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Review

A Review on the Reliability and the Readiness Level of Microalgae-Based Nutrient Recovery Technologies for Secondary Treated Effluent in Municipal Wastewater Treatment Plants

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Abstract: Algae-based wastewater treatment technologies are promising green technologies with huge economical potential and environmental co-benefits. However, despite the immense research, work, and achievement, no publications were found wherein these technologies have been successfully applied in an operational environment for nitrogen and phosphorus removal of secondary treated effluent in municipal wastewater treatment plants. Based on a literature review and targeted comprehensive analysis, the paper seeks to identify the main reasons for this. The reliability (considering inlet wastewater quality variations, operating conditions and process control, algae harvesting method, and produced biomass) as well as the technology readiness level for five types of reactors are discussed. The review shows that the reactors with a higher level of control over the technological parameters are more reliable but algal post-treatment harvesting and additional costs are barriers for their deployment. The least reliable systems continue to be attractive for research due to the non-complex operation and relieved expenditure costs. The rotating biofilm systems are currently undertaking serious development due to their promising features. Among the remaining research gaps and challenges for all the reactor types are the identification of the optimal algal strains, establishment of technological parameters, overcoming seasonal variations in the effluent's quality, and biomass harvesting.

Keywords: algae-based technology; circular economy; Green deal; algae; microalgae; phosphorus recovery; phosphorus removal; review; wastewater treatment

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1. Introduction

Deepening the knowledge about the impact of human activities on the environment reflects on all areas of our lives and leads to setting new, more informed goals. This drive is now more focused than ever on green and energy-neutral technologies. Most of the recent policies recognize this and appeal to scientific developments that are in line with concepts such as the EU Green deal [1] and circular economy plans [2].

In the field of wastewater treatment, algae-based technologies emerged in the 50′s of the 20th century and continuously evolved ever since [3,4]. Nowadays, they are seen as promising green technologies with huge economical potential and a number of environmental co-benefits [5–7].

The wide range of algal groups, the different environments that they inhabit, and the broad spectrum of elements and compounds they consume or accumulate make them suitable for application in the treatment of nutrient dense mediums, such as wastewater [6,7]. That is why the algae-based wastewater treatment technologies are studied for their use for various purposes, such as nutrient removal of phosphorus (P) and nitrogen

(N), organic matter degradation, pathogenic microorganism elimination (disinfection), micropollutants and heavy metals reduction, etc. [8–12]. Most algae wastewater treatment systems use only microphytes (also known as microalgae) with typical cell sizes below 30 µm in contemporary reactor designs [13,14].

Despite the wide field of possible applications for algae in the wastewater treatment process, they are mostly adopted for P and N removal [15–17].

The focus of this paper is precisely on the most researched applications of the algae-based wastewater treatment [18–20]. The microalgae technologies for tertiary treatment aimed at P and N removal after the conventional biological step at the wastewater treatment plants (WWTP) will be the subject of the review, as visualized in Figure 1.

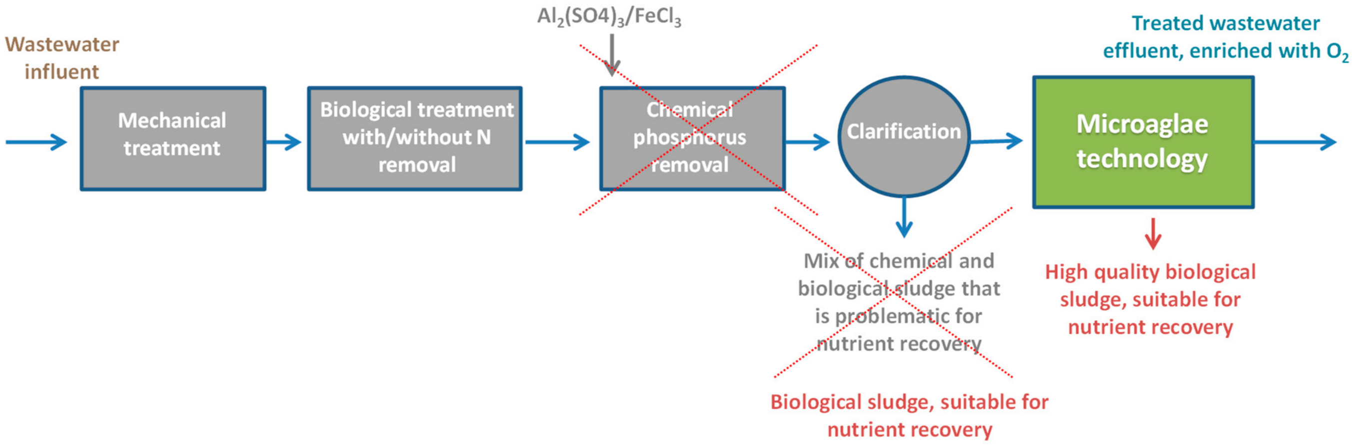

Figure 1. Algae-based wastewater as a tertiary treatment step for P and N removal. Scheme adapted from Valchev et al., 2021 [21].

The use of algae as a tertiary treatment step has many advantages: eliminated need for the use of reagents for P reduction; eliminated recirculation of wastewater for N removal (reduced energy consumption); and improved quality of the generated excess biomass, which allows for its further application through safer nutrient utilization [8,22,23]. Furthermore, the use of algae increases the effluent quality by decreasing the water acidity and increasing the dissolved oxygen (DO) concentration [8,11].

Despite the high number of worth-to-be-considered advantages and the great amount of time for their development, these technologies are still not applied in an operational environment [24]. This paper seeks to identify the main reasons for the failure to reap the benefits of the algae-based technologies for N and P removal and recovery in municipal WWTPs. Two main aspects are discussed: the reliability of the developed technologies and their readiness level. Based on the review of the research achievements and the targeted comprehensive analysis, the paper concludes on the research prospects.

2. Global Achievements

2.1. Algal Cultures

The preliminary selection of appropriate algal cultures is of key importance for achieving the needed wastewater treatment rate.

Despite the major developments and huge progress, there is still no unified optimal algal strain/consortia for wastewater treatment, and novel strains are constantly tested for their efficiency [5,25]. Even though there are no specific criteria for algae selection for N and P removal in WWTPs, the basic considerations include: (1) non-toxic culture or consortia, (2) rapid adaptation to the wastewater medium, (3) intensive growth of the culture in water, and (4) storage of high value substances in the cells [5,8,25–27].

Another major consideration, especially regarding open reactors, is phycoprospecting, i.e., selection of a strain that is local for the geographical location of the WWTP site [28,29].

This way the algal biomass develops faster in the reactor since it is already adapted to the regional climatic conditions (light intensity, seasonal variations, temperature fluctuations, etc.); the accommodation period is shorter and the treatment process is initiated faster. Another advantage of this approach is that the system is more stable and there is less chance of displacement of the preliminary selected culture [21,30,31].

Algal genera, such as Chlorella, Scenedesmus, Spirulina, Micractinium, Actinastrum, Pediastrum, Dictyosphaerium, Coelastrum, Botryococcus, Phormidium, Nannochloris, and Ulothrix, are some of the most commonly used throughout the scientific literature, regardless of the specific geographical location of the site. However, none of them are considered optimal for practical use for wastewater treatment [8,25,26,32].

2.2. Nutrient Removal Mechanisms

The enormous amount of research that has been carried out so far reveals the N and P removal mechanisms, which is a very helpful step towards a better understanding of the nature of these processes and enables the development of different technologies.

In terms of P removal, the most commonly used form of P that is preferred by the algae is inorganic orthophosphates, but they can also adapt to organic or inorganic polyphosphate biochemical substrate by using extracellular substances (enzymes) to degrade them [16,17]. The P removal in wastewater usually occurs in two main mechanisms [17]. One of them is full biological removal, which includes active transport (for cell use), biosorption (to the cell walls), and bioaccumulation (through build-up in the cells of different stock compounds or as parts of different compounds taken up by the algal cells, including phosphate groups) [16,33]. The other mechanism is known as biologically induced chemical/alkaline precipitation of P. It is triggered when the preferable, available inorganic CO_2 in the water is fully taken up by algae (lowering the acidity of water). After that, they switch to HCO_3^- and CO_3^{2-} sources of inorganic carbon, leading to an increase in the OH^- ions concentration and a rise in the pH levels, respectively [34–36]. This process induces the formation of Ca^{2+} and Mg^{2+} phosphate salts, which are then precipitated [34–36].

For N removal, algae can adapt very well to the most common forms of N that are available in municipal wastewaters—NH_4^+, NO_3^-, and NO_2^- ions [16,33]. There are two N removal mechanisms in wastewater [37,38]. The first one is the biological uptake of N for the needs of processes in the cell (in building compounds, nucleic acids, enzymes, hormones, vitamins, stock substances, etc.) [16,33]. The order of preference of nitrogen sources for microalgae is $NH_4^+ > NO_3^- > NO_2^-$ [16,33]. However, if limited or no ammonia is present in the wastewater medium, the algae will utilize the NO_3^- and NO_2^- anions for the mentioned nitrogen needs of the cells. Nitrogen as NO_3^- can be assimilated with the Nit receptor in the plasma membrane. Nitrates are then converted into NO_2^- in the cytosol with the help of a nitrate reductase, after which NO_2^- can be transformed into NH_4^+ in the chloroplast with the use of the nitrite reductase [16,33]. This biological uptake by algae is the main mechanism for N removal in secondary treated municipal effluents since most of the ammonia is transformed into nitrates through the aeration processes of the biological treatment. The second mechanism is known in the literature as "stripping" of the ammonia, which is most common for algae-based reactors with an additional aeration system for external CO_2 supply. It is usually triggered by intensive photosynthesis that induces an increase in the pH levels (above 10–10.5), at which a transformation from ammonia ions (NH_4^+) to ammonium gas (NH_3) occurs. The gas is then carried out into the air through bubbles, which are produced by the aeration system of the reactor [37].

2.3. Technologies for Nutrients Removal

Depending on the environmental conditions, the effluent requirements and the specifics of the existing or newly designed WWTP, the studied algae-based wastewater treatment systems varied across a broad range of technological designs. The treatment systems can generally be divided into three main groups—suspended, algal bead (active immobilization), and attached (passive immobilization) growth [39–41]. Each of these groups has specific reactor design variations (Figure 2).

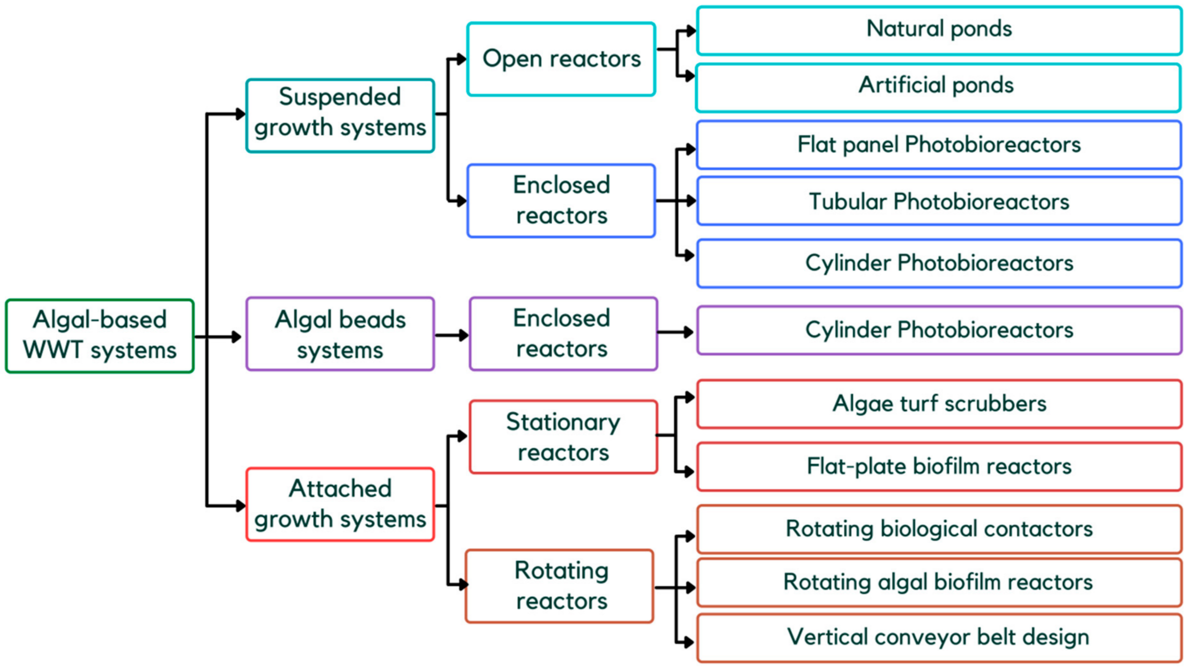

Figure 2. Tree diagram of the most commonly used algae-based wastewater treatment reactor designs.

2.3.1. Suspended Growth Systems

Out of the three main reactor design branches, shown in Figure 2, the suspended growth systems are the most commonly used regardless of the specific climatic conditions. Two major groups of reactor configurations—open and enclosed—are reported (Figure 2).

- Open reactors

The open suspended algal growth reactors include natural or artificial ponds. Perhaps the most investigated reactor configuration is the High Rate Algal Ponds (HRAP) that are usually formed into Raceway ponds [31,40]. These reactor designs consist of artificially constructed water beds that function in a continuous or batch mode [39,42,43]. They are divided into parallel canals in which the wastewater–algae suspension reacts to achieve the full treatment process. Mixing is provided through a slowly revolving paddlewheel, creating a steady flow (approx. optimal flow velocity of 0.3 m s^{-1}) throughout the system and is additionally assisted by the natural wind masses above the water surface (Figure 3) [44]. Microalgal cells are freely floating into the wastewater medium through the shallow ponds (0.15–0.50 m) [39,45,46]. These systems generally use natural solar illumination as a light source. The shallow depth of the HRAP allows the light to penetrate to all layers of the suspension. The algal biomass in these systems needs to be carefully monitored as internal shading inhibition might occur if hyper concentration of algae is reached in the reactor [43,47]. The HRAP reactors are preferred to the wastewater treatment realm, mainly because of the easy and inexpensive construction and operation [31,40].

Some attempts for advanced technological designs of the HRAP include CO_2 addition ($HRAP_W$) to the water through an aeration system to intensify the algal activity and reach better pH management [48,49]. However, this design has not proven its feasibility yet since most of the CO_2 does not dissolve into the water and it flows back into the atmosphere, resulting in higher operational costs with little efficiency and artificially increased CO_2 emissions [31].

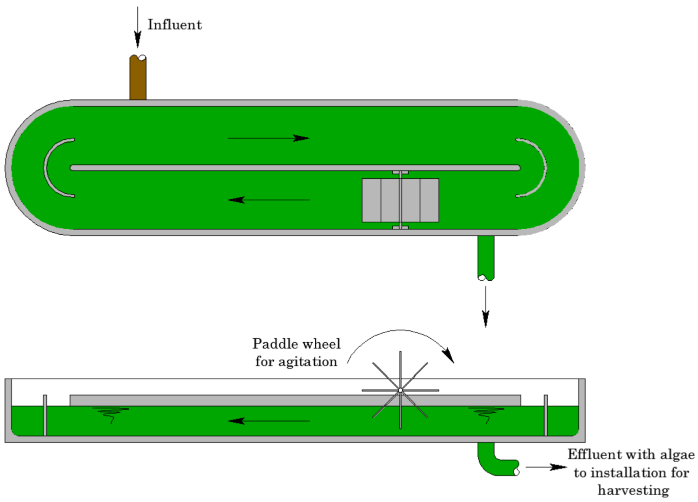

Figure 3. Open Raceway pond scheme adapted from Arbib et al, 2017 and Sutherland et al., 2020 [42,45].

- Enclosed reactors

These reactor configurations are known as Photobioreactors (PBRs). They can also operate in a continuous or batch mode [39,50,51]. The algae–wastewater suspension is separated from the surrounding environment via transparent or semi-transparent barriers, usually made of glass, plexiglass, polycarbonate and plastic bags, etc. [39,52]. Different reactor set-ups are available in order to optimally utilize the light from the source. The most commonly used systems in wastewater treatment applications include tubular, flat panel, and cylinder configurations (Figure 4) [39,53]. A better overall control of all the parameters of the system and superior light utilization are achieved through the insulation of algae in comparison to the open systems [39,52,53]. However, extra supplementary systems are required to provide the appropriate vital medium for algae to achieve such intense processes. Usually, the addition of aeration, pumping, and degassing installations are necessary to achieve CO_2 concentration control, pH regulation, O_2 toxicity prevention, steady flow and mixing conditions, etc. [39,52,53]. The provision of all elements of the additional equipment increases the capital and operational expenditures and leads to complications during exploitation, hindering the development of the enclosed suspended growth algae-based wastewater treatment systems [39,52,53].

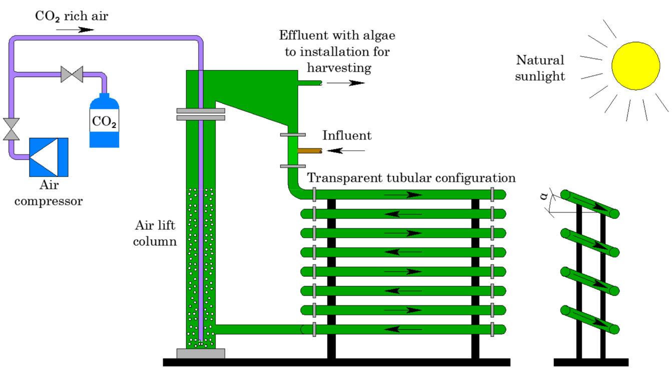

Figure 4. Enclosed tubular photobioreactor scheme adapted from Ting et al., 2017 [39].

2.3.2. Algal Bead Systems (Active Immobilization)

Another widely researched wastewater treatment reactor configuration is the active immobilized algal bead systems. These reactors resemble the enclosed suspended growth systems with the difference that the algal cells do not float freely into the water medium but are encapsulated into separate beads with specific a biomass concentration inside [39,54]. The beads are preliminarily prepared in a laboratory setting, either through covalent bonding, adsorption, semi-permeable membrane encasing, or polymer enfolding (Figure 5) [39,54,55]. The main reason for the development of such technology is the mitigation of the post-treatment algal harvesting and higher control over the algal monoculture contamination [39,54]. Also, these systems allow for the use of higher algae concentrations that induce processes with a higher intensity [39,54]. Nevertheless, as with the enclosed suspended growth systems, the immobilized algal bead reactors require the whole supplementary equipment package with an additional preliminary encapsulation step [39,54].

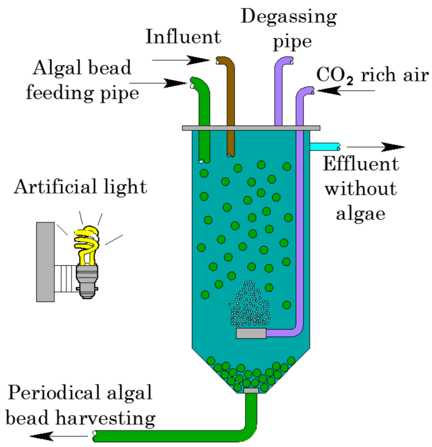

Figure 5. Immobilized algal bead reactor scheme adapted from Ting et al., 2017 [39].

2.3.3. Attached Growth Systems (Passive)

With the attached growth wastewater treatment systems, the algal biomass entwines a carrying media matrix while the influent wastewater provides the sufficient amounts of biochemical substrate [39,56,57]. By controlling the thickness of the biofilm, the excess amounts of algae are harvested easily, allowing for constant effluent and biomass quality results [39,57]. In order to achieve optimal conditions for algal growth, and, respectively, wastewater treatment performance, two main groups of reactor designs have been currently developed: (1) Stationary and (2) Rotating reactors [39,57].

- Stationary attached growth reactors

Stationary biofilm reactors use polymer mats for the carrier matrix and have no moving parts in their configuration. Wastewater is passed over the matrix on a thin layer so that the light can penetrate the system entirely and reach all algal cells [15,39,57]. The biofilm thickness is controlled by scraping [15,57]. The two main reactor configurations used in the literature are (1) Algae turf scrubbers (ATS) and (2) Flat-panel biofilm reactors [39]. ATSs (Figure 6) are usually open systems (canals) with 0.10 to 0.20 m of depth, resembling the open pond suspended growth reactors, but with a thin polymer matrix on the bottom [39]. These systems have a simplified operation but require extremely large amounts of area due to their reduced depth [46]. Flat-panel biofilm reactors are enclosed in single or twin-layer systems that have improved control and better utilization of the light but require all the additional installations for an enclosed reactor design, making their operation much more complicated [58].

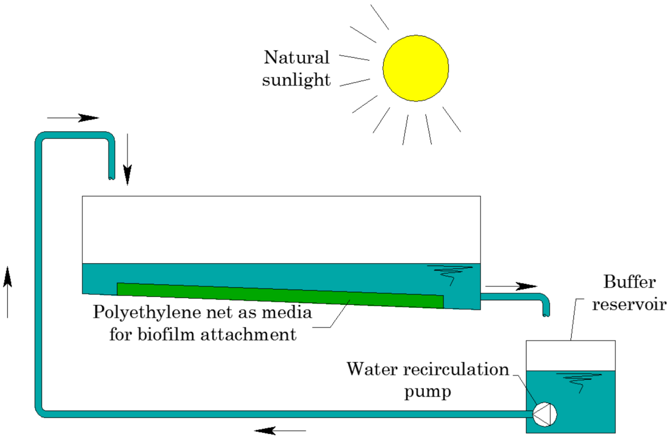

Figure 6. Algae turf scrubber scheme adapted from Shen et al., 2009 and Ting et al., 2017 [39,59].

- Rotating attached growth reactors

In order to save area and to achieve better light, substrate, and air transfer, rotating attached growth systems were developed [15,39,57,60]. In these reactors, the carrier matrix is attached to an electrically driven rotating shaft that interchanges the attached algae between the wastewater and the surrounding air medium [15,39,57,60]. The three main reactor types of this group use similar principles that are executed in various ways. They include: (1) Rotating biological contactors (RBC), (2) Rotating algal biofilm reactors (RABR), and (3) Vertical conveyor belt design (VCBD) [15,39,57,60]. RBCs resemble the biodiscs used for attached growth biological wastewater treatment with activated sludge, but are rarely used since they do not utilize the available light properly [15,39,57,60]. RABRs use natural (cotton, etc.) or synthetic (plastic polymer, etc.) ropes as an attachment matrix, which are wrapped around a rotating cylindrical drum. Excess algal biomass is harvested with the passing of the rope through an opening with a specific diameter, allowing for the maintenance of a constant thickness of the biofilm [15,39,57,60]. These systems perform better than the RBCs and are currently under development [15,39,57,60]. VCBDs use the same principle as RABRs but require lower footprints since the width of the canal for the placement of the conveyor belt is more narrow (Figure 7). This is achieved through the use of wider conveyor belts that provide the same amount of attachment area as a few revolutions of a thin rope around the drum of the RABR [15,39,57,60]. The biomass is directly scraped at the top of the conveyor belt with a paddle [15,39,57,60]. This is perhaps the most promising patented technological design reactor configuration for the attached growth algae-based wastewater treatment.

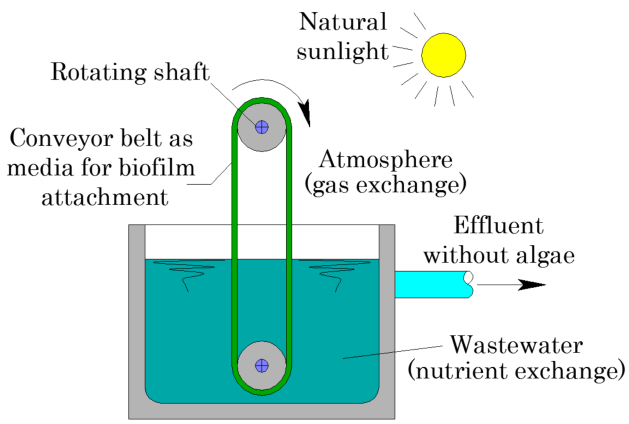

Figure 7. Vertical conveyor belt reactor scheme adapted from Zhao et al., 2018 [60].

3. Reliability of the Algae-Based Wastewater Treatment Technologies for N and P Removal from Secondary Treated Effluent

Four major aspects for the reliability of the algae-based nutrient removal and recovery technologies will be analytically discussed below:

(1) **Wastewater quality**—a review on how sensitive the technology is in regard to the variable wastewater quality at the inlet of the algae-based reactor;
(2) **Operating conditions and process control**—a review on how stable the operation is, i.e., whether constant effluent quality could be ensured;
(3) **Algae harvesting method**—a review on the availability of an appropriate algae harvesting method as well as how reliable the method is;
(4) **Produced biomass**—a review on how safe for re-use the biomass is.

Since this section provides a significant amount of information, it was organized into specific subsections, as shown in the related guiding table (Table 1).

Table 1. Navigation table for Section 3.

Aspects Considered in the Reliability Analysis	Subsections
3.1. Wastewater quality at the inlet of the algae-based reactor	3.1.1. Carbon to Nitrogen (C:N) ratio
	3.1.2. Nitrogen to Phosphorus (N:P) ratio
3.2. Operating conditions and process control	3.2.1. Presence of invasive microalgae, bacteria, protozoa and macro grazers affecting the microalgal growth
	3.2.2. Light utilization, illuminated surface to reactor volume ratio (S_f/V) and algal bio-mass concentration/algal biofilm thickness in the reactor
	3.2.3. Water flow velocity, agitation and shear stress on the algal cells/beads
	3.2.4. Temperature control
	3.2.5. pH variation, transfer of CO_2 and O_2 oversaturation inhibition
	3.2.6. Evaporation control
3.3. Algae harvesting method	-
3.4. Produced biomass	-

3.1. Wastewater Quality at the Inlet of the Algae-Based Reactor

Algae generally need macro concentrations of C, N, and P, and micro concentrations of K, S, Ca, Mg, Fe, Na, and Si. The share of these macro and micro elements varies with different strains or consortia [44,61]. Le et al., 2019 reports that, despite the needed micro elements, the general molecular formula of microalgae can be presented as $CH_{1.83}O_{0.48}N_{0.11}P_{0.01}$. Since hydrogen, oxygen, and the microelements are supplied with wastewater, the limiting elements for the growth of the algae are C, N, and P [61]. The ratios between those three elements may play a significant role in the dominance of the selected algal strain/consortia over the natively grown cultures in the medium [61,62].

The wastewater quality at the inlet of the algae-based reactor, and especially the share of the macro elements in it, influences the treatment process significantly—regardless of the reactor/technology. However, some bioreactors are less sensitive to the variations and achieve more constant results, even with high variability in the inlet wastewater characteristics.

Estimation of the reliability of the available algae-based wastewater treatment reactor configurations regarding the process stability, with respect to the wastewater quality variation, is graphically presented in Figure 8.

This estimation is based on the reported research works, which are discussed below.

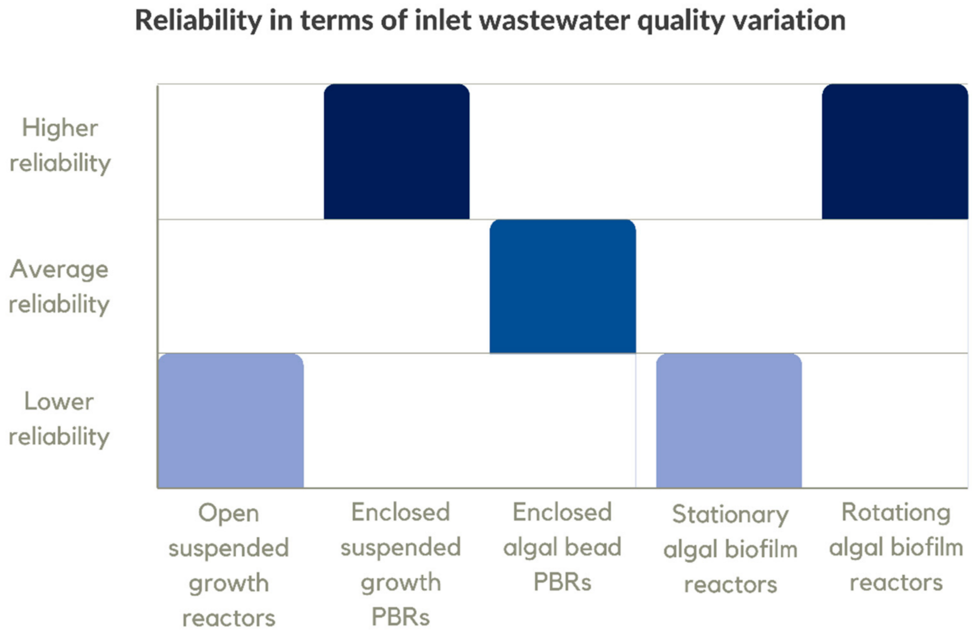

Figure 8. Reliability of the available algae-based wastewater treatment reactor configurations in terms of wastewater quality variation.

3.1.1. Carbon to Nitrogen (C:N) Ratio

The C:N ratio plays a key role in the algae wastewater treatment process. The typically low C:N ratio in most secondary effluents is usually a limiting factor for the wholeness of the treatment process [63]. Zheng et al., 2018 found that, after experimental work with different types of wastewaters, a C:N ratio of 7.9:1 could be considered optimal for high nitrogen and phosphorus removal efficiencies (over 90% on both indicators) when algae-based wastewater treatment is used [63]. In lower C:N ratios, the inorganic carbon in wastewater is usually insufficient for the photoautotrophic biochemical processes of algae, and their activity decreases, leading to both N and P removal deterioration [63]. On the other hand, the organic carbon content of wastewater can hinder the algal activity if a COD:N ratio higher than 20:1 is present in the medium [62,64]. According to Ma et al., 2017, the preferable values of COD:N ratios are 5:1 to 10:1 [64].

Different reactor designs offer various techniques for dealing with the inorganic carbon deficiency in wastewater. Usually the open pond suspended growth system and the stationary fixed film bioreactors have lower control over the C:N ratio since they use simpler equipment and more relieved operation, which often depend on the natural conditions of the environment [46,65]. Respectively, they are more prone to effluent quality compromise due to the suboptimal C:N ratio in the inlet, since the addition of CO_2 from an external source is inefficient in their designs [46,65,66]. Hence, these two reactor designs are the least reliable in terms of C:N ratio control. The RABR systems have improved CO_2 transfer compared to the stationary biofilm or open pond suspended growth systems since the rotating parts of the reactors increase the contact of the algal biomass with the naturally available CO_2 in the surrounding atmosphere. Even though this process is not controlled by any additional sensory equipment, when the algal biofilm thickness is properly maintained, the inlet C:N ratio has a lower impact on the nutrient removal efficiency, making the technology more reliable [67,68]. On the other hand, the enclosed algae-based WWT systems (suspended and immobilized algal growth) are supplied with an exogenous CO_2 source. The aeration system using CO_2 enriched air (1–5% CO_2) controls the inorganic carbon content in wastewater through a sensory system, making the enclosed reactors more stable and reliable when C:N ratios at the inlet are lower [69,70]. Hence, the enclosed suspended growth reactors achieve the highest reliability in terms of C:N inlet ratio variation. The enclosed immobilized algal bead systems also benefit from the aeration system but the insulation of the granules lowers the level of nutrient transfer. Furthermore, the aeration intensity and the algal growth inside the granules should be strictly monitored since tears can occur in the shell, lowering their reliability to an average level [71,72].

3.1.2. Nitrogen to Phosphorus (N:P) Ratio

Inlet N and P loads influence the nutrient removal rates when algae-based wastewater treatment is used. Rani et al., 2021 report in their review that some studies show controversial results [25]. Some authors provide data that higher initial nutrient loads result in higher microbial biomass growth and better treatment than moderate loads, whereas other papers state that better performance of the algal biomass is achieved in the treatment of more diluted wastewaters [25]. The study concludes that the acclimatization of the algal species in general is dependent on the type of wastewater used and an optimum amount of nutrient load for the used strain is required for better performance of the algae-based wastewater treatment system [25].

In this regard, another important ratio of macronutrients for the vital functions of algae that influences wastewater treatment efficiency is the N:P ratio. Even though this ratio is usually balanced by the internal processes of the algal biomass itself, Le et al., 2019 reports a globally applied ratio of 11:1 for optimal microalgal growth [61]. Arbib et al., 2013, on the other hand, reports that for optimal N and P removal, a ratio for N:P of 9:1 to 13:1 should be kept when using the *Scenedesmus obliquus* strain, and the algal behavior changes once this range is compromised [73]. Wang et al., 2010, which was cited by Li et al., 2019 and Mohsenpour et al., 2021, reports a preferable N:P ratio range of 6.8:1 to 10:1 if the biomass is used for nutrient removal [44,62,74]. However, a report by Liu and Vyverman from 2015, cited by Rani et al., 2021, suggests that for filamentous benthic algal families such as *Cladophora*, *Klebsormidium*, *Pseudanabaena*, the optimal N:P ratios for municipal wastewater nutrient removal should be ranging from 5:1 to 15:1 for *Cladophora*, from 7:1 to 10:1 for *Klebsormidium*, and from 7:1 to 20:1 for *Pseudanabaena* [25,75]. In general, the different strains of algae are impacted by the different N:P ratio of different magnitudes. Some strains from the *Cladophora* reach greater nutrient removal efficiencies in waters with lower N:P ratios. Algal families like *Pseudanabaena* have superior performance with higher N:P ratios [75]. The limitation of this parameter in terms of range is dependent not only on the wastewater characteristics but also on the used strain/consortia for the specific study. No general, universal conclusion for the algae-based wastewater treatment as a whole can be made about the lowest value of the N:P ratio at which the nitrogen becomes the limiting factor and, respectively, the highest value of this ratio when the phosphorus is insufficient.

Mohsenpour et al., 2021 report in their review an averagely used secondary treated wastewater C:N:P ratio of 100:34:7 (C:N = 2.94 and N:P = 4.85) [44]. These values are at the low end or even below the overall reported optimal C:N and N:P ratios, meaning that the key consideration in the algae-based wastewater treatment for nutrients removal should be the strain selection and the specific operation of the WWTP. In this regard, the reliability of the system is not based on the specific bioreactor design, but rather on the operation and the specific regime at which the secondary treated effluent is mixed with the algae. Generally, fixed film bioreactors achieve more stable conditions with the algal biomass attached to a carrier, especially when rotating systems, such as RABR, are used. Zhao et al., 2018 reports that when a specific operation of the reactor (HRT, biofilm thickness control, etc.) is reached, algal growth may not be limited by the total nitrogen and total phosphorus, even when ammonia and Ortho-P concentrations reach zero [60]. Also, according to Nur et al., 2018, a continuous or semi-continuous inflow operation for the suspended growth algae-based wastewater treatment systems is superior to batch systems due to a lower variation in the inlet wastewater characteristics, hence algae can adapt their activity more easily with a steady variation in medium conditions [76]. Enclosed suspended algal growth systems with continuous operation of the reactor have better control over the parameters in the reactor and, hence, they reach a higher reliability level in terms of inlet quality variations [39,52,53]. The enclosed immobilized algal bead systems attain only an average level of reliability, due to their limited nutrient transfer through the coating layer of the granules [39,54].

3.2. Operating Conditions and Process Control

Algae-based wastewater treatment relies on the vital functions of the biomass, thus the maintenance of stable operating conditions shall be considered from the point of view of the biotic and abiotic factors, which influence the algal activity in the respective bioreactor configuration. These factors include temperature (of the medium and the environment), illumination (solar or artificial), bacteria and grazers presence, etc. The higher the control over the influencing factors, the higher the reliability of the technology, i.e., the lower the risk is of a compromised treatment process. Some factors can be controlled more easily with established and stable equipment embedded in the respective reactor design, whereas others are either too difficult, expensive, or even impossible to control.

The estimated reliability of the algae-based wastewater treatment technologies in terms of the maintenance of stable operating conditions and process control is shown in Figure 9.

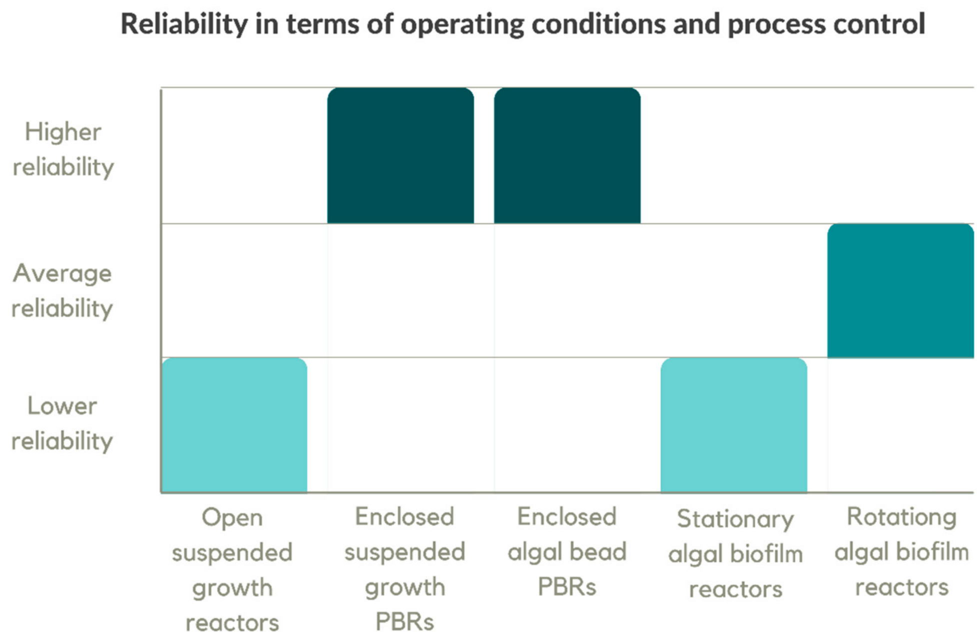

Figure 9. Reliability of the available algae-based wastewater treatment reactor configurations in terms of maintenance of stable operating conditions and process control.

This estimation is based on the analytical review of the research achievements so far. With respect to the specific reactor design, some systems provide a higher level of control over the factors that influence the wastewater treatment process, which ensures stability of the operation and, consequently, more consistent effluent quality. Six operating parameters of the algae-based wastewater treatment systems are analyzed below.

3.2.1. Presence of Invasive Microalgae, Bacteria, Protozoa and Macro Grazers Affecting the Microalgal Growth

One of the main issues in algae-based wastewater treatment is the preservation of an initial projected monoculture or mixed culture integrity throughout the wastewater treatment process. Different invasive algal strains from various evolutionary lines, such as *Chlorella*, *Scenedesmus*, etc., can enter the reactor through the influent or the surrounding environment, leading to a shift in the technological parameters. The native algal strains could reduce the N and P removal efficiencies, nutrient reduction rates, hinder post-treatment algal harvesting, and even deteriorate the effluent quality through extracellular toxic compounds (allelochemicals) [8,39]. Wastewater is a breeding medium for bacteria, protozoa, viruses, etc., that can affect the algae-based wastewater treatment process. The specific conditions in wastewater can lead to the formation of either symbiotic or asymbiotic relationships between microalgae and the other microorganisms. Variations in micro- and metafauna of the influent directly affects the cultivation of microalgae and biomass production. Some microalgal species have antibacterial activity, which could inhibit the growth of bacteria, but, on the other hand, some prokaryotes cause adverse conditions for algal growth with reported data that bacteria can excrete metabolites presenting an algicidal effect. Therefore, the type of biotic consortia that is present dictates the nature of the relationship between the microalgae and other microorganisms [8,25,77]. Also, algae-based wastewater treatment bioreactors can be colonized by different organisms that act like grazers or parasites of the phytoplankton. For example, Ferro 2019 reports members of the groups *Opisthokonta*, *Alveolata* and *Rhizaria*, ciliates (*Oligohymenophorea*, *Spirotrichea*, *Litostomatea* classes), and amoeboids (*Cercozoa* phylum), but also multicellular organisms (*Rotifera*, *Annelida*, *Nematoda* phyla) accompanied by fungi (phyla of *Cryptomycota*, *Chytridiomycota*, *Ascomycota*) that negatively impacted the process in a prolonged operation of a HRAP [78]. In this regard, the control over the bacteria, protozoa, and macro grazers that could potentially affect the microalgal growth is nearly impossible. Rani et al., 2021 report in their review that different authors have applied methods for the prevention of biological contamination in algal cultures, such as acidification, ozone treatment, UV radiation, removal of particulate matter (larger than algae), high ammonia concentration, and diurnal anaerobic conditions. However, additional energy expenditures and costs for effective elimination of harmful microbial populations are needed [25]. Aside from the methods for the full prevention of invasive organisms—either native algal strains or different kinds of bacteria and protozoa—another method for partial prevention is the application of enclosed algae-based wastewater treatment systems over open ponds with suspended or attached biomass. The enclosed reactors actually isolate the algae–water suspension from direct contact with the surrounding environment and reduce the probability of invader development. The only possibility for contamination is through the influent wastewater and its biotic content. This risk is significantly lower since algae create a unique ecosystem inside the reactor with specific parameters, making enclosed bioreactors a more reliable option in this regard, compared to the open systems that are more prone to contamination [39,77].

3.2.2. Light Utilization, Illuminated Surface to Reactor Volume Ratio (S_f/V) and Algal Biomass Concentration/Algal Biofilm Thickness in the Reactor

Light is the main energy source of the photoautotrophic/mixotrophic algae. Light saturation and photoperiod (quality and quantity) may differ between the used species. Mohsenpour et al., 2021 in their recent review report a general illumination saturation point for freshwater algae between 200 and 400 $\mu E\ m^{-2}\ s^{-1}$ [8,44]. Higher light intensities can lead to biomass loss due to photoinhibition (irradiance above 1000 $\mu E\ m^{-2}\ s^{-1}$) and such cases have been previously reported for *Synechococcus*, *Haematococcus*, *Chlorella*, *Phaedolactinum*, and an *Scenedesmus almeriensis* algal strain [79–82]. On the other hand, lower levels of light penetration (below 30 $\mu E\ m^{-2}\ s^{-1}$) can lead to suboptimal nutrient removal with decreased microalgal activity [43,79,81].

The illuminated surface to reactor volume ratio (S_f/V) parameter accounts for the effectiveness of light (natural or artificial) utilization by a certain reactor. Higher S_f/V ratios achieve more intense processes and allow the use of higher biomass concentrations in the reactor. Systems using enclosed configurations with suspended or immobilized bead growth of algae reach the highest S_f/V ratios due to their transparent tubes/walls and, respectively, they can work with the highest biomass concentrations [69,83]. This increases their reliability in terms of light utilization. The attached growth systems, on the other hand, have the lowest illuminated surface to volume ratio, especially the extremely shallow ATS reactors with a typical depth of 0.05 to 0.15 m that require large area footprints to reach the needed light saturation [84]. Improved modifications in the rotating attached growth reactors are aimed at tackling this problem with a revolving mechanism interchanging the algae medium between the nutrient rich water and the atmosphere, utilizing more light than the stationary attached growth systems [85,86]. The reliability, in terms of light utilization, of the revolving systems is not as high as the enclosed suspended growth algae-based WWT reactors, but still better than the ATS systems and the open pond suspended growth bioreactors. The open ponds reach a higher light utilization (with respect to their volume) than the ATS due to their slightly bigger depth—0.15–0.5 m—and the larger length to width ratio of 6 or 7 [39,45–47]. The typical S_f/V ratios for the different reactor types ranges are shown in Table 2.

Table 2. Sf/V ratios of the main branches of algae-based wastewater treatment reactors.

Reactor Type	S_f/V Range	Sources
Open suspended growth reactors	5 to 10 m^{-1} (standard of 6.7)	[83]
Enclosed suspended growth reactors	20 to 400 m^{-1} (standard of 86.7)	[83]
Enclosed algal beads reactors	-	-
Stationary attached growth reactors	1.55 to 6.06 cm^{-1}	[87]
Rotating attached growth reactors	approx. 23 m^{-1} [a]	[88]

[a] Calculated, based on the total rotating surface of the RABR to the volume of the tank reported in Christenson et al., 2012 [88].

Higher values of algal biomass concentration in the system result in faster nutrient removal and lower hydraulic retention time (HRT) of the reactor. The limiting factor affecting the algal biomass concentration is the internal shading that occurs with hyper concentration (in suspended growth systems) or increased algal biofilm thickness (in attached growth systems) [39,86]. Hence, the values of the optimal algal biomass concentration are directly correlated to the S_f/V ratio, discussed above. Bioreactors with higher S_f/V ratios can utilize light better and use higher biomass concentrations, respectively. The limiting algal biomass concentrations/algal biofilm thicknesses for the different types of systems are shown in Table 3.

Table 3. Maximum biomass concentration/biofilm thickness of the main reactor branches.

Reactor Type	Maximum Biomass Concentration/Biofilm Thickness	Sources
Open suspended growth reactors	0.1 to 1.8 g L^{-1} (standard under 1.5 g L^{-1})	[47,89]
Enclosed suspended growth reactors	0.6 to 1.8 g L^{-1} (standard over 1.5 g L^{-1})	[39,90]
Enclosed algal beads reactors	0.1 to 3 g L^{-1}	[39,55,91,92]
Stationary attached growth reactors	-	-
Rotating attached growth reactors	0.00025 m to 0.002 m	[93]

The concentration of the algal biomass that carries out the treatment and accounts for its intensity and rate is directly correlated to the bioreactor's ability to efficiently utilize light. Based on that, the reliability order of the reactor configurations is the same as their order from the segment regarding the S_f/V ratio, and this is also visible from the table above. The most reliable systems, in terms of higher biomass concentration and intensity of the treatment process, are the enclosed reactors with suspended or immobilized (bead) algal growth, followed by the rotating algal biofilm systems. The least reliable with regard to biomass concentrations are the stationary attached growth bioreactors and the open algal ponds.

3.2.3. Water Flow Velocity, Agitation, and Shear Stress on the Algal Cells/Beads

These three parameters are interconnected. The water flow velocity in suspended growth systems should be high enough to prevent biomass settling in the formed dead zones and excess shear stress on the cells/beads [47,89,94]. Furthermore, the flow velocity should ensure mixing of the influent's nutrients. Optimal reported value for open pond systems is between 0.1 and 0.5 m s^{-1} [47,89].

For enclosed photobioreactors with suspended or immobilized algal growth (hermetized systems), the agitation is provided by the aeration system. Suspended growth PBRs can withstand higher aeration intensities (in the range of 1–8 L min^{-1}) than immobilized algal systems since the individual beads' coating is prone to tearing by shear stress from an aggressive air bubble supply [39,55,95,96].

For stationary attached growth systems, the water flow velocity is mainly connected to the water distribution and nutrient transfer to all parts of the system and typical values are around 1 m d^{-1} for ATS [97]. Rotating algal biofilm reactors and vertical conveyor belt attached growth reactors (VCBR) control this transfer through control of the revolutions per minute (RPM) or the rotating speed of the driving shaft [67,98]. The scientific literature reports that the speed of the rotating drum of a RABR is 0.08 m s^{-1} [88] and the rotating belt of a VCBRs is 0.04 m s^{-1} [60].

This parameter decreases the overall reliability of the enclosed algae-based wastewater treatment systems, especially the ones using immobilized algal beads, since a compromising of the bead or the algal cell, due to intense aeration, could lead to a total failure of the treatment process in general.

3.2.4. Temperature Control

The temperature range for freshwater algal growth, regardless of the strain, is typically between 15 and 30 °C (optimal of 20–25 °C). Some thermophilic strains can withstand higher temperatures of up to 56 °C and some psychrophilic strains can live in environments with temperatures of 17 °C [8,25,76,79,99,100]. Generally, the algal activity significantly drops (in some cases with more than 90%) with temperatures under 15 °C, leading to decreased nutrient removal efficiency and removal rate retardation [8,25,76,79]. Di Cicco et al., 2021, for example, report a case study from the Agropoli WWTP (45,000 p.e.) in Italy where the average temperature of 22 °C and the average sub-acid pH values from 5 to 7 in the bioreactor form a potentially suitable medium for greenhouse growth of the emerging extremophilic thermoacidophilic microalgae with high potential for WWT and resource recovery [101].

Temperature control is a very energy and cost intensive process that requires the addition of heating and cooling systems, accompanied by sensory equipment. Depending on the geographical coordinates of the WWTP site, wastewater can heat up, cool down (even freeze), or both (during different seasons), which can lead to a temperature that is out of the optimal range for the specifically used algal strain. Usually in enclosed systems, the main problem, with regard to temperature, is the buildup of excess heat (mainly in the summer seasons) inside the reactor due to solar radiation, higher air temperatures, and its transfer through convection and mixing (aeration). An innovative solution to this problem is studied by di Cicco et al., 2021. They report that the application of a specifically selected thermophilic algal strain, such as microalgae from the *Galdieria* genus, can result in an enclosed algae-based wastewater treatment system that does not require additional equipment for cooling [99,100]. According to Djamal et al., 2019, temperature control for large-scale open pond systems is economically impossible as temperature varies during the day and with seasonal changes. In these cases, the strain should be chosen to be able to withstand temperature fluctuations, especially in regions with temperate and cold climates where climate fluctuations can be significant and ice formations can occur during the coldest months of the year [81]. It could be concluded that in terms of temperature control, all reactor configurations are unreliable and unstable.

3.2.5. pH Variation, Transfer of CO_2 and O_2 Oversaturation Inhibition

The majority of algae grow in mediums with pH levels between 7 and 9 [8,102]. However, some algae are alkalophilic with preferable pH values of 9 or even 10 and above, while other strains, such as the extremophilic thermoacidophilic microalgae, prefer mediums with pH values of 5–6, which were measured in the effluent of real WWTPs [8,101,102].

The value of the pH and the CO_2 quantity in the medium are directly correlated. Since algae, for the most part, are photoautotrophic—or more broadly put, mixotrophic—they mainly depend on inorganic carbon sources for their vital processes. The most preferred form of carbon for the needs of algal cells is CO_2, making its presence in wastewater crucial for their overall activity. Carbonic acid is formed in water with higher concentrations of CO_2, leading to lower pH levels. In cases of CO_2 depletion, algae switch to alternative inorganic carbon sources such as CO_3^{2-} and HCO_3^- anions [103]. This process should be carefully monitored since it has direct correlations to variations in the pH level and dissolved oxygen concentration (O_2 and OH^- anions are final products of the CO_3^{2-} and HCO_3^- anion inorganic carbon depletion). CO_2 deprivation induces an increase in the pH value and the DO concentration in water. If not addressed properly, this process could lead to inefficient nutrient removal due to decreased algal activity and lower effluent quality [8,65,102]. Such dynamics of the three parameters—CO_2, pH, and DO—occur in all algae-based wastewater treatment reactors [8,21,34,104]. Both the preferred pH level of the strain and the CO_2 concentration in the reactor should be synchronized, when this is possible, in order to achieve the most favorable conditions for the specifically used algal strain for the optimal removal of nutrients from the wastewater medium [8,102]. Also, high DO concentrations above the level of air saturation (0.225 mol O_2 m^{-3} at 20 °C) can hinder photosynthesis in most microalgae species, regardless of the concentration of carbon dioxide in the medium. This process is called O_2 oversaturation inhibition, and it should also be monitored during reactor operation in cases in which a high probability of occurrence is present [105].

Open suspended growth systems and stationary attached growth systems most often rely solely on the re-aeration of CO_2 from the large water surface area of the pond/channel. In these cases, an artificial addition of CO_2 from an external air source is inefficient due to the low solubility of CO_2 in water [31]. This leads to a lower intensity of the processes in the open suspended growth and the stationary attached growth systems and decreased control of the three parameters [8,106]. However, the open nature of the algal ponds/channels allows for the release of the excess oxygen into the atmosphere, making the system less prone to inhibition through O_2 oversaturation [70].

Enclosed algae-based systems with suspended or immobilized algal biomass show higher reliability due to the possibility to control pH and oxygen through sensors and the aeration system. When the pH value is out of the designed range, the pH meter signals the aeration system, supplying the reactor with air that is enriched with CO_2. This way the pH levels are constantly restored—the air supply is either stopped (at low pH level) or started (at high pH level) [107]. However, the enclosed design of these systems leads to O_2 buildup inside the reactor which, if not de-gassed properly, could lead to an inhibition of the vital processes of the algae that would result in hindering of the overall nutrient removal process [108].

RABR systems achieve an overall better CO_2 transfer to the algal cells, without the additional aeration system, achieving a middle ground (between open and enclosed suspended growth systems) in terms of the control of this parameter. The results remain stable as long as the preferred biofilm thickness is controlled and all of the available biomass has exposure to the atmosphere and the wastewater medium [86,103].

3.2.6. Evaporation Control

This parameter is relevant only for the open suspended and the open attached growth systems, because the enclosed suspended growth and immobilized algal bead PBRs are isolated from direct contact with the atmosphere. Evaporation in open pond systems can be severe (up to 0.01 m^3 m^{-2} day^{-1} in some regions), especially during the summer months of the year, and, if not addressed and controlled properly, could lead to algal biomass hyper concentrations and, respectively, internal shading, problematic agitation, aggravated nutrient transfer throughout the system, etc. [47,70,81]. Also, salinity increase can reach a point at which it can affect the microalgal growth and composition through osmotic stress, ionic stress, and membrane permeability [8]. This factor can be addressed through an external addition of fresh water to the medium, but this increases the complications during extended periods of operation and decreases the reliability of these two main groups of open system types.

3.3. Algae Harvesting Method

Algal biomass harvesting after the treatment process is currently one of the most difficult, energy intensive, and expensive parts of the whole algae-based technology, regardless of the reactor configuration [7,109,110].

A graphical estimation of the reliability by reactor type for the reliable harvesting methods is shown in Figure 10.

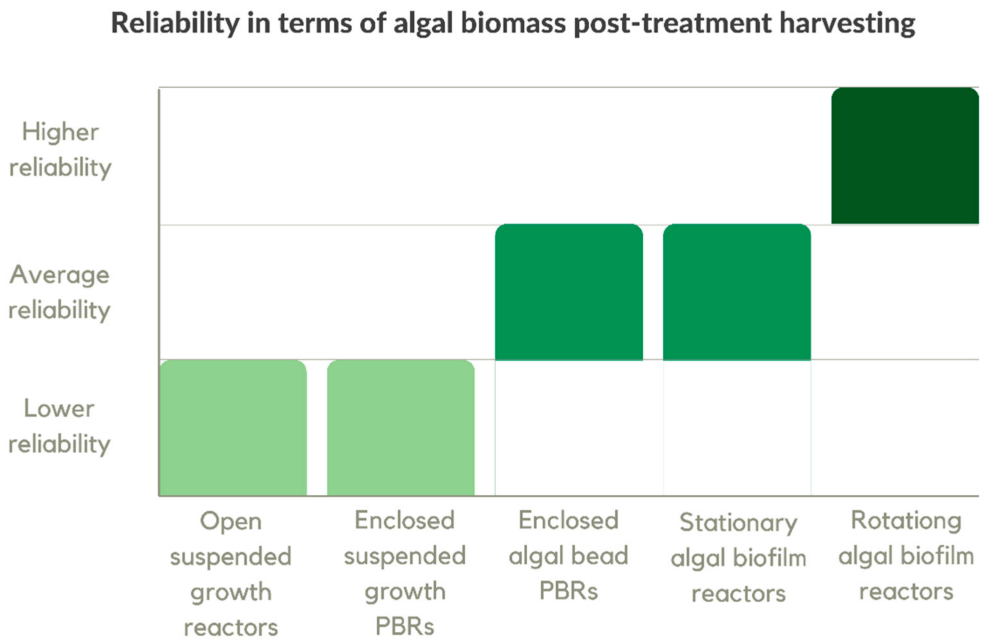

Figure 10. Reliability of the available algae-based wastewater treatment reactor configurations in terms of reliable algal biomass post-treatment harvesting.

This estimation is based on an analysis of the reported research results, which are presented below.

The harvesting methods can be generally divided into three main groups with respect to the specific reactor type—harvesting for suspended, attached, and immobilized (algal bead) growth systems.

- Harvesting for suspended growth systems

Out of the three main groups of bioreactor types, the least reliable and most problematic, in terms of algal biomass in post-treatment harvesting, is the group of suspended growth systems. This is mainly due to three factors: (1) the small size of the typically used microphytes (typically smaller than 30 μm); (2) the microalgal cell density is almost identical to that of water (1.08–1.13 kg L^{-1}); and (3) the net negative charge of the algal cells, which are especially heavily expressed during the exponential phase of growth. The combination of all these factors makes microalgae non-susceptible to free sedimentation (or flotation) and, respectively, this immensely hinders the application of current technologies into full scale wastewater treatment plants [110–113].

The harvesting methods for suspended growth algae-based wastewater post-treatment and their effectiveness are presented in Table 4.

Table 4. Algae harvesting methods for suspended growth systems.

Method Type	Short Description	Dry Solids in the Harvested Algae	Effectiveness of Biomass Separation	Source
Chemical				
Metal coagulant addition	$Al_2(SO_4)_3$ or $FeCl_3$ are generally used for neutralization of the negative algal cell charge and floc formation.	3–8%	above 90%	[110–113]
Organic biopolymer addition (flocculant)	Use of organic polysaccharides such as chitosan, acting as a flocculant.	3–8%	above 90%	[110]
Mechanical				
Centrifuge	Most reliable and commonly used method. It uses centrifugal forces to separate the biomass from water.	5–20%	around 90%	[113]
Tangential membrane filtration	Also very commonly used. Usually, ultrafiltration membranes are used with pore size of <2 µm.	5–25%	70–90%	[111,113–115]
Free sedimentation	Unreliable, depending solely on the gravitational sedimentation.	0.5–3%	10–50%	[111]
Dissolved air flotation (DAF)	Usually applied in combination with coagulant addition in enclosed systems where the aeration induces the dissolved air flotation (DAF).	3–6%	50–90%	[111,113]
Physical				
Electrophoresis	Rarely used in fresh waters. Algal cell's negative charge forces a biomass concentration around an anode.	10–20%	90%	[110]
Ultrasound	Rarely used. The ultrasound either concentrates algae (MHz wavelengths) or tears the cells directly (KHz wavelengths).	-	-	[110]
Biological				
Autoflocculation	Extremely unreliable. Flocculation occurs either due to pH increase and Ca^{2+} and Mg^{2+} salts formation, or either through extracellular polymeric substances (EPS) accumulation.	1–6%	can reach up to 90%	[110]
Bioflocculation (microbial flocculation)	Rarely used. Through addition of floc forming organisms such as fungi, bacteria or protozoa. Usually requires addition of acetate, glucose, etc. for the faster occurring heterotrophic processes.	3–8%	above 90%	[110]

The currently used algae harvesting technologies for suspended growth algae can reach high efficiency levels, especially the most often used centrifugation, membrane filtration, and coagulation/flocculation methods. However, their application in commercial use for large scale WWTPs is still very limited since they require either precise dosage of external reagents, excessive amounts of energy, or both, thereby decreasing the overall reliability of the suspended growth reactors in general [70,109,110].

- Harvesting for immobilized (algal bead) growth systems

The preliminary separation of the algal biomass into separate algal beads neutralizes the negative factors affecting the post-treatment harvesting of the free algal suspension.

The individual beads enfold high concentrations of algae (up to 3 g L^{-1}), leading to denser agglomerates of biomass with a larger diameter (4–6 mm for each bead) [91,92]. Also, through the coverage of such large groups of cells (1.5×10^6 cells in a bead), the charge of the individual cells is no longer a factor in the harvesting [91]. The overcoming of all the negative factors through a preliminary preparation of the algal beads leads to a much easier biomass post-treatment separation. This can be achieved either through free gravitational sedimentation or coarse sieving [39,54,116]. The facilitated algal harvesting with the immobilized bead algal technology is a main advantage in these systems and makes them more reliable in terms of downstream processing. However, it should be noted that very strict control of the aeration intensity and agitation in the reactor must be kept. Also, the velocity at which the treated wastewater passes through the sieve should be closely tracked. These two considerations are needed in order to avoid shear stress on the beads. There is a decreased chance of leakage of biomass outside of each individual bead when the two considerations above are executed.

- Harvesting for attached growth systems

A main advantage of the attached algal growth systems is the facilitated biomass harvesting [7,93,117]. The specific algae separation method varies with the different reactor configurations but generally includes variations of scraping for the reduction of the biofilm layer to the desired thickness. The highest reliability in terms of harvesting is achieved with the rotating algal biofilm systems where the separation process is completely automated. Usually, with the RABR, when the layer of algae grows beyond the optimal level of nutrient, air, and water transport, the rope of the reactor (biofilm media) is pulled through a scraping opening with a specific diameter that removes the excess biomass and keeps a constant level of entwinement of the material [93]. VCB systems, on the other hand, also solve the problem of biofilm thickness control with scraping, but with a specifically designed scraper blade that is installed at the desired distance from the conveyor belt to keep an optimal layer width [60]. Even though ATS biofilm systems provide easier algae harvesting, they have lower reliability in this regard since they need to be scraped manually periodically [46,118]. The limiting factor of all these harvesting methods of the attached growth bioreactors is the matrix's wholeness and the durability of the material, since with every scrape, the biofilm media also gets shaved off [67,88]. Nevertheless, biomass separation is much easier and less energy intensive in comparison to the suspended growth systems, which leads to a higher reliability of the attached growth systems in this regard.

3.4. Produced Biomass

The generated excess biomass of microalgae in the wastewater treatment process can be extremely valuable and finds applications in fields like fertilizer and biofuel production, pigment extraction for paints and colorants, bioplastics manufacturing, etc. [24,119,120]. However, since the algal biomass in wastewater treatment bioreactors is prone to contamination and remains in contact with different kinds of bacteria and protozoa for prolonged periods of time, not all applications are widely common. Due to algae's high nutrient content (mainly N and P), its ability to fix nitrogen (some strains), its biomass' high calorific value, and its capacity for lipid storing, the two main post-treatment applications are in the fields of fertilizer and third generation biofuel production (ethanol, methanol, propane, biodiesel) [113,120–123].

The safe application of the generated excess biomass is usually connected to the possibility of contamination of algae during the wastewater treatment processes. A lower risk of contamination of the preliminary cultivated culture/consortia leads to more constant parameters of the harvested biomass, hence a more stable nutrient and lipid content. Since invasive algae have the ability to release extracellular allelochemicals that can be toxic to other organisms in high concentrations, the contamination risk actually can amplify the chances of potential soil toxicity magnification if a harsh native mix of algal strains is directly applied as fertilizer [8].

The estimated reliability of all reactors in the aspect of harvested biomass application is shown in Figure 11.

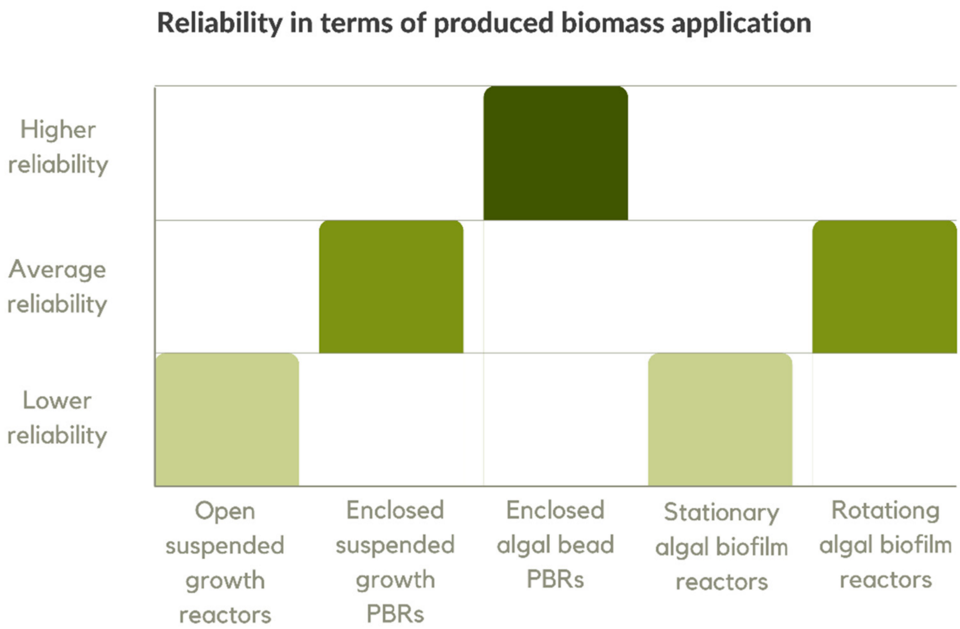

Figure 11. Reliability of the available algae-based wastewater treatment reactor configurations in terms of safe excess algal biomass applications.

The estimation shown in Figure 11 is based on the understanding that the technologies that offer more stable operating conditions, better control over the algal culture integrity, and produce more constant effluent quality results actually become more reliable in terms of post-treatment applications of biomass. As mentioned in the previous points, these types of systems are generally considered to be the enclosed immobilized growth reactors, due to their double isolation of the biomass from the environment—firstly, through the enclosing of the system to the surrounding atmosphere, and, secondly, through the isolation of the algal cells inside each individual shell of the separate beads. Enclosed suspended growth algae also achieve stable operation and more constant effluent results with enhanced control over the parameters in the reactor. Even though they are not as reliable as the immobilized algal beads, a high reliability rating is still reached. Algal biofilm technologies have attached biomass that is generally more stable and resistant to changes in the environment, but these technologies normally use an open reactor that has a direct connection to the surrounding environment, increasing the chances for grazers and contaminants to reach the biomass. Out of all systems, the open ponds have the simplest reactor configuration that has the least control over the technological parameters of the system and the highest probability of culture contamination, leaving them with the lowest reliability score.

Another aspect of the safe application of the harvested biomass is the accumulation of heavy metals and toxic compounds in the algae during the treatment processes. This is almost impossible to control since this parameter is mainly dependent on the secondary treated effluent quality of the respective WWTP. In this regard, all bioreactor configurations reach the same reliability, although some level of control can be achieved by the algal bead shell in the immobilized algal growth systems, since there is a higher level of insulation of the cells with respect to the medium. The scientific literature is still very limited for the larger scale application of this specific branch of algae-based wastewater treatment technology, hence the immobilized algal bead technology also cannot be described as reliable enough in terms of the safety of the post-treatment algal biomass application.

3.5. Conclusions on the Reliability

The estimated reliability presented in Figure 8 to Figure 11 are compiled in a spider chart (Figure 12) for a better general overview.

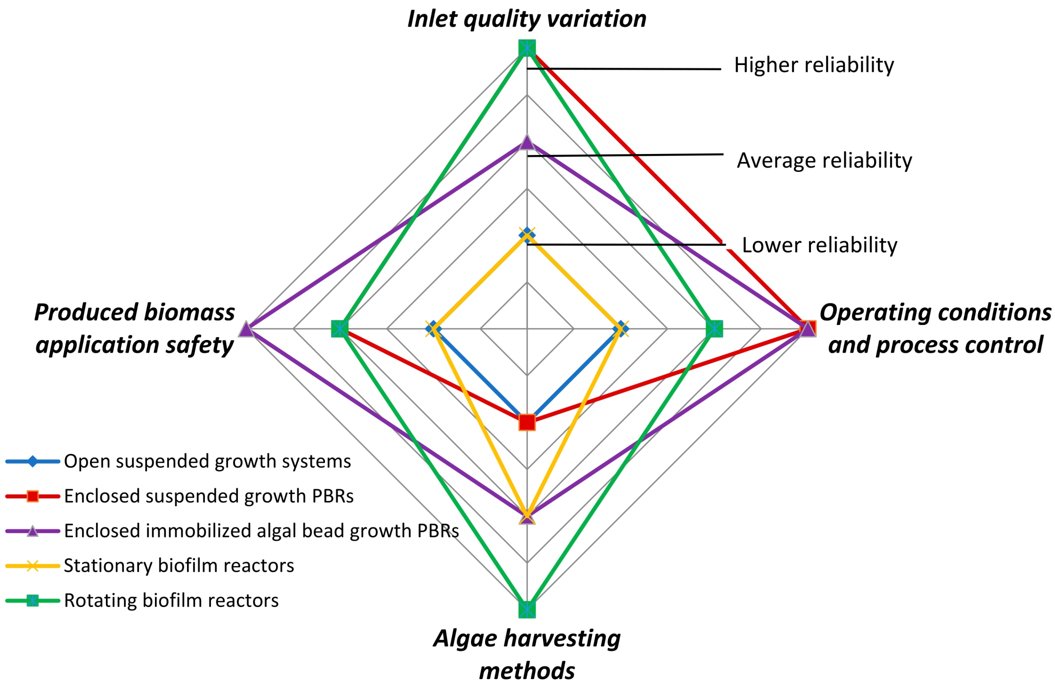

Figure 12. Overview of the four analyzed aspects of the reliability of the compared systems for secondary treated effluent.

As discussed before, the open pond reactors lack control over most of the parameters in the treatment process due to their extremely simplified design and operation. The graphical visualization in Figure 12 shows that the open suspended algal growth configuration appears as the least reliable option in regard to ensuring a stable wastewater treatment processes.

The main strengths of the enclosed suspended growth PBRs are the control over the operating conditions and the resilience of the processes in the reactor. They also achieve a decent level of consistency in terms of the safety of the generated excess algal biomass. Nevertheless, since this technology includes a suspended growth of algae, its main deficiency is the algal biomass harvesting technique as shown in Figure 12.

The high level of insulation of the algal biomass from the surrounding environment that is achieved through the application of enclosed immobilized algal bead PBRs, results in one of the highest reliability ratings. The complicated setup and the preliminary preparation of the separate beads leads to a significant level of control over the whole system in all aspects (Figure 12).

Stationary biofilm reactors, just like the open pond systems, also have simplified configurations and less control over the system's components. Even though the attached biomass facilitates the harvesting aspect, these technologies still have a relatively low reliability rating (as it is visible from Figure 12) and are unstable in their operation.

The rotating biofilm reactors combine the best aspects from the algae-based wastewater treatment systems. Even though they have an open configuration, the stability of the biofilm and the innovative technique for its harvesting, enhanced nutrient and air transfer, and improved light utilization actually increase the total reliability of these types of bioreactors.

In terms of the current reliability of the available algae-based wastewater treatment technologies, without taking into account the economical aspect of the systems, the following general conclusions can be formulated:

(1) The highest overall reliability rating of all reactor types that are currently discussed in the scientific literature is achieved by the rotating biofilm reactors (RABR and VCBR) and the enclosed immobilized algal bead systems;
(2) The lowest overall reliability rating belongs to the open suspended growth algal ponds and are closely followed by the stationary algal biofilm systems;
(3) Enclosed suspended growth PBRs reach an average overall reliability compared to all the other reactor configurations.

It should be noted that these conclusions are based on the current state of the technology. Further improvements in any of the reactor configurations may lead to different ratings.

4. Technology Readiness Level of the Main Algae-Based Wastewater Treatment Technologies for Secondary Treated Effluent

The technical maturity of each technology can be generally estimated with the use of the NASA or European Commission (EC) documents (Horizon 2020—Work Programme 2014–2015; Straub 2015) [124,125]. According to them, a system needs to go through a total of nine technology readiness levels (TRLs) of research, development, and deployment in order to reach a proper full-scale application. This systematic method is established and recognized internationally.

The different reactor types/technologies for algae-based nutrient removal have reached different TRLs. Based on the reviewed publications, the current degree of development of each specific technology is presented in Figure 13.

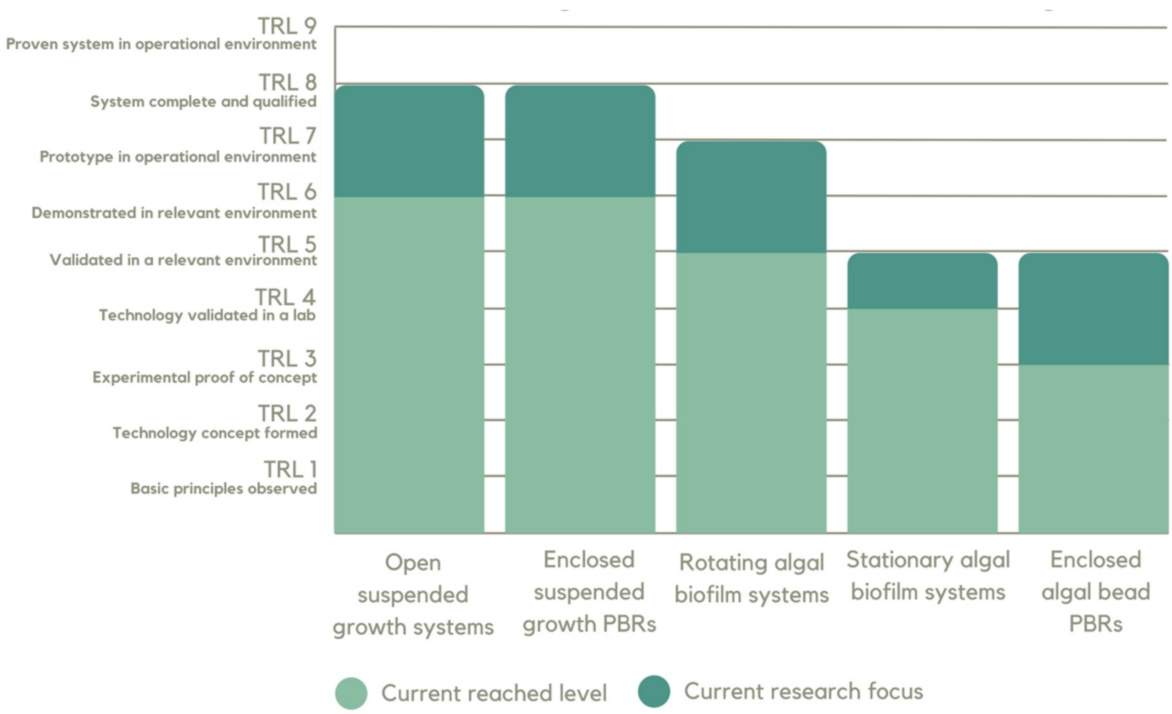

Figure 13. Technology readiness level of the main algae-based wastewater treatment technologies for secondary treated effluent.

No reports were found that any of the algae-based wastewater treatment technologies subject to this review have successfully reached a TRL higher than 6–7 (i.e., verified prototype demonstration in operational environment). Perhaps this is due to the fact that the drivers for the research and development for any reactor type are not only their reliability but also the economic feasibility and the simplicity of the components' operation.

Regardless of the fact that the open suspended growth algae-based wastewater treatment systems have one of the lowest reliability scores among all systems, they still have the highest level of development, reaching a TRL of 6–7 [24,41,57]. The minimal amount of additional costs for the construction and operation of the WWTPs using such systems and their uncomplicated configurations facilitate and promote their use, research, and further development [24,41,57]. Although the full-scale application of the open algal ponds for wastewater treatment is still unreliable and provides fluctuating results, some companies offer the design and construction of these systems for commercial use [24,41,57]. These system applications in real operational environments will be the object of future research that will help with the verification of the open reactors as a suitable method for WWT, taking the next step towards their qualification for full scale application.

A high TRL (6 to 7) is reached also in regard to the floating or stationary enclosed suspended growth algae-based WWT systems [41,57]. The higher control of the system's parameters provides more constant results, but the more complicated reactor configuration, the increased difficulty in the operation processes, and the additional costs seem to lower the overall interest for the use of the enclosed reactors for algae-based wastewater treatment. Instead, these systems are more commonly used in the field of pure monoculture algae cultivation for biofuel and valuable compound production [41,44].

The rotating biofilm systems are one of the promising future algae-based wastewater treatment reactor configurations. Currently undertaking serious development, these systems are used for the treatment of lower volumes of wastewater [41]. Although not as many research papers as the suspended growth systems are available yet, some companies aim at commercializing the rotating biofilms with different patent pathways and design solutions [41,44,57]. Out of all the algae-based biofilm wastewater treatment technologies, presently VCBDs and RABRs appear to be the most appropriate and footprint saving technologies for scaling up and reach the highest TRL of around 5 (technology validated in relevant environment).

The focus of development in the realm of algae wastewater treatment has been shifted away from the stationary biofilm systems and the immobilized algal beads in recent years, which was likely due to complications with their application in larger than laboratory scale or low volume-controlled environments. Recent advancement attempts of the stationary systems use the evolved twin-layer reactor designs, for example, to enhance the treatment process, but the technology is still emerging [41,44,58,126]. Still, the high amounts of area and the problems with naturally born macro grazers that inhibit the processes remain unsolvable problems, thereby preventing the technology from expanding. Enclosed immobilized algal bead systems offer a great amount of control over all the parameters of the system but require immense costs, substantial volume of preliminary work, and complicated operation in order to achieve a stable process. These are the main reasons that this technology is still at laboratory phase (TRL of 3) and has difficulty with any attempts at system escalation [44,57].

5. Conclusions and Prospects

Immense research has been carried out in the algae-based wastewater treatment field, especially in the last ten years. The broad spectrum of the various advantages of this technology pushes its development. Nevertheless, as with every entirely biological process, the main setbacks remain in the sphere of the delicate balance between the control of the technological parameters of the reactor, the energy input of the system, and the facilitation of the operation and the expenditures related to it.

The present review shows that the most reliable systems are those with the highest level of control over the technological parameters, namely the enclosed suspended and active immobilized growth, and the rotating algal biofilm reactors. Enclosed suspended growth reactors still have issues with the algal post-treatment harvesting and the costs and complications related to their operation. For these reasons, they remain a more viable option for clean monoculture cultivation rather than for wastewater treatment. Active immobilized algal beads require enormous effort in the preliminary preparation and have a highly specific and complex operation, making them extremely problematic for larger scale practical application currently. Rotating algal biofilms, on the other hand, present a promising future for the algae-based wastewater treatment with their ease of harvesting, operation, and process stability. However, there is still a lack of sufficient research on the topic due to the novelty of the reactor designs. Future research is needed for the development of durable matrix materials, unified technological parameters and strains, and verification of the results at a larger scale.

Even though the reliability of technologies such as the open suspended growth systems and the stationary biofilm reactors is lower, their development continues. The non-complex operation and relieved expenditure costs, as well as the lack of specific preliminary preparation, make these technologies appealing for research and practical application. Among the remaining research gaps and challenges are the identification of the best performing algal strains, establishment of technological parameters, overcoming seasonal variations of the effluent quality, biomass harvesting, etc.

Some algae-based wastewater treatment technologies are commercialized but more research is needed for the verification of the application of these systems for nutrient removal from secondary treated effluent in real operational environment.

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Abbreviations

ATS	Algae turf scrubber
C	Carbon
COD	Chemical oxygen demand
DAF	Dissolved air flotation
DO	Dissolved oxygen
EC	European Commission
EPS	Extracellular Polymeric Substances
HRAP	High rate algal ponds
$HRAP_W$	High rate algal ponds with aeration system
HRT	Hydraulic Retention Time
N	Nitrogen
NASA	National Aeronautics and Space Administration
p.e.	People Equivalent
P	Phosphorus
PSBR	Photo-Sequencing Batch Reactor
PBR	Photobioreactor
RABR	Rotating algal biofilm reactor
RBC	Rotating biological contactors
RPM	Revolutions per minute
SABR	Stationary algal biofilm reactor
SBR	Sequencing Batch Reactor
S_f/V	Illuminated surface to reactor volume ratio

TN	Total Nitrogen
TP	Total Phosphorus
TRL	Technology readiness level
TSS	Total Suspended Solids
VCB	Vertical conveyor belt
VCBD	Vertical conveyor belt design
VCBR	Vertical conveyor belt reactor
WW	Wastewater
WWT	Wastewater treatment
WWTP	Wastewater Treatment Plant

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