KNOWLEDGE IN ALGAL CULTIVATION TECHNOLOGY

1 ALGAL POND TECHNOLOGY

1.1 The terminology clarification

Let us start with a terminological note: Although the project uses the term HRAP or High rate algal pond, we are refraining from this term. The term HRAP is indeed used frequently in the literature, but we attribute this to simple inertia. Why *high rate*? High compared to what? The term was invented in early days of algal technology as a contrast to “low rate” lagoons that were alternatively used for wastewater treatment. As this term is at least misleading, if not completely wrong, we will use a very general term *Algal pond* instead. When we want to limit the conversation to raceway shaped algal pond we will use *Raceway algal pond* and abbreviate it as *RAP*. Many claims that we make are true also for *Photobioreactors* (abbreviated *PBR*) which could also be used very generically to mean any vessel containing algae with purpose of their cultivation (i.e. including algal ponds). But common usage has developed into a distinction that PBR means closed systems with usually short optical path and pumping as a means of mixing while RAP means an open system, typically raceway shaped with paddlewheel mixing. But some ponds are placed in a greenhouse, some are very tightly covered, so the distinction between RAP and PBR is really small.

When we are at terminology, we should also justify our misuse of the term *algae*. Algae in modern taxonomy denote Eukaryotic, mostly aquatic organisms, similar to plants, unicellular, or colony forming with non-differentiated (or just slightly differentiated) tissue. The term used to include (some decades ago) Prokaryotic blue green algae that are now consistently classified as bacteria. Algae are further divided into microalgae and macroalgae simply on their size. Some microalgae form multicellular colonies, some macroalgae are just large enough colonies of non-differentiated (microalgal) cells; some macroalgae have differentiated tissues. Aquatic organisms are further classified as planktonic (free floating) and benthonic (attached). The emerging field of algal technology (which is more arts and crafts area of exploiting scientific knowledge of algae; the science of algae is frequently referring to itself as phycology) is not concerned much with taxonomic details or algae, cyanobacteria, macro or micro and similar. For technological purpose we will talk about *algae* in a very general term – this includes cyanobacteria, eukaryotic algae, some macroalgae. We occasionally call our organisms *microalgae*, only if we have to distinguish them from *macroalgae*, but we usually do not care much. So we can say that we want to call with the name *algae* any photosynthetic aquatic organism that we can cultivate and apply to it some of our cultivation tools like pumping, filtering, centrifugation, and similar.

1.1.1 Paddlewheel

The idea of paddlewheel associates with traditional water mill. This paddlewheel system works now for hu0ndreds of years due to its simplicity. As the water pushes each paddle in the direction that causes the wheel to rotate – as it turns around, paddles are lifted out of the water on the downstream side, and new ones enter on the upstream side. The turning force (‘torque’) is formed by the paddles resisting the flow of water (Moore, and Boyd, 1992). In general RAP`s paddlewheel is a reverse hydroelectric plant, where electrical energy is converted to kinetic energy of water by paddlewheel. Hydroelectric plants convert the kinetic energy of moving water into mechanical energy where a generator converts the mechanical energy from the paddle into electrical energy.

Since their first development, RAP systems have been rapidly spreading worldwide due to their simplicity and cost-effectiveness (Mustafa, Phang and Chu, 2012). Compared to the conventional electromechanical systems used in activated sludge waste water treatment, construction costs are lower than half, they need less energy and have much lower operational costs (Park et al., 2011). Consequently, various RAP designs, aiming at better optimization of WWT and biomass production, are still being developed (Mehrabadi, Craggs and Farid, 2015).

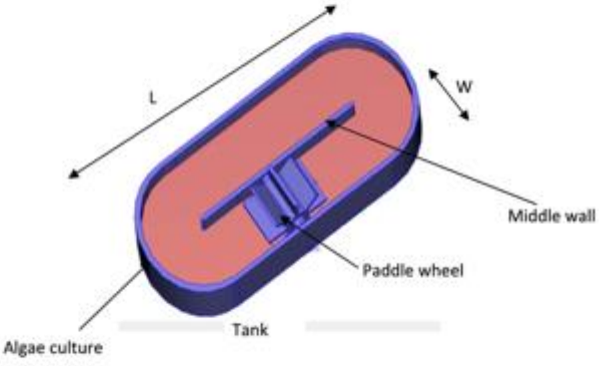

Figure 1: The geometry of a single unit raceway pond (Hadyanto et al., 2013)

While photobioreactors are capable of producing algal biomass at much higher concentrations than in RAPs, earth-lined RAPs are considered a more viable option for commercial-scale biofuel production due to their simpler design and construction as well as reduced capital and operational costs (Park & Craggs, 2011; Hadiyanto et al., 2013).

In RAP, a single-unit raceway pond consists of a tank and paddlewheel to generate circulation flow in each channel and to ensure that the turbulence is achieved (Figure 1). The turbulent flow is important for enhancing homogeneity of light–cell interaction as well as avoiding settling of cells at the bottom of pond. Moreover, turbulent flow prevents thermal and oxygen stratification in the pond. The common velocity used in such ponds is in the range of 10–50 cm/s. The depth ranges from 0.05 to 1.0 m (Archimede, Park et al., 2011) to ensure homogeneity of mixing and sunlight penetration along the culture.

Optimisation of the algal pond environment is critical for the maintenance of a balanced bacterial/algal community enabling effective wastewater treatment. Organic nutrient loading rate, hydraulic retention time, pond depth, CO_2 supply, as well as vertical and horizontal mixing rates are the main operational parameters that can be modified in an algal pond (Craggs et al., 2014). Although RAPs have already been utilized for several decades, the specific optimisation of these parameters for both, wastewater treatment and biomass production is still an on-going process (Rawat et al., 2011). Given that reported photosynthetic efficiency is 1/5 of the theoretical maximum at best (by some authors; this is a topic of discussion in itself), further research is necessary to understand how the operational parameters impact on the physiology, photosynthetic capability and productivity of algae cultivation. While it remains to be seen if the theoretical maximum is actually achievable, there may be opportunities to further enhance algal biomass production and wastewater nutrient removal through improved operation of algal ponds (Sutherland et al., 2014).

1.1.2 Mixing

Algal ponds have been traditionally mixed by paddlewheels. This configuration is so classical that alternative designs are usually not considered. Here we are trying to reconsider this basic design by looking at other fields that were using paddlewheels and have replaced them with alternatives and looking at other implementations of mixing. This is for now a mental exercise, as all demonstration sites in Saltgae project will use paddlewheel mixing. But in parallel we might analyze alternative designs and implement a pilot scale test pond if one of the approaches will seem promising.

Paddlewheels were used as early water turbines in mills, saws and other early installations harnessing water energy into mechanical motion. Paddlewheels have been used for maritime applications 200 and more years ago and they were replaced by propellers in the time between 1827 and 1880. It seems that these applications are very similar: in both cases we are propelling the water relative to the paddlewheel. Detailed examination shows that beside the similarity there are also very deep differences in the requirements: marine applications are typically used at much higher speeds: water speed in algal ponds is at maximum around 0.5 m/s (approx 1 knot) while ship propulsion focuses at speeds in the order of 10 – 120 knots (for large freighters to racing boats). In marine applications seaworthiness (usability in harsh weather and sea conditions) is of primary importance and they also highly value manoeuvrability, flexibility, low weight and many other factors that are less important for algal ponds. Marine applications give no consideration to shear forces (except as their influence on cavitation), they do not consider efficiency of vertical mixing.

Mixing technologies are very common in bioreactor of all sizes from small axenic reactors used to produce genetically engineered products, to large scale anaerobic digestors. As seen in bioreactors, mixing might be implemented by pumps and also by bubbling. The last alternative seems particularly interesting in case of algae since we have to bubble with CO_2 anyway. However, it has turned out that using bubbling in order to provide fluid motion is inherently less efficient in terms of energy required. There are occasions where such bubble based motion is advantageous (in some types of PBR, in mammoth pumps used in deep underwater applications), but no known algal pond application claims that bubbling is advantageous as a means of mixing.

Alternatives have been proposed: Aqualia in their All-Gas project has developed a low RPM propeller mixing that they claim is up to 60% more energy efficient than paddlewheel mixing. This approach was designed in analogy to the paddlewheel vs. propeller propulsion for ships in early 19th century. Although the results by innovative mixing by Aqualia are good (patented), the large pond for the All-Gas projects have been built using conventional paddlewheels. Reasons for that are difficulties in preparing the propeller ducts at large scale and the fact that energy consumption for mixing is just a small fraction of total energy consumption [personal communication].

Other alternatives have been used in different implementations: PBR cultivation of algae was developed at various locations and with various shapes and geometries, early developers in this area include group of Emilio Molina at University of Almeria, group of Rene Wijffels at Wageningen University, group of Mario Tredici, Giusepe Torzillo and Graziella Chini Zittelli at CNR and/or University of Florence, and many others. Some other concepts include vacuum lifting and releasing of a water column (Heliopur/Aqualia/CENTA), hydrofoil induced eddies and mixing by slitted board that is pulled from side to side inducing primarily vertical vortices (Vonshak, 1997). While PBR cultivation seems to be effective, but not cost effective, there are no good reports from the other approaches but “good results in the pilot installation”.

We cannot omit mentioning John Benneman, a collaborator of prof. Oswald who is advocating open ponds with paddlewheels from early 1960 to today as the most optimal and cost effective solution for wastewater treatment and (possibly) algal biofuels production.

Cultivation in PBR and many other attempts were focused to algal biomass growth and less so to wastewater treatment. Objectives and boundary conditions in these two use-cases are different, so might be the optimal solutions.

1.2 Algal production - Parameters influencing algal physiology

Fundamental to success of both, the enhanced wastewater treatment and biomass productivity is the optimisation of the performance of the algae. Despite RAPs being an established technology, algal photosynthesis and productivity is still limited in these ponds (Sutherland et al., 2014). A theoretical maximum photosynthetic efficiency (defined in this instance as the conversation of sunlight energy into biomass) is predicted as maximally 5% (Benemann, 2008), although some authors predict even higher: 10–13% of solar irradiance (Sutherland et al., 2014 and the references within). Actual photosynthetic efficiencies achieved until now are between 1% and 2% (Stephenson et al., 2011 and references within).

1.2.1 Light availability

Light limitation is usually the main growth limiting factor of algal performance in RAPs. Light reaching the surface of the pond varies on diurnal and seasonal scales. The high biomass concentration and high concentration of non-algal particulate matter in the wastewater affect the amount of light that can reach lower layers of the pond, often with up to one third of the water column receiving insufficient light to support net photosynthesis (Borowitzka, 1998). For example, an algal concentration of 300 mg TSS/L will absorb almost all of the available light (PAR) within the top 15 cm of the RAP, leaving the rest of the pond depth in the dark (Park et al., 2011). As a result, cells near the surface are exposed to supersaturating light and photoinhibition or even photodamage, whereas cells near the bottom of the water column receive little to no light. Consequently, photosynthesis is suboptimum, negatively impacting biomass yield. Light limitation, temperature and nutrient concentration can all modify the chlorophyll content of a cell, which affects the chlorophyll specific light absorption and ‘‘package effect’’ (internal self-shading) (Beardall et al., 2013).

From practical experiment as we can see it on Figure 2 it is clear that in a reality a 2% algae concentration means that a light can hardly pass more than 2cm in to the medium. In addition, we should bear in mind that Figure 2 illustrates pure water with chemicals as nutrients, and Spirulina. In case we add wastewater as nutrient source this situation is even worse. This situation can suggest us two things. First is that horizontal mixing is unnecessary, second is that great deep is irrelevant if we can't ensure fast and economical vertical mixing.

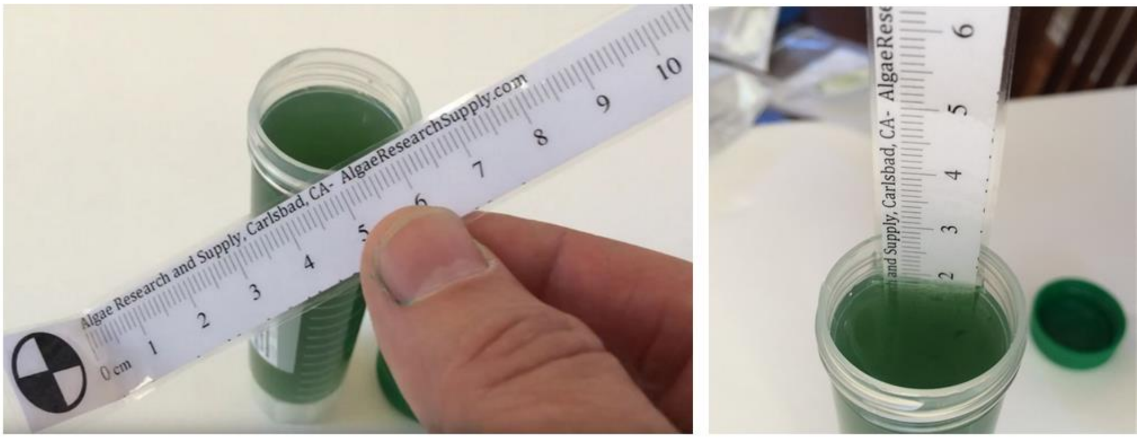

Figure 2: In reality 2 g/L concentration means that light can hardly pass more than 2cm in to the medium (image source: algaeresearchsupply.com)

1.2.2 Temperature

Water temperature varies on diurnal and seasonal scales, affecting photosynthesis and respiration rates. Algal productivity increases with increasing pond temperature up to an optimum temperature above which increasing algal respiration and photorespiration reduce overall productivity (Park et al., 2011). Most algal species have an optimum temperature range between 15 and 35 degrees C (Sutherland et al., 2014), but can be higher, depending on nutrient and light limitations (Park et al., 2011).

1.2.3 Carbon availability

Heterotrophic metabolism of organic matter in the wastewater treatment RAP, resulting in the generation of CO_2, supplies an estimated 25–50% of the dissolved inorganic carbon (DIC) required by the algae (Borowitzka, 1998). In some wastewater cases, algal productivity is nevertheless carbon limited due to the low carbon/nitrogen ratio of wastewater (municipal wastewater typically 3:1) compared to algal biomass (typically 6:1) (Park & Craggs, 2011). In such cases it makes sense to provide additional carbon in the form of CO_2.

1.2.4 pH

pH in algal ponds varies over the day, increasing with photosynthetic drawdown of carbon and decreasing overnight with respiration (Sutherland et al., 2014). pH shifts the DIC equilibrium with a reduction of available CO_2 with increasing pH. The pH optimum for many freshwater algae is around 8 and aerobic heterotrophic bacteria that oxidize organic matter in wastewater treatment RAP have an optimum pH of 8.3, above which bacterial activity is increasingly inhibited (Park et al., 2011). The elevated pH can act to enhance NH_3-N removal from the pond liquid via ammonia volatilization and phosphorus removal through phosphate precipitation (Park et al., 2011). Additional factors that influence pH and mineral balance are nitrification and denitrification processes and ions that are introduced to water with flue gases – if they are used as CO_2 control. In reality (with naturally occurring algae) we have observed periods of naturally occurring very low pH values in the range of 5-6, without introduction of any CO_2 [results from AlgaeBioGas-Slovenian demo centre].

1.2.5 Dissolved oxygen concentration

Intense photosynthesis in algal ponds can increase dissolved oxygen levels to >200% saturation during daytime (saturation levels are decreasing with the temperature). High dissolved oxygen levels in excess of normal air saturation are believed to impact on algal productivity (Park et al., 2011 and the references within).

In some cases [paper mill wastewater pilot, Albaqua project, Algen unpublished results] we have observed relatively slow release of oxygen from the algal cells providing high oxygen levels well into the dark period. In such cases we were tempted to increase the volume of algal bacterial treatment vessel also with a dark section where excess oxygen could be used by bacteria. Such dark sections could also be used for ‘classical” aeration during the period of low light conditions or if the microbial community leans to bacteria only (which may happen from time to time).

1.2.6 Nitrogen and phosphorus concentration

Nitrogen and phosphorus are both limiting growth factors. N:P ratios above 30 suggest P limitation and below 10 N limitation (Dodds, 2003). In some cases, improved nutrient removal and significantly greater biomass yields were shown when N and P starting concentrations were stoichiometrically balanced (Sutherland et al., 2014). Another potential problem that may arise is the ammonia toxicity. Toxic levels are widely different for different algae (and presumably also bacteria). For some species ammonia toxicity start at concentrations as low as 100 mg/L, some species easily survive 2000 mg/L. There are two processes that lower ammonia levels: volatilization at higher pH and nitrification by nitrifying bacteria.

The ratio of N:P in algal biomass can vary from about to 4:1 to almost 40:1 depending on algal species and nutrient availability in algal culture, therefore, high productivity may be achieved even at relatively low N:P ratios in algal bacterial ponds (Park et al., 2011).The exact ratio N:P and ammonia concentration of course depends on the microbial community at work in the algal pond, or said better, microbial community adapts to the available levels. This is also true for the toxicity of ammonia – only tolerant species survive.

1.2.7 Zooplankton grazers and pathogens

Grazing by herbivorous protozoa and zooplankton (e.g. Rotifera and Cladocera) as well as fungal parasitism and viral infections can significantly reduce the pond algal population within a few days (Park et al., 2011 and the references within). In many cases diverse algal community is much more immune to quick collapse than any monoculture. We have observed wildly different threatening grazer communities: in some instances, zooplanktonic ciliate, rotiferae and other macrozooplankton were dominant; in other cases, we had attacks of Chironomidae larvae or Soldier fly larvae. These insects seem to be very effective in destroying algal culture, so we are using insect nets over all openings of the greenhouse and an additional one over inoculation pond to prevent massive insect attacks.

1.3 Pond design upgrades and modeling

Due to the capability of RAP to produce algae biomass at a large scale, the design of these ponds is still a major issue in this field. Besides environmental parameters (pH, temperature, light intensity, CO_2 concentration, nutrients), the hydrodynamic characteristics are critical to obtain high algae productivity (Hadyanto et al., 2013). Hydrodynamic mixing is required to ensure frequent exposure of algae cells to light, to avoid the settling of algae cells, to homogenize the nutrient distribution and to enhance the utilization of CO_2 in the pond (Hadyanto et al., 2013). Current RAP designs still result in the appearance of dead zones where the flow is stagnant, and non-uniform velocity (or said better low vertical velocities) throughout the pond, which present major problems due to the negative impact on algae growth (Hadyanto et al., 2013).

RAP is an endless loop shape pond system with shallow water, generally 20-30 centimetres deep (Figure 3), but with implementations as low as 5 cm (Archimede) and as high as 1 m. Fluid content is gently moved by electrical driven paddlewheel, with typical flow speeds of around 10-50 cm s^{-1} (de Godos et. al., 2009) to prevent algal settling and allow sufficient illumination for optimal algal growth.

Figure 3: Typical RAP as endless loop with water moved by electrically driven paddle wheel. Depth of roughly 20-30 centimeters (www.wikiwand.com/en/Algae_fuel)

The basic geometry design of raceways depends on the ratio between the length and width of the pond's channels (L/W) besides other factors such as economic aspect, hydrodynamic mixing and carbon dioxide uptake.

The turbulent flow has an important effect on algae growth and therefore needs to be considered in the pond design. Depending on the type of the algae, turbulent flow may improve the productivity by enhancing mass transfer, enhancing algae cell exposure to the sunlight, decreasing the effect of the photo-inhibition, preventing cell settling and reducing exchange transfer barriers around the cells [see references no. 64-68]. An increase of turbulence is normally achieved by increasing the rotation speed of the paddle wheel. Richmond (1980), showed that doubling of the velocity could increase algae productivity by 50%. However, the increase of velocity also requires more mixing power. Besides velocity, the dimension of the pond affects the power consumption.

1.3.1 Mixing

The performance of mixing can be determined by variation of the hydrodynamic properties (circulation velocity, presence of dead zones and shear stress) which are influenced by the geometry of the pond and the paddlewheel (Cornet et al., 1998). If the mixing velocity is not sufficiently high, the cells will accumulate in the areas with the low flow. In these ‘‘dead zones’’ (stagnant areas) anaerobic conditions are developed, resulting in propagation of anaerobic bacteria and, to a limited extent, a reduction of the physical volume of pond, thereby decreasing the residence time and cultivation efficiency (Shilton, 2006). Under certain conditions, this area is also a potential place for formation of toxic compounds (Becker [69] in Hadyanto et al., 2013) or at least unwanted or even pathogenic bacteria (Algen experience). We have examined mixing performance within this project with a CFD model.

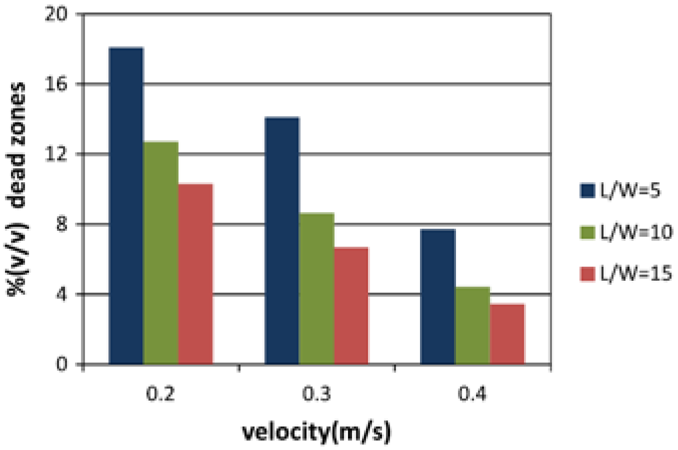

Figure 4: Dead zone in RAP (water depth 25 cm) at the different L/W and water velocities (Hadiyanto et al., 2013)

1.3.2 Shear stress

The hydrodynamic stress conditions directly influence the productivity of algae by shear stressing – sensitive algae (Musgrove and Heaven., 2014). High degree of mixing, and subsequently a high fluid velocity, is known to present a limitation on the growth of sensitive algae cells by damaging the cell structure [70-72]. The yield of algae production initially increases with the increasing turbulence, probably due to the improved supply of CO_2 or dark-light cycle frequency, but after reaching the optimum value yield deteriorates rapidly with further increase of turbulence (Hadiyanto, et. al., 2013). While shear stress presents a great problem in photobioreactors, it hardly gives significant contribution to the cell damage in large open pond systems (Hadiyanto et al., 2013). According to Hadiyanto et al. CFD modelling (2013), shear stress in larger ponds results in lower shear forces (Figure 5).

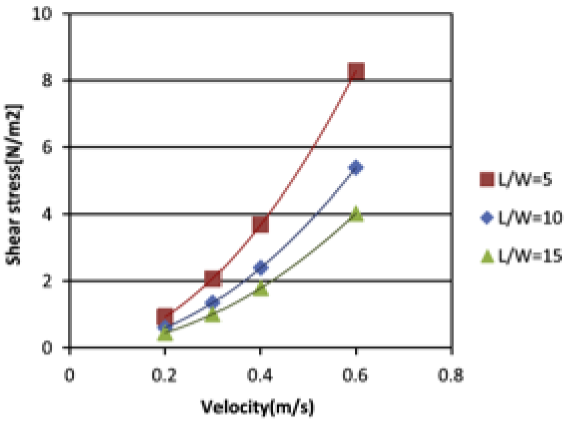

Figure 5: Shear stress for different velocities and pond sizes based on CFD (Hadiyanto, et. al., 2013)

Some shear stress calculation have been done within this project.

1.3.3 Power consumption

For any energy application power consumption is a primary design consideration; paddlewheel performance may be important part of this. (Passos et. al., 2014). In wastewater the power consumption is slightly less important, but it is still an important part of the cost. The power consumption is mostly determined by the hydraulic power to generate liquid velocity in the channel (Hadiyanto, et. al., 2013). The actual power for a raceway is dependent on the raceway channel length (Figure 6). Presumably, more energy is consumed when moving the water along a longer channel. Hadiyanto and co-workers (2013) suggested that a constant depth the larger length / width (L/W) ratio results in less power consumption per unit area than smaller L/W ratios. On the other hand, relationship among L/W ratio and essential energy use per area is not linear and is reached at L/W> 15. As a result, making raceway with a higher L/W ratio is energetically more beneficial. At the same L/W ratio, the increase of energy needed for paddlewheel becomes exponential function of water velocity. The optimal L/W ratio is at least 10, while the power consumption is approximately cubic function of the applied velocity. Additionally, the shape of bends significantly affects affects the required energy to circulate the water flow as much of the energy is lost in (horizontal eddies) (Hadiyanto et. al., 2013, Liffman et al., 2013). We have performed some CFD calculations within the project.

RAPs have the central dividing wall for splitting the streams. Diverse designs seek to diminish the energy consumed in moving water around the raceway pond and to ensure there are regions with vortices that separate a region from the main flow. Such division zones are not optimal for algal growth because they can isolate algae from nutrients, diminish their exposure to light and consequently increase the algal death rate. Some engineers use a thicker divider as mounds, while others use asymmetrical or rounded turning vanes at the ends of raceway (Hadiyanto et. al., 2013, Liffman et al., 2013).

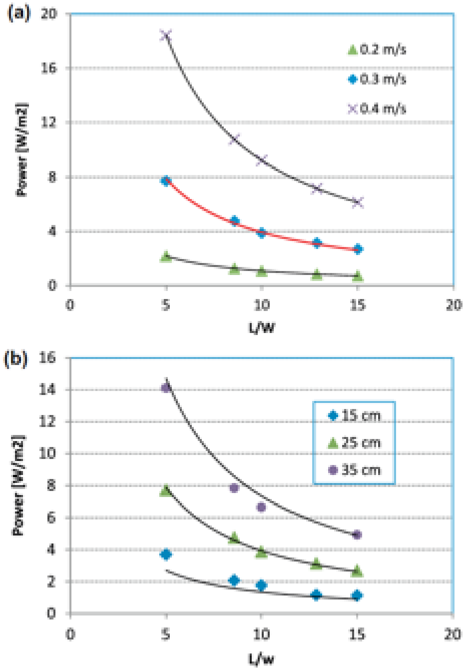

Figure 6: Power consumption for different L/W ratios of the pond with (a) variation of velocity at constant pond depth of 25 cm; (b) variation of pond depth at constant velocity 30 cm/s; points represent CFD calculations, lines represent head loss calculation

1.4 Possible modifications

1.4.1 Biological

The ideal attributes of algal species for use in wastewater treatment RAPs are: (1) high growth rate (high productivity) when fed with wastewater nutrients which are predominantly NH_3-N and phosphate-P; (2) tolerance to seasonal and diurnal variation in outdoor growth conditions; (3) form aggregates thereby enabling simple gravity harvest (Park et al., 2011). More than 1000 algal taxa have been reported one or more times as pollution tolerant which include 240 genera, 725 species and 125 varieties and forms (Abdel-Raouf et al., 2012). The most tolerant genera include eight green algae, five blue-greens, six flagellates and six diatoms (Abdel-Raouf et al., 2012). From different sources, cited by Abdel-Raouf and co-workers (2012), (1) the most frequent genera in a waste stabilization ponds were *Chlorella*, *Ankistrodesmus*, *Scenedesmus*, *Euglena*, *Chlamydomonas*, *Oscillatoria*, *Micractinium* and *Golenkinia*; and (2) the most tolerant eight genera were found to *be Euglena*, *Oscillatoria*, *Chlamydomonas*, *Scenedesmus*, *Chlorella*, *Nitzschia*, *Navicula* and *Stigeoclonium*. The widely used algae cultures for nutrient removal are species of *Chlorella*, *Scenedesmus*, and *Spirulina* (Rathod, 2015 and the references within). Many of the algal species (*Scenedesmus* sp., *Micractinium* sp., *Actinastrum* sp., *Pediastrum* sp., *Dictyosphaerium* sp., *Coelastrum* sp.) that dominate wastewater treatment RAPs often form large colonies (50–200 lm) (Park et al., 2011).

Attempts to grow introduced algal species in RAP as monocultures for periods greater than 3 months have all failed due to contamination by other native algae and/or zooplankton (Park et al., 2011). However, Bhattacharjee and Siemann (2015) successfully utilized monocultures of *Spirulina platensis* UTEX LB 1926 (Cyanobacteria), *Chlorella* sp. UTEX 2248 (Chlorophyta), and *Scenesdesmus obliques* UTEX 393 (Chlorophyta) for WWT and biomass production in open tanks for 14 weeks.

Zooplankton grazers may be controlled through physical (filtration, centrifugation, low DO concentration/high organic loading) and chemical treatments (application of chemicals/invertebrate hormone mimics, increase in pH and free ammonia concentration) (in Park et al., 2011). Presently there are no general treatments to control fungal infections.

1.4.2 Chemical

(1) **Addition of CO_2 gas** has been shown to enhance biomass production and nutrient removal in wastewater algal ponds through co-beneficial carbon supplementation and reduced pond water pH (Park and Craggs, 2010, 2011; Sutherland et al., in press). While CO_2 addition is standard practice in commercial algal production, it is not currently used in wastewater RAPs, except for a few small scale experimental trials (Park & Craggs, 2011). CO_2 addition in pilot-scale wastewater treatment RAPs (4-day HRT) was shown to improve algal/bacterial biomass production 30% (Park & Craggs, 2011).

Since the C:N ratio in wastewater is less than that of algal biomass, algal production and wastewater nutrient removal in algal ponds could be enhanced by daytime CO_2 addition (Park & Craggs, 2011). A consequence of carbon limitation of algal growth is the daytime increase of the pond water pH. Adding CO_2 to control pond water pH between 7.5 and 8 is regarded as sufficient to overcome carbon limitations in wastewater (Park & Craggs, 2011; Craggs et al., 2013). Sutherland et al. (2014d) showed that, due to rapid uptake of carbon by the algae in summer, CO_2 additions at least every 30 minutes were required to maintain adequate pH control. For full-scale systems with circuit times greater than 30 min this would require multiple CO_2 addition points to ensure carbon limitation is adequately mitigated.

One of the main challenges is to apply sufficient CO_2 cost-effectively. A CO_2 addition sump can be included in the full-scale wastewater RAP design (Figure 8) to allow for carbon augmentation of the culture (Craggs et al., 2012; Musgrove & Heaven, 2014) CO_2 can be added into a counter current gas sparging sump (1.5 m depth) creating turbulent flow within the pond (Figure 7).

While nutrient removal by physico-chemical processes such as ammonia volatilization and phosphate precipitation may be reduced by CO_2 addition to a wastewater treatment RAP, it has been shown that this reduction in treatment can be offset by the increased algal production and associated nutrient assimilation into this biomass (Park & Craggs, 2011).

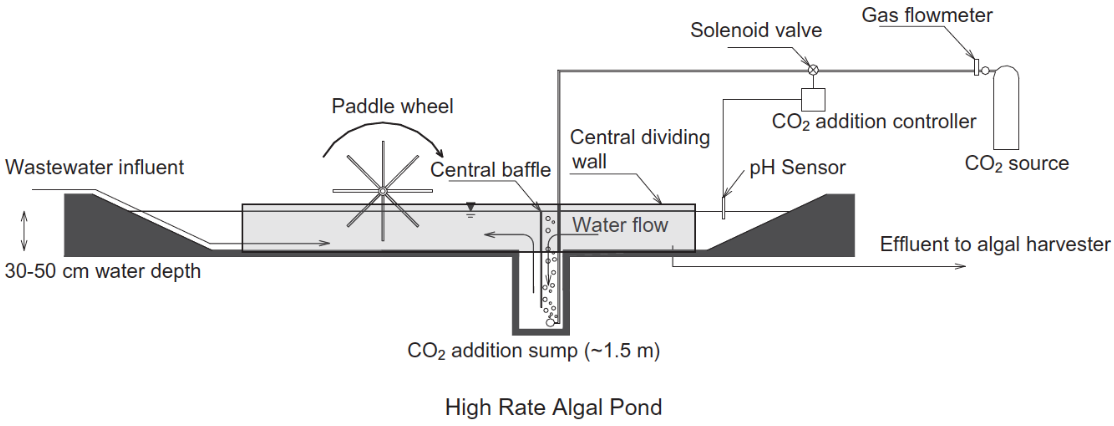

Figure 7. CO_2 addition sump in RAP (Park et al., 2011)

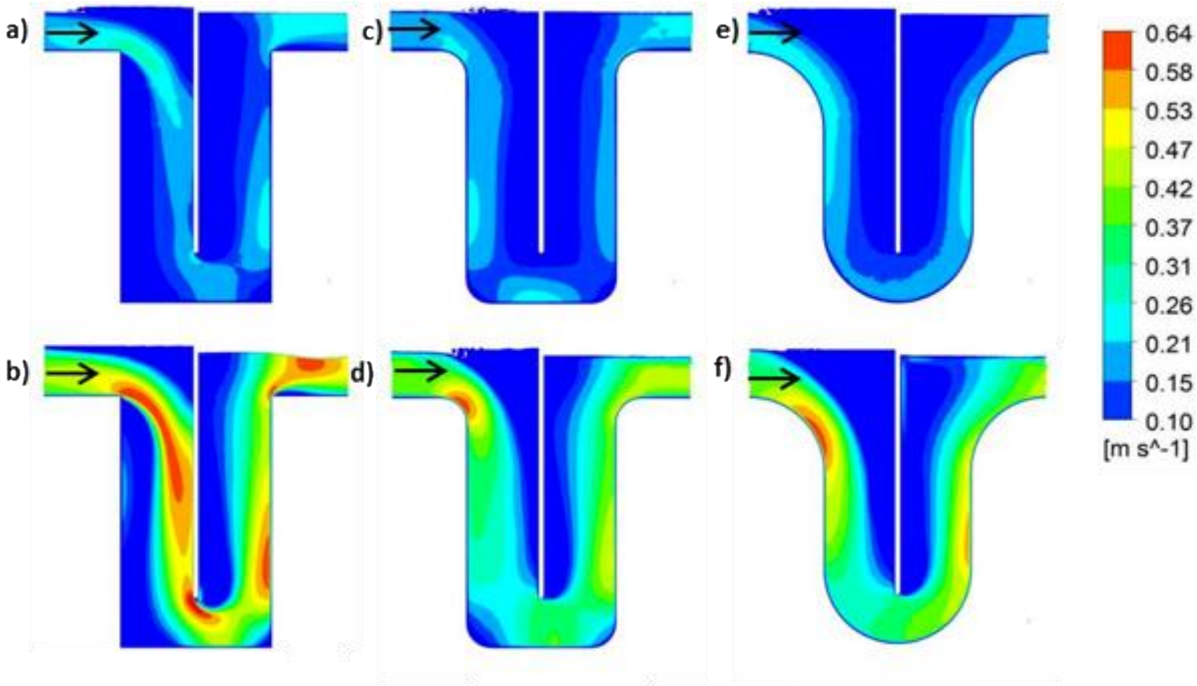

Figure 8: Flow velocity profiles of different sups with varying inlet velocities (Musgrove & Heaven, 2014)

Figure 8 shows flow velocity profiles of different sumps with varying inlet velocities. a) Basic with inlet velocity of 0.15ms-1, b) Basic with inlet velocity of 0.35ms-1, c) Radii of 0.1m with inlet velocity of 0.15ms-1, d) Radii of 0.1m with inlet velocity of 0.35ms-1, e) Radii of 0.3m with inlet velocity of 0.15ms-1, f) Radii of 0.3m with inlet velocity of 0.35ms-1. The black arrows indicate the direction of flow. The dark blue areas indicate where velocity has dropped below 0.1ms-1.

(2) **Nutrient load:** Addition of the limiting nutrient to optimise N:P ratios is cost prohibitive for most wastewater treatment systems. Some manipulation of N:P ratios can be achieved through practises such as dilution of influent with harvester effluent return, particularly if removal rates between N and P differ, however, this is unlikely to be sufficient enough to fully address limitations (Sutherland et al., 2014).

Nutrient load is influenced by the wastewater quality and hydraulic retention time. Lower nutrient loads result in higher effluent water quality, but at the expense of photosynthetic efficiency and biomass yields (Sutherland et al., 2014). Nutrient loads can be altered also through changes in hydraulic retention time.

1.4.3 Physical

(1) **Pond depth:** Depth is crucial for modifying the pond light environment and governing biomass concentration. Nevertheless, guidelines for RAP operational depth are unclear (Sutherland et al., 2014). Optimum depths for RAPs reported in the literature range from 15 to 100 cm (Park et al., 2011), while some propose the RAP should be as shallow as possible to provide maximum illumination, neglecting the photodamage effect and thermal instability. A balance between improved light climate and thermal stability is therefore likely to be one of the key drivers for improving algal photosynthesis (Sutherland et al., 2014).

Depth is limited by technical requirements, including the precision of ground levelling, the energy required by the paddlewheel, land requirements, pond construction costs, pond operation and harvesting costs (Sutherland et al., 2014).

(2) **Mixing:** Vertical mixing determines both, the amount and frequency of light exposure an individual cell experiences (Sutherland et al., 2014). In RAPs, mixing enables that all cells are at least briefly exposed to the saturating light at frequently enough for high productivity. Ideally, cells should be optimally exposed to light and then moved into the dark zone, while replaced with the cells from the bottom. Typically, RAPs are designed with a depth of about 30 cm, however, turbulent eddies (resulting from water flow around the pond) and paddlewheel mixing provide a degree of vertical mixing through the pond depth thus ensuring that the algal biomass is intermittently exposed to light (Park et al., 2011).

Increased vertical mixing have been shown to increase algal photosynthesis and productivity due to increased light/dark cycles in photoreactors, while the effects of enhanced vertical mixing on photosynthesis and productivity in RAPs have been less conclusive so far (Vonshak 1997, Sutherland et al. 2014 and the references within). Laws et al. (1983; in Sutherland et al., 2014) recorded a doubling in photosynthetic conversion efficiency with the use of multiple foils to generate and sustain sufficient turbulence in a shallow (<100 mm) outdoor pond.

Regarding harvesting, larger and heavier colonies/floccs are more desirable due to their relative ease of harvesting with simple gravity sedimentation (Park et al., 2011). The challenge is to provide sufficient vertical mixing to ensure these heavy colonies remain entrained in the water column in the pond (Sutherland et al., 2014). Colony size can result in the ‘‘package effect’’, with decreasing light absorption efficiency with increasing colony size, while nutrient and gas exchanges decrease (Sutherland et al., 2014).

In full-scale wastewater RAPs, with water velocity of 0.2 m/s, the circuit time can be up to 90 min and the single paddlewheel mixes a parcel of water once for each circuit pass. Long circuit times coupled with potential for laminar flows and shallow pond depth may favour smaller more buoyant species. The colony size and harvestability were shown to be directly related to the frequency of mixing events, increasing with increasing mixing events (decreasing circuit time) (Sutherland et al., 2014). Understanding how the frequency of mixing events affects the performance of algae, including photosynthesis, productivity, nutrient removal efficiency, physiological and morphological adaptations, is important for enhancing wastewater treatment and biomass yields (Sutherland et al., 2014).

(3) **Hydraulic retention time** (HRT) modifies the biomass concentration in the pond by allowing or preventing biomass accumulation on longer or shorter HRT, respectively (Sutherland et al., 2014). HRT in wastewater RAPs typically varies between 3 and 9 days, depending on the season and latitude. HRT can be altered by modifying pond depth, which will further modify the light climate in the pond, or through dilution with harvester effluent (Sutherland et al., 2014).

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