

# Review on Challenges and Limitations for Algae-Based Wastewater Treatment

Aigars Lavrinovičs<sup>1,2</sup>, Tālis Juhna<sup>3</sup>

<sup>1,3</sup> *Department of Water Engineering and Technology, Riga Technical University, Riga, Latvia*

<sup>2</sup> *Institute for Environmental Solutions, Riga, Latvia*

**Abstract** – Microalgae biomass production is recognized as a cost-effective and sustainable alternative to currently used approaches to tertiary wastewater treatment. However, such limitations, as algae biomass separation from water, process efficiency in cold climate and the algae biomass ability to reduce micropollutant content in wastewater hamper this method from full-scale use. This review discusses the identified drawbacks and offers possible improvements and modifications for wastewater phycobioremediation.

**Keywords** – Cold climate, harvest, microalgae, micropollutants, tertiary treatment, wastewater.

## I. INTRODUCTION

The increasing global population and its economic activity lead to elevated pollution loads to natural surface waters. High nutrient concentrations in a water body promote a eutrophic ecosystem state, thus providing favorable conditions for rapid algal growth. When the algal biomass decays, dissolved oxygen content is depleted, resulting in aquatic animal death and overall deterioration of ecosystem health and the services it provides. Estimations show that the economic losses from surface water eutrophication can exceed 2 billion US dollars annually [1], affecting the real estate, recreation, and fishing industries. Among other sources, municipal wastewater treatment plants significantly contribute to freshwater eutrophication. Substantial role is played by small WWTPs. According to Regulation No. 34 by the Cabinet of Ministers of the Republic of Latvia there is no requirement for phosphorus content reduction in WWTPs that operate for less than 2000 person equivalent.

To reduce the WWTP input to eutrophication, their management must be reconsidered. Currently phosphorus content in the WWTP effluent is mostly reduced by chemical precipitation [2] at the tertiary treatment phase. However, the use of chemicals is associated with high costs and results in excess waste sludge production increasing the risk for secondary water pollution [3], [4]. These shortcomings of conventional tertiary wastewater treatment have led to introduction of more sustainable approaches. Numerous studies have demonstrated that phycoremediation, i.e. application of algae biomass for wastewater treatment, is an efficient measure for reducing nutrient concentration by up to 95 % [5]–[7]. Besides wastewater treatment, algae biomass is perceived as a raw material for bioenergy production, nutrition and perfume products as well as high value substance extraction [8]. Thus, with successful downstream processing, algal biomass not only can provide a low-cost wastewater treatment, but is also potentially profitable wastewater management approach. However, despite the promising application possibilities, certain obstacles hamper

algae-based wastewater treatment from full-scale operation and from becoming a cost-effective alternative for conventional methods. Current technology for algae biomass separation from water significantly increases the total treatment costs [9]. Therefore, a lack of rapid and inexpensive biomass harvest method has been identified as the major limitation for algae-based wastewater treatment. Moreover, efficient operation with minimal energy requirement is possible at certain climate conditions. Thus, algae-based wastewater treatment is limited to finite geographical locations. Finally, the everchanging chemical content of wastewater raises new challenges, requiring treatment of wastewaters with problematic and unknown contamination [10], while their influence on algal growth is often obscure.

This review aims to point out and discuss the identified limitations and ambiguities, as well as offers possible improvements and modifications for wastewater phycobioremediation that could overcome the currently known limitations.

## II. ALGAE-BASED WASTEWATER TREATMENT

Microalgae is an autotrophic unicellular organism that can perform photosynthesis. Powered by light, algae convert water and carbon dioxide into oxygen and carbohydrates, thus providing energy for its biomass growth [11]. Also, nitrogen and phosphorus are consumed as nutrients for algae cell reproduction. The ability to produce oxygen and take up nutrients from water have made microalgae biomass cultivation prospective for cost-effective wastewater treatment.

Initially the use of microalgae for wastewater bioremediation was proposed by [12]. They demonstrated that algae biomass production in domestic sewage works as an aeration technique with low energy requirement. It resulted in biochemical oxygen demand reduction by more than 85 % in a pilot scale waste stabilization pond. Afterwards, algae-based nutrient removal and recovery from wastewater was successfully demonstrated [13], [14]. Further, wastewater was found to be a suitable growth medium for low-cost microalgae biomass cultivation for energy production and high value product extraction [15]. Present algae-based wastewater treatment studies have evolved to various scales and techniques, covering diverse contamination removal [16], [17] and optimizing algae growth conditions to reach utmost water treatment and biomass production performance [18].

Algae metabolism is the mechanism behind wastewater phycoremediation. Nutrients are taken up and transformed within the algal cell where they can be assimilated into nucleic acids and proteins for algae biomass growth [19], [20]. Nitrogen is present

in wastewater in the form of organic nitrogen, or inorganic compounds such as ammonium and nitrate. Ammonium is the preferred form for algae uptake, while nitrate within the algae cell is reduced back to ammonium and assimilated into amino acids for the synthesis of proteins [21]. Inorganic phosphorus is utilized for ribosome RNA synthesis. In addition, microalgae can consume extra phosphorus through a luxury uptake, store it in the form of polyphosphate and utilize it at low external phosphorus availability conditions [22].

[23] have estimated that there are roughly 72 500 known and described species of freshwater, marine and terrestrial algae. Still, only few of them have been tested for the tolerance in wastewater [24], [25]. Among others, *Chlorella* and *Scenedesmus* species algae are far more often used for wastewater treatment [5], [26], [27].

Reviews done by [20] and [28] show the efficiency of algae in nutrient concentration and biochemical oxygen demand reduction, and depending on the treatment setup, scale, algae species and environmental conditions, nearly complete contamination removal can be achieved. Wastewater treatment using microalgae is performed in open or closed systems. Open systems such as natural lagoons, pond and artificial ponds and reservoirs are simple to construct and maintain, and therefore are the preferred installations for algae-based wastewater treatment [29]. However, the treatment process is less controllable. Closed systems, which mostly are photobioreactors, provide more control over the treatment process, but are expensive to install and maintain. The major controllable parameters include the photosynthetic active radiation (PAR), which is considered the key factor for algae production with the optimal range of photon flux being between 30 to 400  $\mu\text{mol m}^{-2} \text{s}^{-1}$  [30]. Additional  $\text{CO}_2$  supply can maximize the algae biomass production [31], it prevents development of alkaline pH level. Suitable pH values for algal growth are reported to be between 7 and 9 [32]. The role of temperature for algae biomass production is rather unclear and is highly variable between species and their origin [33]. Still, the optimal range is reported to be between 15–30 °C [34]. The role of nutrients is expressed by optimal molar ratio of the available nutrients. The empirical ratio between nitrogen and phosphorus is determined to be 16 : 1 [35], however, it is highly variable between species and is affected by the environmental conditions [36]. Finally, successful algae growth and resulting water treatment efficiency depend on the presence of other organisms. Although bacteria are often seen as a competitor to algae for nutrients, consortium of these two organisms is used as a source for both natural aeration and  $\text{CO}_2$  supplementation [37]. Presence of algae grazers, such as zooplankton or rotifers, has a negative effect on algae production rate and water treatment performance.

Additional benefit to low energy wastewater treatment comes from the algae biomass suitability for added value product extraction. Due to rapid biomass production rate and high lipid content, algae have the potential to become a major raw material for biofuel production [38]. Although the estimated production costs of algae-based biofuel are uncompetitive with currently used biomass [39], diligent research is carried out on optimized algae biomass production for higher lipid yield [18]. Other high-value products derived from microalgae are  $\beta$ -carotene,

astaxanthin, docosahexaenoic acid, eicosahexaenoic acid, phycobilin pigments and algal extracts for use in cosmetics [8], with a multi-million global market value.

Extensive research done on wastewater phycoremediation clearly demonstrates the potential of microalgae production for sustainable wastewater treatment. However, despite the massive knowledge generated throughout the past six decades, various shortcomings have been identified for this wastewater treatment approach. In further sections of this review, major obstacles that hamper a global algae-based wastewater treatment implementation are discussed.

### III. LIMITATIONS FOR ALGAE-BASED WASTEWATER TREATMENT

#### A. Algae Biomass Harvest

Algae biomass harvest has been identified as one of the major drawbacks for cost-effective wastewater treatment and its downstream processing. Main factors that complicate algae harvest include very small portion (up to 0.05 %) of algae dry weigh in the total suspension, microscopic size of a single cell, negative cell surface charge that prevents them from forming larger and easily harvestable particles as well as rapid growth rate [9]. These aspects significantly increase the total costs of both algae biomass harvest and its application for wastewater treatment. It is estimated that cost of algae biomass harvest can constitute up to 30 % of the total production expenses [40], which is due to high energy consumption, which, depending on the chosen method, varies between 0.1 and 15  $\text{kWh m}^{-3}$  [41]. Moreover, up to 90 % of the total inventory costs are attributable to the harvest and dewatering devices [42]. Although various algae biomass harvest techniques have been developed and are widely used, each of them has its advantages and drawbacks, which encourages research on finding more economically feasible, universal and simpler methods.

#### Physical and Chemical Methods

One of the simplest algae biomass harvest methods is sedimentation, which offers an inexpensive solution for algae biomass harvest. It is done using gravitational force, where algal biomass is separated from liquid due to differences in their densities. Still, the difference between algae cell and water density is relatively small, making the process rather slow. Moreover, sedimentation rate is affected by a series of biotic and abiotic factors. Different sedimentation rates are suggested for various algal functional groups, reaching 3.6  $\text{m d}^{-1}$  for the wastewater tolerant *Chlorella sp.* [43]. The water temperature affects its viscosity, so algae sedimentation rate is likely to increase at higher temperatures. Also, elevated pH levels promote algae cell floc formation leading to more rapid sedimentation. As sedimentation is a relatively slow process, it is often coupled with other harvest techniques or is modified for more rapid performance.

Centrifugation can be viewed as a derivation of sedimentation, where instead of gravitational force centrifugal force is employed. Centrifugation provides simple and rapid algae biomass separation from the liquid, and is efficient for all algae species and cell sizes [44]. However, due to large investment and operating expenses, it becomes cost-inefficient for large scale

algae-based wastewater treatment systems [45], [46], leading to fourfold increase in total treatment costs [47].

To neutralize the negative surface charge of algae cells and promote formation of larger particles that easily settle, electrolytes and synthetic polymers are used. Usually, aluminum sulfate and ferric chloride are used to stimulate flocculation [45], and their use can result in more than 90 % of algal biomass recovery [46]. On the other hand, use of chemical flocculants can lead to secondary pollution and it limits the downstream processing of algae biomass. In addition, for large scale algae-based wastewater treatment systems, flocculant use for algae biomass harvest becomes economically detrimental, and it is likely to become more expensive than precipitant use for tertiary treatment alone.

Certain algae species can form flocs naturally under environmental stress, such as elevated pH level or changes in nutrient and dissolved oxygen concentrations [48]. Manipulation of these parameters in controlled environment offer a relatively cheap flocculation method. However, it is still viewed as slow and unreliable flocculation technique, with limited possibilities for application [49].

Another method for neutralizing the algae cell surface charge and promoting their flocculation is the use of electrophoresis [45]. Although this method does not require addition of chemicals, flocculation using electric field is associated with high energy consumption and maintenance costs [48], especially for large scale performance.

Flotation can be viewed as the next step after flocculation. Most commonly dissolved air floatation is used. It employs decompression of pressurized fluid that generates microscopic bubbles and promotes easily harvestable algae mat formation [50]. Although this method has been recognized as effective for large scale use, it is still an energy-intensive process [51].

Filtration is another simple and effective algae harvest method, which gives up to 90 % recovery of algal biomass [52]. There are several types and designs available for filters, but their application is mainly determined by the variable algae cell size. In addition, the algae cell diameter is inversely proportional to the expenses of this method. Macrofiltration methods with lower energy requirement are applicable for macroscopic filamentous algae species, such as *Spirulina* [53]. For species like *Chlorella* and *Scenedesmus* the cell diameter varies between 5  $\mu\text{m}$  and 20  $\mu\text{m}$ , therefore membrane microfiltration is applied for their harvest [54]. However, microfiltration is associated with slow performance and is not suitable for large scale. Micro and ultrafiltration methods are also related to high performance and maintenance costs, due to rapid membrane clogging that needs to be frequently changed as well as high energy demand due to micro-pore membrane pressure resistance [45].

Different harvest approach is offered by immobilization of algae cells into a polymeric matrix, which provides a convenient and cost-effective alternative for conventional algae biomass harvest methods [55]. The immobilization matrix can be either synthetic or naturally derived polymer and must meet certain requirements such as phototransparency, non-toxicity and stability in the algae growth medium [56]. Besides simple biomass harvest, immobilization matrix has the advantage of

hyperconcentrated culture use and protection of algae cells against hazardous bacteria or natural grazers. Among other natural immobilization matrixes like agar, alginate or collagen, chitosan obtained from chitin is a frequently used material [56], [57]. The natural properties of chitosan include considerable uptake rate of nutrients which can be done in parallel with immobilized algae biomass hyper-concentrate. [56] obtained 70 % nitrate and 94 % phosphate uptake using immobilized *Scenedesmus sp.* cells. However, various studies show that the choice of material for immobilization matrix can play an important role in overall wastewater treatment efficiency. [58] used alginate bed as immobilization matrix and found that free-cell cultures show better wastewater treatment performance over immobilized cultures. [59] used *Chlorella vulgaris* and *Azospirillum brasilense* bacteria as algae growth-promoting organism co-immobilized in alginate bed to treat synthetic wastewater. Co-immobilized culture showed 32 % higher removal rate of ammonium than single algae culture alone. Phosphorus reduction was not observed, though. The existing studies show that immobilized algae cultures are a good solution for biomass harvest, and thus, can significantly reduce harvest costs. Still, its performance mostly has been studied in bench-scale under controlled conditions. Thus, assessment of pollutant removal efficiency and economic viability of immobilization polymer use on pilot-scale is required.

#### Biological Methods

A perspective, yet undeveloped technique for microalgae biomass separation from water is the application of natural algae predators for biofiltration. In natural ecosystems algae cells are consumed by filter feeding organisms from higher trophic levels of the food-web, which include zooplankton, mussels and certain filter feeding fish species. Such an approach for wastewater treatment is attractive due to low energy consumption under optimal conditions for biomass cultivation. Contaminant removal is done by algae in accordance with the previously described mechanism. Electrical energy may be required for aeration, additional CO<sub>2</sub> feeding, mixing and water pumping. Algae harvest performed by zooplankton and fish basically require food resource which is ensured by the filterable algae biomass. Although the provisional energy consumption of such wastewater treatment approach would be relatively low, it requires trained personnel for regular monitoring and maintenance, as this treatment method is a living system not mechanical process.

The potential use of filter feeders for algal biomass removal has been studied in lab-scale systems [60] and mezocosms [61], [62]. [60] used a chain of *Scenedesmus sp.* algae, *Daphnia sp.* zooplankton and *Notemigonus crysoleucas*, *Pimephales promelas* and *Notropis lutrensi* filter feeding fish as a tertiary water treatment system. Each of the organism groups was isolated forming an artificial aquatic food-web (AAFW). It was reported that such a system could remove up to 78 % of nitrogen and 98 % of phosphorus compounds from secondary treated domestic wastewater. Similar results were achieved by [61] and [62]. However, they excluded filter-feeding fish from the treatment system. [63] showed that continuous light regime promotes algal biomass growth and thus increases nutrient removal efficiency.

The latest study, using AAFW for water treatment, demonstrated reduction of TN and TP concentration by 28 % and 47 % respectively in a eutrophied subtopic river [64].

Although proved to be efficient on a laboratory scale and demonstrating significant pollutant reduction rates in outdoor conditions, such systems still need to be tested under various climate conditions. The influence of seasonal temperature variations, fluctuating water inflow rate, as well as natural light regime are location-specific. Thus, similarly to other wastewater bioremediation systems [65], [66] variable response to performance efficiency can be expected in different climate zones. Also, biotic factors, such as species selection for AAFW organisms is likely to affect performance. For instance, zooplankton preference for green microalgae species can reduce algae harvest efficiency, especially when open cultivation systems with mixed algae strains are used.

Another important factor affecting AAFW based wastewater treatment efficiency is the content of waste stream. Besides nutrients and organic matter, that are the target parameters in conventional wastewater treatment, micropollutants such as heavy metals, coliform bacteria and a variety of pharmaceuticals, personal care products, prescription and illicit drugs and other substances, their compounds and residuals, all together classified as emerging contaminants, are present in wastewater. Variable efficiency in reduction of these pollutants has been demonstrated by algae-based wastewater treatment systems [67]. Yet, it is unclear how these micropollutants would affect the filter-feeding organisms in the AAFW and its overall water treatment performance.

### B. Removal of Micropollutants

Along with nutrients and organic compounds, wastewater contains a large variety of micropollutants that often pass the conventional wastewater treatment and are released in natural water bodies. Substances like heavy metal ions, pathogenic bacteria as well as compounds and residuals of pharmaceuticals, personal care products, household chemistry, drugs and others can cause adverse effects on aquatic organism development and human health [68]. For the reduction of their content advanced treatment is used, which, however, is associated with high energy demands and performance costs. Although several studies have demonstrated the applicability of algae for micropollutant removal from wastewater [16], [69], [70], limitations and knowledge gaps exist to rely on algae biomass production as an effective means for micropollutant content reduction.

#### Heavy Metals

With growing industrialization, heavy metal ions are becoming more common pollutant in the urban sewage [71], thus increasing the load to natural aquatic ecosystems. Unlike organic contaminants, heavy metals are not biodegradable and tend to accumulate in living organisms. It is known that many heavy metal ions are toxic or carcinogenic, causing dysfunction of the organism containing them. Therefore, heavy metal ions, such as zinc, copper, nickel, mercury, cadmium, lead and chromium are of major concern when it comes to wastewater treatment. Methods for heavy metal content reduction in water include chemical precipitation, ion exchange, adsorption, membrane

filtration, coagulation and flocculation, floatation and electrochemical treatment [72]. Still, more economical and sustainable ways for heavy metal content reduction are being searched for, and algae application seems to be a promising alternative [73].

The ability of algae to remove metals from wastewaters have been broadly studied. It is known that algae cells are capable to adapt to toxic environment and take part in heavy metal uptake. Both living and dead cells were found to contribute to metal sorption in wastewater [74]. [73] studied the capability of *Cladophora fracta* to remove metals from stock solutions and achieved 85–99 % removal of Cu, Zn, Cd and Hg. Gao et al. (2016) used *Chlorella vulgaris* for domestic wastewater treatment in membrane photobioreactor and achieved complete reduction of Fe and Mn ions, while Cu, Zn and Al ions were reduced by 65 %, 80 % and 93 %, respectively. [75] studied metal removal from textile wastewater using lab-scale algae pond system under different flow and light conditions. They achieved 98 % reduction of chromium (Cr) concentration regardless the loading rate and light regime, while for zinc (Zn) the removal rate was higher (80 %) at high loading rate under continuous 24 h lighting. For other metals (Pb, Cd and Cu) the removal rates were between 20 % and 30 %.

Despite the evident efficiency of algae-based metal-ion removal from wastewater, there are still ambiguities regarding efficiency for certain metal ions. The abovementioned studies show significant contrast in removal rate between different metal ions at equal conditions. Yet, the key factors for unequal metal ion removal remains unclear. In addition, algae biomass use for metal-ion reduction in wastewater clearly limits its further application due to the negative effects of metal ion accumulation in living organisms.

#### Coliforms

Coliform bacteria are pathogenic microorganisms that are found in gastrointestinal tract of all warm-blooded animals. They are used as an indicator for fecal contamination in water. Although considered to be a harmless organism, certain strains can cause gastroenteritis [76]. Due to the negative effects on human health, coliform content in the effluent wastewater becomes relevant when it is reused, for instance, in public swimming pools.

To prevent outbreaks of waterborne diseases, advanced tertiary wastewater treatment for coliform removal is performed. Common methods for coliform and other bacteria removal are chlorination, sand filtration or ultraviolet disinfection [77]. Although chlorination is the most widely used water sterilization method, certain shortcomings of this method, such as formation of toxic and potentially cancerogenic by-products have been identified [78]. Therefore, development of novel and sustainable treatment methods is required.

There are several studies proposing application of microalgae for coliform content reduction in wastewater. It has been demonstrated that in open algae-based wastewater treatment systems photosynthetic growth of algae develops adverse conditions for pathogenic organisms [79], [80], forming high pH and dissolved oxygen concentration. Also, auxiliary effect is

given by received light intensity [81], [82]. However, certain limitations are known for algae-based coliform removal. A study by [83] showed that algae decay can produce significant amount of dissolved organic carbon, which promotes bacterial survival. Such conditions can be formed by extended hydraulic retention time, insufficient mixing and algae sedimentation. In addition, it was observed that elevated algae cell density reduces light attenuation, thus lowering their contribution to coliform neutralization [84]. Also, it is hypothesized that algae are promoting decay of fecal coliforms and producing a neutralizing substance. This phenomenon is attributed to observations in natural eutrophic lake in tropical climate [85] as well as in laboratory experiments with different types of wastewater [69]. Still, this mechanism is not fully understood, thus a relevant topic for further research remains open.

Additional research topic regarding algae-based coliform removal from wastewater is the influence of climate on outdoor treatment facility performance. Depending on the latitude, earth surface receives different solar radiation. Also, location-specific temperature is likely to influence algae growth and resulting coliform inactivation. However, to support these assumptions, detailed case studies are required.

#### *Emerging Contaminants*

Over the last decades there has been an increasing concern about the content of emerging contaminants (ECs) in wastewater. They include a vast variety of organic and inorganic micropollutants, such as pharmaceuticals, prescription and illicit drugs, personal care products, nanomaterials, perfluorinated compounds and other substances [86]. With effluent wastewater, ECs are delivered to natural waters and can also be found in drinking water. Although these pollutants are mostly present in trace concentrations ( $\mu\text{g l}^{-1}$ ), they are known to have adverse effect on aquatic and terrestrial organisms as well as on human health [87], [88].

The main pathways of ECs to the wastewater include domestic use of personal care products and household chemicals, use of prescription and illicit drugs and subsequent excretion of their residuals, disposal of expired medicine as well as waste disposal from pharmaceutical industry, chemical labs or hospitals. Despite the awareness of EC presence, there are no legal regulations for their removal from wastewaters. In addition, conventional wastewater treatment plants mostly are not designed for EC removal, which results in continuous EC loads to natural aquatic ecosystems [87].

Several advanced wastewater treatment methods have been applied for ECs removal. However, the success of their removal largely depends on the chemical properties of certain micropollutants. A study by [89] showed that advanced treatment methods like coagulation, flocculation and lime softening could not sufficiently reduce the total EC content, mainly due to pollutant competition for sorption surface. [90] demonstrated that more substantial EC reduction can be achieved by methods used for drinking water preparation, with powder activated carbon and chlorination being more effective than others. Still, the removal rate was variable between certain substances. In addition, chlorine by-product formation from its reaction with ECs makes

it unsuitable for this purpose. Among others, membrane filtration technology such as reverse osmosis and nanofiltration have shown the most promising results with nearly complete EC removal [91]. Still, the main disadvantage of membrane filtration is its high consumption of energy.

Bioremediation has been proposed as an energy-efficient technique for EC content reduction in wastewater. Different studies on removal of ECs like pharmaceuticals, personal care products and pesticides have been done in constructed wetlands [92], [93], all showing highly variable substance-specific removal rates. Removal of ECs is also studied in algae-based wastewater treatment systems. A comprehensive review by [67] shows that similarly to constructed wetlands, EC removal rate in HRAPs varies from insignificant to complete reduction. Also, results from studies conducted in closed algae reactors [94] indicate that removal efficiency of emerging contaminants depends on chemical properties of each substance individually, and is largely determined by treatment system scale, operational regime and design as well as climatic conditions. Thus, it can be concluded that the unpredictable and selective performance makes algae production rather unsuitable for emerging contamination reduction, and likewise is the applicability of AAFW.

#### *C. Algae-Based Wastewater Treatment Efficiency in Cold Climate*

The microalgae and aquatic vegetation use for wastewater treatment in higher latitudes is limited by the short vegetation season as well as low temperatures and shorter daylight hours in seasons other than summer. Moreover, low temperature becomes a serious concern if filter feeding organism use is considered for algal biomass harvest. Due to seemingly less efficient performance determined by low temperature and PAR, algae use for contaminant removal from wastewater in cold and temperate climates has not been extensively investigated. Still, the existing studies highlight certain conditions for successful wastewater phytoremediation in cold climate. Operation under low temperature also becomes a challenge if biofiltration is to be applied for algal biomass harvest.

The potential for successful algae-based wastewater treatment at low temperature is shown in a study by [33], which indicates the importance of algal strain origin. Their results demonstrated that *Clamydomonas sp.* isolated in cold climate zone lost productivity and the resulting nutrient uptake rate at temperature above its natural environment. Further, a study by [95] showed that at cold climate conditions phosphorus removal rate was significantly affected by the available PAR, while the role of temperature was minor. The importance of sufficient light availability over temperature and origin of algal strain was also shown by [96]. They used marine cyanobacteria (*Oscillatoria sp.*) and diatom (*Phaeodactylum tricoratum*) strains from temperate climate in a mesoscale corrugated raceway and achieved continuously complete removal of both ammonium and orthophosphate even at temperatures as low as 4 °C. [97] demonstrated the capability of polar cyanobacteria (*Phormidium sp.*) strain in nutrient content reduction in growth medium at 5 °C and constant illumination of 225  $\mu\text{mol m}^{-2}\text{s}^{-1}$ . However, due to the low temperature, reduced nutrient removal pace was observed.

The abovementioned studies that used microalgae showed reduced biomass growth rate at lower temperatures. Still, considerable nutrient removal rates were observed. An indication for causes of such relation can be found in a study by [95]. During their experiment, most of the phosphorus was reduced by algal luxury uptake and stored as polyphosphate which is not used for biomass production. However, it is not clear whether this is a temperature driven mechanism. In addition, reduced biomass growth also points to indirect nitrogen compound removal, while direct utilization of nitrogen would result in protein synthesis and algal biomass production [20].

Despite the promising contamination removal rates in experimental scale studies, algal bioremediation efficiency show decrease with system scale-up. In a comprehensive study by [98], it was conformed that at temperature below 10 °C and PAR below 200  $\mu\text{mol m}^2 \text{s}^{-1}$  substantial reduction of both BOD and COD (90 % and 65 % respectively) can be achieved in a pilot-scale HRAP. However, a lower reduction of total nitrogen and phosphorus by 47 % and 20 %, respectively, was observed.

Even though nutrient reduction by algae has been successfully demonstrated at low temperatures, it is apparent that outdoor treatment facilities cannot be used during winter at negative temperatures, when most biological processes stop and no contaminant removal takes place. As a solution, greenhouse treatment plants can be used. The performance of such an approach was studied by [99], who used a hybrid consisting of conventional wastewater treatment plant and AAFW for additional treatment. This setup showed reduction of total nitrogen, total phosphorus and heavy metal concentrations by 39 %, 28 % and 47–98 %, respectively. Additionally, pathogen content was reduced close to bathing standards. However, it was concluded that the estimated energy costs required to run such a system in cold climate cannot compete with conventional wastewater treatment, unless valuable biomass is produced for profit. Similar study was conducted by [100], who achieved higher nutrient reduction rate. However, the resulting energy consumption led to the same conclusions.

#### IV. CONCLUSIONS AND RECOMMENDATIONS

The acquired knowledge on algae-based wastewater treatment marks a promising alternative for conventional wastewater treatment as cost-effective and sustainable technology. Still, certain limitations should be overcome before implementing microalgae as a key organism for the wastewater treatment process. Algae biomass harvest is recognized as the major obstacle for competitive wastewater treatment performance. All the currently known harvest methods have their drawbacks related to economically detrimental large-scale use and downstream processing limitations. Although understudied, artificial aquatic food-web for wastewater treatment is an attractive alternative for existing harvest techniques due to its low energy consumption and sustainable performance under optimal conditions. Nevertheless, certain limitations exist also to this harvest approach. Firstly, its applicability in colder climate, where additional energy is necessary for optimal performance conditions makes this method noncompetitive with conventional wastewater treatment. Similarly to arctic algal strains, the use of

filter feeding organism species originating from cold climate might result in satisfactory performance under low temperatures. However, this assumption needs to be confirmed in a case study. Another consideration for testing different species is related to AAFW application in open systems, where natural shift of dominant algal specie is likely to develop. Thus, biofiltration stage of the AAFW system might lose its efficiency due to grazer preference of food.

Since algae-based wastewater treatment is viewed as a raw material source in bioenergy production and valuable substance extraction, biofiltration is not applicable as a biomass harvest method. Because of biofiltration, valuable substances produced by algae are used for filter feeder metabolism. To cover the performance costs and gain profit, AAFW for wastewater treatment is recommended to be oriented on high-valued organism aquaculture.

Even though algae-based wastewater treatment leads to efficient reduction of coliforms, the mechanism and its drivers are still not completely clear. Also, not all metal ions and emerging contaminants can be reduced using algae. Therefore, if filter feeding organisms are used for algae harvest, they can be exposed to harmful and toxic substances, which can limit the use of aquaculture products and ultimately collapse the AAFW system. Thus, before the impact of microcontaminants on AAFW organisms and their further biomass use is investigated, this wastewater treatment method has limited applicability with respect to wastewater origin and its chemical content.

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**Aigars Lavrinovičs**, PhD student at the Water Research laboratory in Riga Technical University and researcher at the Institute for Environmental Solutions. Fields of research: Water pollution control.  
Address: Kīpsalas Street 6a – 263, Riga, LV-1048, Latvia  
E-mail: aigars.lavrinovics@rtu.lv

**Tālis Juhna**, Dr. sc. ing., Professor of Riga Technical University (RTU), Department of Water Engineering and Technology, Institute of Heat, Gas and Water Technology, the Faculty of Civil Engineering. Since 2012, he has been a Vice-Rector for Research at Riga Technical University.  
Main research interests: drinking water treatment; removal of natural organic matter; iron and manganese removal from groundwater; bacterial growth in water distribution networks; rapid methods for identification of pathogens in drinking water.  
T. Juhna is the author of more than 100 scientific publications.  
E-mail: talis.juhna@rtu.lv