrients in wastewater treatment plants (Govarthanan et al., 2020).

— Microalgae Chlorella in the treatment of zootechnical wastewater

Piggery wastewater contains high concentration of suspended solids and nutrients, small amounts of heavy metals and organic matter, antibiotics and hormones, so its insufficient treatment can cause serious environmental pollution and risks to human health. The typical composition of piggery wastewater exhibits the following concentrations: nitrogen 800–2300 mg/L, phosphorous 50–230 mg/L, biochemical oxygen demand 2000–30000 mg/L, and N/P ratio is about 12–17 (Chen et al., 2020).

A common feature of piggery wastewater is the high-strength ammonium, which is toxic for microalgae and therefore it should be mitigated during piggery wastewater treatment by microalgae, by the addition of glucose to adjust C/N ratio of the wastewater (Lu et al., 2018). Recognizing the potential benefits of microalgae cultivation incorporated into the piggery sewage systems, studies into the use of microalgae as a treatment for piggery wastewater have been ongoing for the last decades.

Lv et al. (2018) used Chlorella vulgaris selected from five freshwater microalgal strains of Chlorophyta for nutrients removal from undiluted cattle farm wastewater by two-stage processes of microalgae-based wastewater treatment. By the end of treatment, 62.30%, 81.16% and 85.29% of chemical oxygen demand, ammonium and total phosphorus were removed.

The two two-stage processes included a setup of double biological treatment by Chlorella vulgaris, respectively a setup of biological treatment by Chlorella vulgaris followed by activated carbon adsorption. After 3–5 days of wastewater treatment by the two processes, the nutrients removal efficiency of chemical oxygen demand, ammonium and total phosphorus ranged between 91.24–92.17%, 83.16–94.27% and 90.98–94.41%.

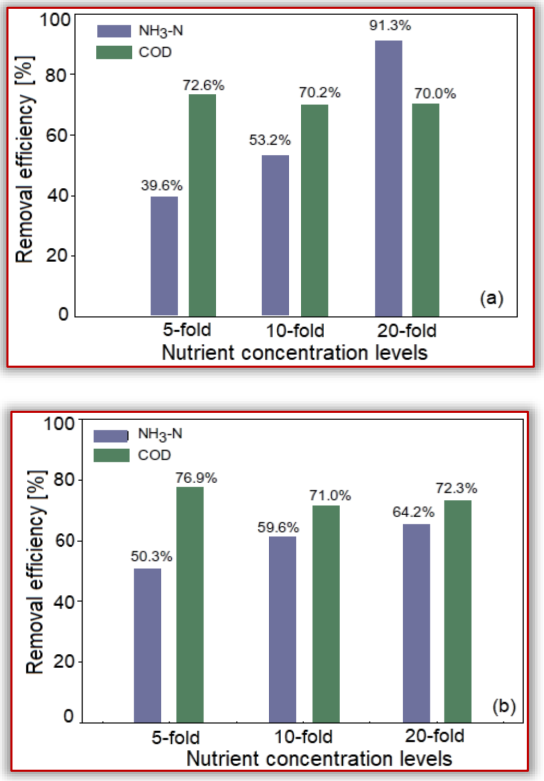

Figure 5 – Removal efficiency of NH_3-N and COD at different nutrient concentration by Chlorella vulgaris JSC-6 grown mixotrophically (a), respectively heterotrophically (b) in piggery wastewater (Whang et al., 2015)

Whang et al. (2015) tested an isolated carbohydrate-rich microalga Chlorella vulgaris JSC-6 to treat piggery wastewater. They obtained between 70–77% COD removal and 40–90% NH_3-N removal in mixotrophic and heterotrophic conditions, depending on wastewater dilution ratio, with the highest removal percentage obtained for 20-fold diluted wastewater (Figure 5).

Chlorella vulgaris can grow well in pretreated fresh pig urine (at 8-fold dilution, pH=6, $MgSO_4 \cdot 7H_2O$ dosage of 0.1 mg/L). About 1.72 g/m²·day of microalgal biomass could be produced, and 98.20% of NH_4^+-N and 68.48% of total phosphopous could be removed in the batch mode at cultivation in light-receiving-plate enhanced raceway pond. A hydraulic retention time of 7-9 days is optimal for the efficient removal of nutrients and microalgae biomass production in continuous regime (Zou et al., 2020).

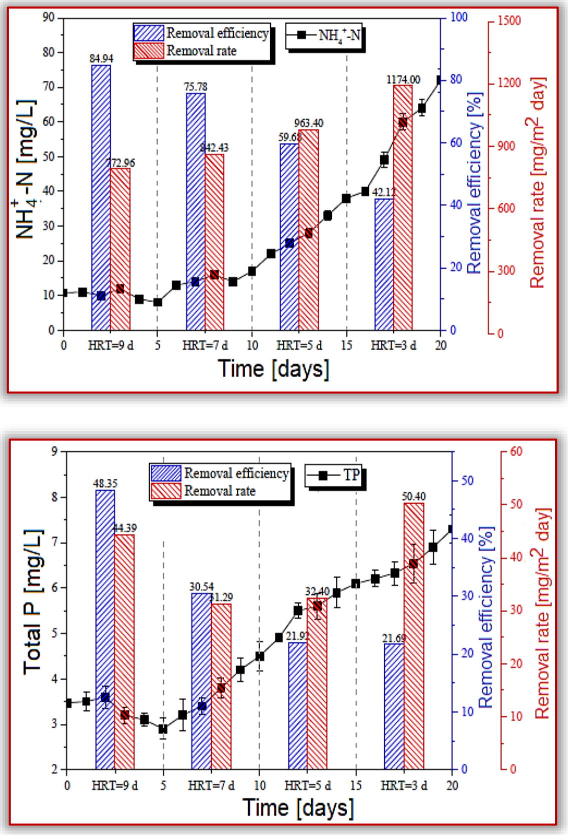

Figure 6 - Removal efficiencies and removal rates of NH_4^+-N and total phosphorous (Zou et al., 2020)

Zhou et al., (2018) cultivated Chlorella zofingiensis in municipal wastewater and also in municipal wastewater mixed with 8 % pig biogas slurry. The latter contains abundant mineral nutrients after anaerobic treatment; hence it had a significant effect on microalgal growth (2.5 g/L biomass).

Figure 7 illustrates that in municipal wastewater (MW), nitrogen and phosphorous concentrations decreased continuously in the first 24 hours, and then remained stable, with Chlorella zofingiensis showing a good removal efficiency of nutrients. Nitrogen decreased continuously within the first 60 hours then remained stable, whereas phosphorus concentrations decreased rapidly in the first 36 hours.

Overall, the removal rates for nitrogen and phosphorus in MW were 21 mg/L·day, respectively 4.6 mg/L·day, achieving

90 % total phosphorus removal and 93 % total nitrogen removal.

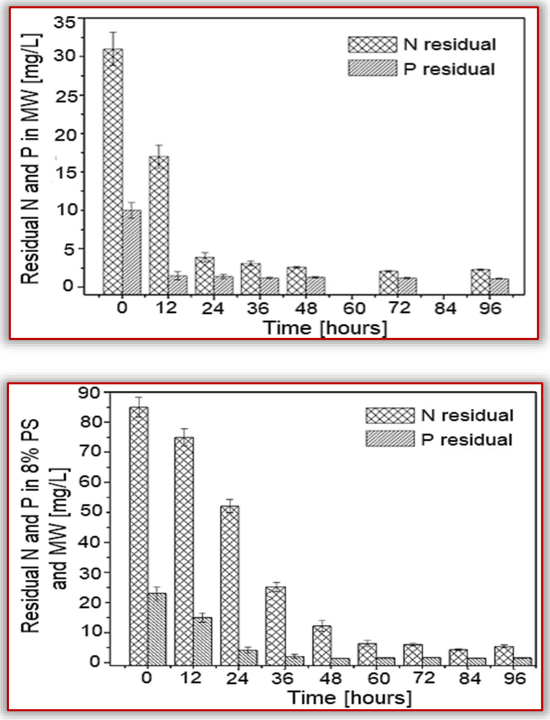

Figure 7 – Removal of nutrients by Chlorella zofingiensis in municipal wastewater and in 8 % pig biogas slurry in municipal wastewater (Zhou et al., 2018)

Zhu et al., (2013) treated piggery wastewater by the freshwater microalgae Chlorella zofingiensis, a protein-rich algae, and obtained removal rates of 68–81 % for nitrogen, 90–100 % for phosphorous, respectively 65–76 % for COD.

CONCLUSIONS

Conventional methods are widely employed for the treatment of municipal and zootechnical wastewater, which is based on biological and chemical systems and is costly. Hence, the use of microalgae for wastewater treatment got increased attention in recent years. In the past few decades, tremendous advances have been made in the field of algal technologies for combating numerous disadvantages of techno-economic order and improving biomass production, and the research is ongoing worldwide.

Chlorella vulgaris is widely cultivated in different types of wastewater and it proved its efficiency in the removal of nutrients (nitrogen and phosphorous) that would otherwise cause eutrophication, and in the removal of chemical oxygen demand, biochemical oxygen demand, heavy metals, and even pathogens.

Besides their important role in the sustainable treatment of wastewater and in the reduction of greenhouse gases, microalgae represent an abundant biomass with potential to be capitalized in obtaining biofuels of real interest in the current climatic conditions.

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