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WASTEWATER TREATMENT WITH MICROALGAE – A LITERATURE REVIEW

Avloppsrening med mikroalger – en litteraturstudie

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Abstract

Microalgae can be used for tertiary treatment of wastewater due to their capacity to assimilate nutrients. The pH increase which is mediated by the growing algae also induces phosphorus precipitation and ammonia stripping to the air, and may in addition act disinfecting on the wastewater. Domestic wastewater is ideal for algal growth since it contains high concentrations of all necessary nutrients. The growth limiting factor is rather light, especially at higher latitudes. The most important operational factors for successful wastewater treatment with microalgae are depth, turbulence and hydraulic retention time.

Key words – Microalgae, wastewater treatment, phosphorus, nitrogen, light, operation, ponds, growth

Sammanfattning

Mikroalger kan användas för tertiär rening av avlopp på grund av deras goda förmåga att assimilera näringsämnen. När algerna växer höjs dessutom pH-värdet i vattnet, vilket inducerar fosforutfällning och ammoniakavgång till luften, och det kan även ha en desinficerande verkan på vattnet. Hushållsspillvatten är idealiskt för alger att växa i eftersom det innehåller höga koncentrationer av alla nödvändiga näringsämnen. Tillväxten begränsas istället av ljus, speciellt på högre breddgrader. De viktigaste driftparametrarna för framgångsrik avloppsrening med mikroalger är djup, omrörning och uppehållstid.

Introduction

To use microalgae for wastewater treatment is an old idea, and several researchers have developed techniques for exploiting the algae's fast growth and nutrient removal capacity. The nutrient removal is basically an effect of assimilation of nutrients as the algae grow, but other nutrient stripping phenomena also occur, e.g. ammonia volatilisation and phosphorus precipitation as a result of the high pH induced by the algae [1]. Some reports reveal that a large part, sometimes up to 90 %, of the phosphorus removal is due to this effect [2–4]. In addition to tertiary treatment, microalgae may provide heterotrophs in secondary treatment with oxygen, and can also be used to absorb e.g. metals from mine wastewater. The increase in pH during photosynthesis also has a disinfecting effect on the wastewater [5].

The term microalgae refers to all algae too small to be seen properly without microscope, and often includes both eukaryotic microalgae and the prokaryotic cyanobacteria [6]. In this report microalgae refers to both types, and cyanobacteria refers to cyanobacteria in particular. The most important common feature of all eukaryotic microalgae and cyanobacteria is that they have oxygen-evolving photosynthesis and that they use inorganic nutrients and carbon. Microalgal biomass can be used for hydrogen gas production, bioenergy conversion and production of pharmaceutical substances or food just to give some examples [7–17].

This paper is a compilation of reported experiences from wastewater treatment with microalgae. The aim is mainly to explain the most important factors that affect microalgal growth and to give some advice on design and operation of algal treatment steps.

Algal growth

Successful treatment of wastewater with microalgae requires good growth, and understanding of the factors that affect growth is therefore essential. The growth rate of algae and cyanobacteria is influenced by physical, chemical and biological factors (Table 1). Examples of physical factors are light and temperature. Chemical factors can be availability of nutrients and carbon dioxide, and biological factors are e.g. competition between species, grazing by animals and virus infections. Operational factors affect the factors mentioned above, and basically concerns bioreactor design, mixing and dilution rate.

Carbon and nutrients

Algae are autotrophs, i.e. they can synthesise organic molecules themselves from inorganic nutrients. A stoichiometric formula for the most common elements in an average algal cell is $C_{106}H_{181}O_{45}N_{16}P$, and the elements should be present in these proportions in the medium for optimal growth [18]. High ratios between nitrogen and phosphorus, about 30:1, suggest P-limitation, whereas low ratios of about 5:1 suggest N-limitation. According to the ratios most often found in wastewater, phosphorus is rarely limiting algal growth, but nitrogen may be [19]. Though, since wastewater often exposes the algae to nutrient concentrations of up to three orders of magnitude higher than under natural conditions, growth is more likely limited by carbon and light [5].

The rate at which an algal cell takes up a specific nutrient depends on the difference between the concentration inside and outside the cell, and also on the diffusion rates through the cell wall. The thickness of the unstirred layer of water just outside the cell wall also plays a role, where thicker layers give slower diffusion rates. To avoid such thick boundary layers in order to enhance mass transfer rates of nutrients and metabolites, turbulence in the water is essential [8, 19].

Carbon

Microalgae assimilate inorganic carbon in the photosynthesis. Solar energy is converted to chemical energy with oxygen (O_2) as a by-product, and in a second step the chemical energy is used to assimilate carbon dioxide (CO_2) and convert it to sugars. The overall stoichiometric formula for photosynthesis is:

$$6\,H_2O + 6\,CO_2 + \text{light} \Rightarrow C_6H_{12}O_6 + 6\,O_2$$

The inorganic carbon species normally used by microalgae are CO_2 and HCO_3^-, the latter requiring the enzyme carbonic anhydrase to convert it to CO_2 [18, 19]. Beside these, some algal species are able to use organic carbon sources as well, such as organic acids, sugars, acetate or glycerol [19–23]. This heterotrophic metabolism is probably significant in waste loaded ponds, where the standing crops of algae can be very high and consequently exhausted on carbon dioxide [19, 23]. Some studies have indicated that about 25–50 % of the algal carbon in high rate algal ponds is derived from heterotrophic utilisation of organic carbon [19]. The organic carbon sources can be assimilated either chemo- or photoheterotrophically [19, 22, 23]. In the first case, the organic substrate is used both as the source of energy (through respiration) and as carbon source, while in the second case, light is the energy source. In several algal species, the mode of carbon nutrition can be shifted from autotrophy to heterotrophy when the carbon source is changed; this is the case with e.g. the green algae *Chlorella* and *Scenedesmus* [11].

Table 1. *Factors that influence algal growth in a high rate algal pond [16].*

Abiotic factors, physical and chemical	Light (quality, quantity) Temperature Nutrient concentration O_2, CO_2 pH Salinity Toxic chemicals
Biotic factors	Pathogens (bacteria, fungi, viruses) Predation by zooplankton Competition between species
Operational factors	Mixing Dilution rate Depth Addition of bicarbonate Harvesting frequency

Atmospheric carbon dioxide may be provided to algal cultures by means of aeration [9]. However, since the ambient atmospheric concentration (0.033 %) is far below optimum for algal growth, supply of extra carbon dioxide may be necessary [11, 24]. This can be accomplished by providing the cultures with air enriched with 1 to 5 % CO_2 [21].

The amount of CO_2 dissolved in water varies greatly with pH, and addition of CO_2 results in a pH decrease (Figure 1). At higher pH values, e.g. at pH greater than 9, most of the inorganic carbon is in form of carbonate (CO_3^{2-}) which cannot be assimilated by the algae [19]. The decreased availability of CO_2 may act limiting on the algal growth, however, this effect is not often very pronounced. Since CO_2 addition is one of the most costly items in high-density mass algal cultures, it can for that reason be economically beneficial to sacrifice some production in order to save CO_2 [25].

Nitrogen

Besides carbon, nitrogen is the second most important nutrient to microalgae since it may comprise more than 10 % of the biomass [11]. Nitrogen exists in many forms, and the most common nitrogen compounds assimilated by microalgae are ammonium (NH_4^+) and nitrate (NO_3^-) [26]. The preferred compound is ammonium, and when this is available, no alternative nitrogen sources will be assimilated [27]. However, ammonium concentrations higher than 20 mg NH_4^+-N per litre are not recommended due to ammonia toxicity [19]. In addition to these nitrogen compounds, urea ($CO(NH_2)_2$) and nitrite (NO_2^-) can be used as nitrogen sources. However, the toxicity of nitrite at higher concentrations makes it less convenient [11]. Cyanobacteria are also able to assimilate the amino acids arginine, glutamine and asparagine and some species can fix nitrogen gas (N_2) [27]. Of all nitrogen sources, this nitrogen fixation is the most energy demanding and only occurs in some cyanobacteria when no other nitrogen compounds are available in sufficient amounts [28]. Several microalgae can take up nitrogen in excess of the immediate metabolic needs, so called luxury consumption. This can be used later in the case of nitrogen starvation.

Phosphorus

Phosphorus is another macro-nutrient essential for growth, which is taken up by algae as inorganic orthophosphate (PO_4^{3-}). The uptake of orthophosphate is an active process that requires energy. Organic phosphates can be converted to orthophosphates by phosphatases at the cell surface, and this occurs especially when inorganic phosphate is in short supply. Microalgae are able to assimilate phosphorus in excess, which is stored within the cells in the form of polyphosphate (volutin) granules. These reserves can be sufficient for prolonged growth in the absence of available phosphorus. The growth rate of an alga may therefore not respond at once to changes in the external concentration of phosphorus, in opposite to the immediate responses to temperature and light [21, 26].

Mostert and Grobbelaar (1987) found that the phosphorus concentration in cells varied with supply concentration, from a maximum of 1170 mg dry mass per mg P at a supply concentration of 0.1 mg P l^{-1} to as low as 10 mg dry mass per mg P at supplies of 5 mg P l^{-1} and greater [8]. Algae cultivated in wastewater may hence contain much higher amounts of phosphorus than is normally needed.

Other nutrients

Nitrogen and phosphorus are macro-nutrients, which are needed in high amounts for growth. Other macro-

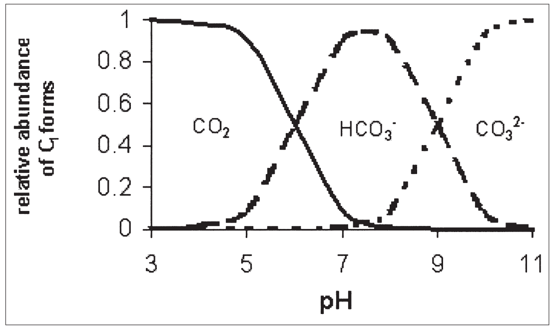

Figure 1. *The relative abundance of inorganic carbon forms as a result of pH in seawater.*

nutrients are sulphur, potassium, calcium and magnesium. Micro-nutrients, which are needed in smaller amounts, are manganese, molybdenum, copper, iron, zinc, boron, chloride and nickel. In addition, some other elements can be essential for certain algal species, like sodium, silicon, cobalt, iodine, vanadine and selenium. To prevent growth limitation by micro-nutrients, these are often added to commercial algal cultures together with a chelating agent, e.g. EDTA. [21, 26]

Light and temperature

Light

Microalgae are phototrophs, which means that they obtain energy from light. However, some algae are able to grow in the dark using simple organic compounds as energy and carbon source (see also carbon section). The light energy is converted to chemical energy in the photosynthesis, but large parts are lost as heat. Oswald (1988) reports that in outdoor ponds, more than 90 % of the total incident solar energy can be converted into heat and less than 10 % into chemical energy [18]. Fontes (1987) reports a conversion efficiency of sunlight energy into chemical energy of only 2 % [9].

There are several strategies used by microalgae to remain near the water surface in order to catch enough light. These strategies aim to decrease the specific gravity and thereby minimise the sinking rate. Examples of this are fat accumulation, mucilage production, selective accumulation of ions (monovalent ions have a lower specific gravity) and buoyancy among some cyanobacteria which float due to gas vacuoles [21].

However, not all microalgae are able to float at the surface, and algae in deeper parts of a culture vessel may for that reason be light limited since water absorbs photosynthetic active radiation (PAR). Moreover, in dense cultures the algae themselves can decrease the light availability due to internal shading [19, 23, 29]. When cultivated in raw wastewater, this shading effect can also be further aggravated by high contents of particulate matter [19]. To prevent this, turbulence is essential since it exposes all cells to light for at least short periods, thereby making high productivity possible.

The easiest way to prevent algal cultures from light-limitation is to decrease the depth of the culture vessel. According to Oswald (1988), the productivity in light limited ponds is inversely correlated to the depth [24]. Generally, depths of between 15 and 50 cm are recommended [9, 28]. However, during winter shallower depths are recommended due to the lower light conditions, and depths greater than 20 cm markedly decreases production [9].

However, even though light is most often limiting the growth of microalgae, too much light may also cause lowered photosynthetic effectivity, which is known as photoinhibition [19, 26, 30]. In order to prevent algae at the surface from exposure to inhibiting levels of light, also in this case accurate mixing is crucial.

Temperature

Increased temperature enhances algal growth until an optimum temperature is reached [6, 7, 9, 19, 31]. Further increase in temperature leads to a rapid decline in growth rate. Overheating of algal cultures is a problem especially in humid climates where evaporation is inhibited [6], but in Sweden, the problem is rather growth limitation caused by low temperatures if cultivating outdoors. At low temperatures, microalgae easily get photoinhibited by high light intensities. This sensitivity to bright light at low temperatures may pose an operational constraint on outdoor wastewater treatment in cold climate. At temperatures near optimum for growth, microalgae can better tolerate high light intensities before getting inhibited [19]. Generally, temperatures around 15–25°C seems to suit most algal species, even those which are adapted to growth at colder temperatures. To enable higher temperatures in algal cultures, greenhouses may be a solution at higher latitudes [5, 19].

pH

Microalgal growth rate and species composition may also be affected by pH. As an example, Fontes et al (1987) found that optimal productivity of the cyanobacterium *Anabaena variabilis* were obtained at pH 8.2–8.4, being slightly lower at 7.4–7.8, decreasing significantly above pH 9, and at pH 9.7–9.9 the cells were unable to thrive [9]. However, many algal species accept higher pH values than that. In algal cultures, pH usually increases due to the photosynthetic CO_2 assimilation [19, 31]. pH values above 10 is not uncommon when no CO_2 is supplied [25], and pH can reach 11 or more if CO_2 is limiting and bicarbonate is used as a carbon source [21]. In high rate algal ponds, this pH increase can be compensated by respiration deeper in the ponds, and the pH can then be regulated by letting in more organic material and thereby enhancing the respiration [19]. pH also affects the availability of inorganic carbon; even if pH is high for other reasons than photosynthetic CO_2-exhaustion, the pH regulates what species of inorganic carbon that is available (Figure 1).

Nitrogen absorption by the algae also affects pH in the medium. Assimilation of nitrate ions tend to raise the pH, but if ammonia is used as nitrogen source, the pH of the medium may decrease to as low as 3, which is too acid to support growth [11, 21].

High pH can lead to precipitation of phosphate in the medium by formation of calcium phosphates, but these may redissolve if pH drops, e.g. during night [19, 31]. If the concentration of ammonia is high at high pH, the photosynthesis will be inhibited [19, 22]. High pH may also induce flocculation of some algae, which in turn lead to reduced nutrient uptake and growth, but this flocculation can, on the other hand, facilitate harvesting [19].

In order to avoid extreme pH values, turbulence can promote the gas exchange between water and air which in turn regulates pH somewhat in the water [18].

Inhibitory substances

Many substances can act inhibitory on photosynthesis and algal growth if present in too high concentrations. Examples of such substances are heavy metals, herbicides, pesticides, substances in detergents, household cleaning products and personal care products.

High concentrations of ammonia act inhibitory on algal growth at high pH, and this toxicity is intensified at higher temperatures when a higher proportion of the ammonia occurs as free ammonia which may freely diffuse over membranes into the cells [19, 22]. As already mentioned, total ammonium concentration should not exceed 20 mg NH_4^+-N. Too high levels of organic compounds can inhibit the nutrient uptake by microalgae [22], and acetate can be toxic to some species, due to the un-ionized molecule, which can penetrate the cell membrane and damage the cell interior by ionization [23]. Some algae also produce substances toxic to themselves in the course of their metabolism. These eventually accumulate to concentrations high enough to inhibit growth; a phenomenon called autoinhibition [21].

Biotic factors

Not only physical and chemical factors affect algal growth. In nature, species have to compete with each other for space and nutrients, and this can be reflected in algal cultures as well. Some species inhibit the growth of others in mixed culture; e.g., some cyanobacteria can produce inhibitory substances to the growth of eukaryotic algae, and some eukaryotic algae can produce antibacterial substances [19, 21]. The later may indirectly affect competition among algal species by their effects on associated bacteria.

In pure monocultures, infections by parasites, predators or competing species can be deleterious, and protozoa and rotifers present the greatest threat [7, 8]. In open wastewater treatment systems, infections are hard to avoid, but by keeping optimal conditions for the algae the cultures are less susceptible. Methods to avoid infections can be acidification of the cultures to pH 2 for a short period or by daily removal of particulate matter larger than 100µ with a small porosity screen, which removes mainly zooplankton but not the algae [7]. The acidification is adequate to kill most rotifers and protozoa; however, this is difficult in large ponds. Establishing a pond regime that leads to diurnal anaerobic conditions for a short period can also prevent the development of animal and fungal populations [19], and short periods of high ammonia concentration can eliminate contamination by zooplankton [11]. Alternatively, biocides can be applied, but this can spoil the quality of the product, is expensive and is not environmentally sound [6].

Cultivation methods

The two main groups of systems for cultivation of microalgae are closed and open systems. Closed systems allow greater control of growth conditions, whereas open systems largely depend on external factors and have contact with the open air [25]. However, the open systems are often simpler to construct and operate, and may therefore be preferred from economical reasons. A third, totally different solution for phytoplankton culture is immobilisation, where the cells are trapped in a solid medium [32].

Open systems – ponds

For commercial cultivation of algae, shallow raceway ponds and circular ponds with a rotating arm to mix the cultures are usually used [14]. The raceway pond is set in a meandering configuration with paddle wheel mixers that exert low shearing forces (Figure 2). For wastewater treatment, facultative ponds and high rate algal ponds (HRAP) are the most commonly used. A facultative pond is usually deeper than one meter, has algae growing in the surface water layers and is anoxic near the bottom. An HRAP, on the other hand, is usually less than a meter deep, is continuously mixed by gentle stirring and is aerobic throughout its volume [18]. In HRAPs, microalgae supply oxygen to heterotrophic bacteria, and the nutrients in the wastewater are converted into algal and bacterial biomass [1]. Like in facultative ponds, the raised pH causes ammonia stripping and phosphate precipitation, and most studies about the role of algae in HRAPs point out that this indirect nutrient removal is often more important than direct uptake. The denitrification that occurs in facultative ponds should be considered negligible in an HRAP though, because of the aerobic environment [33]. According to Oswald (1988), properly designed and operated HRAPs are capable of removing more than 90 % of the biochemical oxygen demand (BOD) and up to 80 % of the nitrogen and phosphorus [24].

Closed photobioreactors

Closed photobioreactors can be grouped into two major classes: covered raceways and tubular reactors [14]. Closed photobioreactors usually have better light penetrating characteristics than open ponds; the light path is usually less than 30 mm, which make it possible to sustain high biomass and productivity with less retention time than is possible in ponds [19]. However, since they are more technically complicated, often need expert personnel and require more energy than open systems; the operating cost is higher [34].

By using transparent pipes for cultivation, the internal shadowing effect between the algae is minimised, and the cells can be illuminated from more than one direction. The light refraction will create shaded areas in the tubes though, and sufficient turbulence is therefore needed to provide all cells with light [35]. Tubular reactors can be placed vertically or horizontally, and be constructed of several materials, rigid or soft. In a vertical column reactor, aeration and agitation can be provided by injection of CO_2-enriched air at the bottom of the column [14]. A drawback, however, is that these reactors are more or less parallel to the sun's rays and a substantial amount of solar energy is thus reflected in the summer.

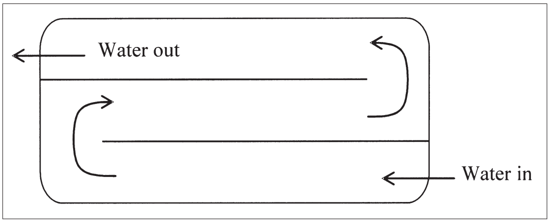

Figure 2. *Schematic picture of a raceway pond.*

Immobilised algae

By trapping the algae in a solid medium, the problem of harvesting can be solved [5, 32, 36]. The medium, that can be e.g. alginate or synthetic polymers, immobilises the algae but let substances in the water diffuse to the cells [32]. The algae-medium mixture is often shaped as beads, but can even cover screens or surfaces [19]. Immobilised algae have been tested for several wastewater treatment purposes, e.g. uptake of metals, nitrogen and phosphorus, and the nutrient uptake rates has been shown to be similar for free and immobilised cells [5].

It should be noted that almost all studies on immobilisation have been carried out in lab-scale, which limits the knowledge of how such methods would work in larger scale. Studies on immobilised algae have been conducted both for living and dead cells. The living cells are studied mainly for nutrient uptake purposes, while dead cells are studied mainly for adsorption of metals [32].

Harvesting

Algae growing in open waste ponds can reach biomass levels of up to 300 mg dry weight per litre [24]. Harvesting of microalgae is therefore crucial for wastewater treatment in order to separate both nutrients and BOD from the water [19]. However, this is not easily achieved, and is consequently a cost expensive part of the cultivating process [5, 12]. Even though harvesting effectively can be accomplished by e.g. filtration or centrifugation, such methods may be too difficult or costly to implement [5, 37, 38]. Some studies have suggested that chemical flocculation with e.g. the polysaccharide chitosan, biological filtration (see below) and even to use immobilised systems should be more advantageous [5].

Sedimentation and flotation

Using sedimentation or flotation, the biomass can be concentrated already in the water, which in turn can be decanted. Sedimentation without addition of chemicals is the most common method in full-scale facilities [33]. Flotation processes operate more efficiently and rapidly than sedimentation and achieve a higher solids fraction (up to 7 %) in the concentrate, but these on the other hand can be more expensive [38].

To facilitate sedimentation or flotation, previous flocculation is desirable. Many algal species are particularly difficult to sediment without treatment due to their natural tendency to float in order to catch enough light. Flotation of unicellular algae without flocculation may also be very difficult due to the hydrophilic cell surface on which air bubbles will not attach (personal unpublished experiences). Algae can be flocculated by addition of various chemical flocculants such as alum, lime, $FeCl_3$, cationic polyelectrolytes, and $Ca(OH)_2$ [5]. A major disadvantage of adding these chemicals, however, is that they can cause secondary pollution. Some toxically safe flocculating agents recognised are e.g. potato starch derivatives and chitosans, and these are suitable for initiating sedimentation [5, 38].

Some microalgae may flocculate naturally, so-called bioflocculation. The process is often induced by turbulence stress and results in formation of biopolymers by extracellular enzymes. The polymers can also be produced by bacteria associated with the algal cells, but in both cases this leads to changes in the surface charge that in turn causes the cells to aggregate [38, 39]. Especially some species of cyanobacteria forms flocs spontaneously. This allows easy harvesting of the biomass. Some cyanobacteria are also able to form gas vacuoles that makes them accumulate at the surface, and some tend to settle under certain circumstances, and both these features makes them easy to harvest [28].

Filtration

Filtration can be carried out at all scales, from coarse screening to sand filters to diaphragm filter presses where 100 % of the algae is harvested [33, 38]. A process known as microstraining can be applied to large colonial or filamentous algae, such as the cyanobacterium *Spirulina* or the nitrogen-fixing species. Microstrainers are devices consisting of a rotating fine-mesh screen and a backwash to collect the algae. They achieve about 20-fold concentration, or higher, and can be complemented with a final more expensive concentration of the biomass [28].

Biological filtration

Biological filtration means feeding of easily harvested filter feeders with algae, and is consequently a form of aquaculture. Well known filter feeders are mussels and cladocerans like *Daphnia* spp. [5]. Complete food chains starting with wastewater have been studied in order to develop integrated systems able to generate useful biomass simultaneously with effluent purification [40]. Pathogen safety of such biomass does not appear to be of major concern although more complete and systematic monitoring of pathogens should be made before the final edible biomass (fish in general) is available for human consumption [5].

General advice on operation

Domestic wastewater is very nutrient rich, and basically all nutrients needed for algal growth are present [24]. The factors limiting algal growth, and hence treatment efficiency is therefore more likely to be light and carbon. Light is the most important parameter to optimise, and hence culture depth and turbulence are vital for good performance. To avoid temperature limitation in northern climate, greenhouses would be recommended in order to have functioning treatment during longer periods than just during summer. To increase the performance during winter, artificial light may also be needed, however that demand extra costs for energy.

The easiest way to start a microalgal wastewater treatment process is to inoculate with water containing a large variety of algae, e.g. water from outdoor ponds. This will create a mixture of algae and other organisms, where the best suited species will grow fastest and dominate the treatment step. Other microalgae will also be introduced eventually, partly from the wastewater itself, partly from algal particles in the air dust. This approach requires less supervision and operation than if a particular alga is chosen to be cultivated for any purpose. A drawback is that the fastest growing microalgae are most often unicellular green algae (*Chlorophyceae*) which are difficult to harvest [37].

Depth

Depths of between 15 and 50 cm are generally recommended. During winter, however, shallower depths than 20 cm should not be used to account for the decreased incident light intensity. [9, 28]

Turbulence

Turbulence can be achieved in many ways, e.g. with air-bubbling, propellers or paddle wheels. Air injection provides CO_2 and N_2 for nitrogen fixation and may be complemented with extra CO_2 (1–5 %). One study reports optimal growth at an air flow of 60 litres liter^{-1} h^{-1} [9], however, if turbulence is the only purpose for the bubbling, lower flows may be enough as well.

A mild and economic method is the use of paddle wheels. According to Oswald (1988), microalgae stirred with paddle wheels also tends to agglomerate and settle when removed from the mixing field, a tendency never observed in shallow ponds mixed by other methods [18]. Mixing velocities of 5–20 cm s^{-1} are common in e.g. raceway ponds, and too high turbulence can be damaging to the algae [19, 28].

Hydraulic retention time

The hydraulic retention time (HRT) should be long enough to prevent the treatment step from wash-out effects, i.e. it should not be shorter than the minimum generation time of the algae (i.e. the dilution rate should not exceed the maximum algal growth rate, μ_{max}). On the other hand, too long HRT allows the algae to grow slower due to nutrient limitation and increased internal shading, and should also be avoided. The effluent concentrations of nitrogen and phosphorus will, on the other hand, be lower at longer HRTs. Between 2 and 7 days HRT are common in microalgal wastewater treatment [12, 28]. During winter, longer retention times would probably be necessary than during summer as a result of the lower growth rate.

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